# ULTRAFAST NONRADIATIVE DECAY AND EXCITATION ENERGY TRANSFER BY CAROTENOIDS IN PHOTOSYNTETIC LIGHT-HARVESTING PROTEINS

By

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# A DISSERTATION

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#### ABSTRACT

# ULTRAFAST NONRADIATIVE DECAY AND EXCITATION ENERGY TRANSFER BY CAROTENOIDS IN PHOTOSYNTETIC LIGHT HARVESTING PROTEINS

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This dissertation investigates the photophysical and structural dynamics that allow carotenoids to serve as efficient excitation energy transfer donor to chlorophyll acceptors in photosynthetic light harvesting proteins. Femtosecond transient grating spectroscopy with optical heterodyne detection is employed to follow the nonradiative decay pathways of carotenoids and excitation energy transfer to chlorophylls. It was found that the optically prepared  $S_2$  (1<sup>1</sup>B<sub>u</sub><sup>+</sup>) state of  $\beta$ -carotene decays in 12 fs fs to populate an intermediate electronic state, S<sub>x</sub>, which then decays nonradiatively to the S1 state. The ultrafast rise of the dispersion component of the heterodyne transient grating signal reports the formation of S<sub>x</sub> intermediate since the rise of the dispersion signal is controlled by the loss of stimulated emission from the S2 state. These findings were extended to studies of peridinin, a carbonyl substituted carotenoid that serves as a photosynthetic light-harvesting chromophore in dinoflagellates. Numerical simulations using nonlinear response formalism and the multimode Brownian oscillator model assigned the S<sub>x</sub> intermediate to a torsionally distorted structure evolving on the S2 potential surface. The decay of the S<sub>x</sub> state is promoted by large amplitude out-of-plane torsional motions and is significantly retarded by solvent friction owing to the development of an intramolecular charge transfer character in peridinin. The slowing of the nonradiative decay allows the S<sub>x</sub> state to transfer significant portion of the excitation energy to chlorophyll a acceptors in the peridininchlorophyll *a* protein. The results of heterodyne transient grating study on peridinin–chlorophyll a protein suggests two distinct energy transfer channels from peridinin to chlorophyll a: a 30 fs

process involving quantum coherence and delocalized peridinin–Chl states and an incoherent, 2.5 ps process involving the distorted  $S_2$  state of peridinin. The torsional evolution on the  $S_2$  state is accompanied by the formation of an ICT character and dynamic exciton localization, which controls the mechanism of excitation energy transfer to chlorophyll *a* acceptors in the peridinin–chlorophyll *a* protein.

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# KEY TO ABBREVIATIONS

ATP	adenosine triphosphate
BChl	bacteriochlorophyll
CCD	charge-coupled device
Chl	chlorophyll
ESA	excited-state absorption
FC	Franck–Condon
ICT	intramolecular charge-transfer
LHCII	light-harvesting complex II
LO	local oscillator
Ν	conjugation length
$\mathbf{NADPH}^{+}$	reduced nicotinamide adenine dinucleotide phosphate
NPQ	nonphotochemical quenching
OPA	optical parametric amplifier
РСР	peridinin–chlorophyll a protein
PSB	protonated Schiff base
SE	stimulated emission
TPM	triphenylmethane

### OVERVIEW OF THE DISSERTATION

The goal of this dissertation is to determine the structural and photochemical mechanism that control and optimize excitation energy transfer from carotenoids to chlorophyll (Chl) acceptors in a light-harvesting protein. The dynamics of nonradiative decay and excitation energy transfer ot chlorophylls have been studied using transient grating spectroscopy with optical heterodyne detection. The heterodyne detection allows a complete characterization of the third-order nonlinear optical signal emitted by a sample in terms of two components in quadrature, the absorption and dispersion components. As discussed in the dissertation, the ultrafast rise of the dispersion signal and detection of different ground state recovery timescales observed in the absorption and dispersion channels provide direct evidence for the formation of conformationally distorted intermediates in the nonradiative decay pathway of carotenoids. The experimental findings are corroborated by numerical simulations using nonlinear response function theory and the multimode Brownian oscillator model. The rest of the dissertation is organized as follows:

Chapter 1 presents a brief overview of light harvesting by carotenoids. The design principle of light harvesting antenna complexes and the mechanisms of energy transfer are introduced first. The complexity in understanding carotenoid photophysics and energy transfer pathways in the peridinin–chlorophyll *a* protein are discussed next. A hypothesis based on conformational dynamics of the conjugated polyene is presented, which could answer many outstanding questions about carotenoid photophysics and energy transfer pathways in light-harvesting proteins. This chapter concludes with a brief discussion of heterodyne transient grating technique that has been employed to address these questions.

Chapter 2 reports results from heterodyne transient grating spectroscopic studies on  $\beta$ carotene in benzonitrile. We show that a simultaneous modeling of the absorption and dispersion components of the heterodyne transient grating signals leads to an unambiguous detection of the  $S_x$  intermediate in the  $S_2 \rightarrow S_1$  decay pathway. Our results also rule out any contributions from the double quantum coherence to the observed signal.

Chapter 3 reports the detection and assignment of a similar  $S_x$  intermediate in case of peridinin in methanol. Numerical simulation of the heterodyne transient grating signals using nonlinear response theory and multimode Brownian oscillator model rules out the possibility of  $S_x$  being a vibrationally excited or hot  $S_1$  state. Rather the  $S_x$  state is best assigned to a twisted conformation of the  $S_2$  state.

Chapter 4 discusses the results of heterodyne transient grating studies of peridinin in a series of polar solvents. It is found that the ICT character in peridinin is developed by out-of-plane torsional motions of the conjugated polyene backbone. The decay of the  $S_x$  state is substantially slowed by solvent friction due to the formation of an ICT character as twisting happens.

Chapter 5 focusses on the energy transfer mechanisms in the peridinin–chlorophyll *a* protein. The results from heterodyne transient grating suggests that the  $S_2$  state of peridinin serves as the donor for two channels of energy transfer to chlorophyll *a* acceptors: a 30 fs process involving quantum coherence and delocalized peridinin–Chl states and an incoherent, 2.5 ps process involving the  $S_x$  intermediate state. The coherent to incoherent transition is promoted by torsional motions which lead to dynamic exciton localization.

Chapter 6 summarizes the overall impact of the dissertation, and future prospects are discussed.

# **Chapter 1: Introduction to Photosynthetic Light Harvesting by Carotenoids**

### 1.1 Photosynthesis: An Overview

Photosynthesis is the fundamental biological process that can harness the power of sun. Organisms like plants, bacteria and algae employ photosynthesis to capture and store solar energy into chemical bonds, which is essential to power life.<sup>1</sup> Though photosynthetic organism varies widely in terms of their habitat, they have similar basic constituents for the photosynthetic apparatus. They all use a light harvesting antenna system that is coupled to a photosynthetic reaction center. Solar energy is absorbed by an array of pigment protein complexes in the light-harvesting antenna and then transferred over hundreds of Ångströms to the photosynthetic reaction center. After receiving the excitation, an electron transfer is initiated in the reaction center and the absorbed energy is harvested as stable charges across a cell membrane. This generates a trans-membrane electrochemical gradient, which is used in the formation of energy storage molecules like adenosine triphosphate (ATP) and/or reduced nicotinamide adenine dinucleotide phosphate (NADPH<sup>+</sup>).

An efficient light-harvesting process is crucial to the success of photosynthesis. Absorption of solar photons by the antenna complex excites the pigment molecules from their ground state to an excited electronic state. Before various radiative and nonradiative processes quench the excited states, excitation energy must be harvested. This is ensured by the ultrafast timescales of the energy transfer and charge separation. Excitations migrate through different pigment-protein complexes and reach the reaction center in just 10–100 ps. The primary charge separation takes place in about 3 ps. The overall quantum efficiency of light-to-charge separation is >90 %.<sup>2</sup> The

design principles that makes photosynthetic light harvesting so efficient, however, remain to be elucidated.



**Figure 1.1** Schematic description of light harvesting and energy transfer to reaction centers in photosynthetic organism. From reference 1. Copyright (2014) John Wiley & Sons, Ltd, used with permission.

## 1.2 Architecture of the Light Harvesting Antenna

The availability of high-resolution X-ray crystal structures has allowed us to determine the structural organization of the light-harvesting antenna complexes surrounding a reaction center. Figure 1.2 shows the structure of main light-harvesting protein found in purple bacteria, Light-Harvesting 2 (LH2) complex<sup>3,4</sup> from *Rhodopseudomonas acidophila*. The LH2 consists of nine circular arrays of  $\alpha\beta$ -heterodimers, with each subunit binding three bacteriochlorophyll *a* (BChl) molecules and one carotenoid. The BChls are arranged in two concentric ring-like structures in LH2. Based on the position of the characteristic Q<sub>y</sub> band of bacteriochlorophylls, the rings are denoted as B800 and B850. The B800 pigments are approximately 21 Å apart from each other and are weakly coupled. In contrast, the distance between individual BChls in the B850 ring is

9 Å. As a result, the excited states of the BChls are strongly coupled and delocalized over the B850 ring forming an exciton manifold. Carotenoids in the LH2 complex are also found to be in



**Figure 1.2** The nonameric structure of the peripheral light harvesting complex (LH2) from *Rhodopseudomonas acidophila*.(1NKZ.pdb)<sup>5</sup> From reference 1. Copyright (2014) John Wiley & Sons, Ltd, used with permission. (a) and (b) views perpendicular to the membrane plane with and without the protein scaffold. (c) and (d) views parallel to the membrane plane with and without the protein scaffold. B800 ring is shown in red and B850 ring is shown in blue. The  $\alpha$  polypeptide is colored yellow and the  $\beta$  polypeptide is colored green. Carotenoids are shown in orange.

close proximity (3.4–3.7 Å) to both the B800 and B850 pigments Carotenoid which plays the role of an accessory pigment, absorb in the blue-green part of the solar spectrum and transfer

energy to BChls. As a result, energy flows downhill from higher energy carotenoids to lower energy bacteriochlorophylls and is delivered irreversibly into the reaction center, the so-called energy funnel.<sup>1</sup>

The use of an energy funneling antenna system becomes critical at low-light conditions due to the low photon flux density of sunlight. As a result, less than one excited states are generated per second. The photosynthetic reaction center, however, requires multiple excitations for a biochemical activity to take place. Accumulating photons at multiple sites and then funneling it to a single reaction center allows the light intensity to concentrate. Moreover, by employing different chromophores, light harvesting complexes can absorb at different parts of the solar spectrum and allows better utilization of the solar spectrum than ever possible by the reaction center alone.<sup>6</sup> Because of their wide range of absorption, the chromophores need to share the excitation energy amongst themselves so that it can reach the reaction center even from the farthest absorbing chromophores. This is ensured by the protein scaffold, which holds the chromophores in proper orientation and distances and thereby, facilitates excitation energy transfer. The structural arrangement of the chromophores and interactions between them and the protein environment often determine the spectral properties and energy transfer mechanisms in the light-harvesting complex.

### **1.3 Energy Transfer Mechanisms in Light-Harvesting Proteins**

The mechanism of energy transfer involves coupling of chromophores via electric dipoledipole interaction, which depends on the relative orientation and distance between the chromophores. The rate of excitation energy transfer is governed by the Fermi golden rule expression

$$k_{DA} = \frac{1}{\hbar^2 c} \left| V_{DA} \right|^2 J_{DA}$$
(1.1)

where  $k_{DA}$  is the rate of energy transfer from the donor (D) to acceptor (A).  $V_{DA}$  denotes the electronic coupling strength between the chromophores. Here  $J_{DA}$  is the overlap integral between the emission spectrum of the donor and absorption spectrum of the acceptor molecule and ensures conservation of energy. Depending on the relative coupling between the pigments themselves and with the surrounding environment, the mechanism of excitation energy transfer can be either be incoherent or coherent types.

### **1.3.1 Incoherent Energy Transfer**

Incoherent type energy transfer happens in the weak-coupling limit in which the dipoledipole coupling between the chromophores are weaker than their coupling to the environment. In this scenario, the excitation essentially gets localized prior to energy transfer. As a result, excitation energy transfer occurs by hoping from one chromophore to another. In this scenario, the Fermi golden rule takes the form of Förster theory and the incoherent hopping rate is given by<sup>7</sup>

$$k_{DA} \propto \frac{\kappa^2}{\tau_D R^6} \int f_D(v) \varepsilon_A(v) \frac{\mathrm{d}v}{v^4}$$
(1.2)

where  $\kappa$  is the mutual orientation factor of the transition dipoles, *R* is the distance between them and  $\tau_D$  is the radiative lifetime of the donor. The integral includes spectral overlap between the emission spectrum of the donor  $f_D$  and the absorption spectrum of the acceptor  $\varepsilon_A$ . The incoherent Forster energy transfer rate, therefore, strongly depends on the transition dipole strength of the donor. The Förster theory, however, is inadequate to describe excitation energy transfer in photosynthetic systems because of close-packed packing of the chromophores within the light-harvesting proteins.

## 1.3.2 Coherent Energy Transfer

The close proximity of the chromophores in the light harvesting proteins results in relatively strong coupling amongst themselves and the coupling strength varies from several tens to several hundred wavenumbers. If the electronic couplings between the chromophores are stronger than their coupling to the surrounding bath, then the excitations are delocalized over the excited state of the chromophores. Formation of such delocalized excited states or excitons allow rapid transfer of energy not limited by the Forster rate.<sup>8</sup>

Indeed, the protein has the ability to tune the electronic couplings amongst the chromophores by changing their electronic properties of the chromophores and thereby determines influence the mechanism of excitation energy transfer.<sup>8</sup> Experiments by Fleming et al.<sup>9,10</sup> have suggested that relatively long lived electronic coherences, delocalized excited states spanning several chromophores, may enhance the quantum efficiency of light harvesting. These and other results<sup>11–14</sup> suggest that the energy-transfer and nonradiative channels determining the fate of a captured photon in a light-harvesting protein are exquisitely balanced by the microscopic details of the electronic interactions between light-harvesting chromophores and by the structure and dynamics of the surrounding protein and solvent medium. A proper understanding of carotenoid-to-chlorophyll energy transfer, therefore, requires a detailed knowledge of the excited state state

### 1.4 Carotenoids as Light Harvesting Chromophores: Ultrafast Photophysics

Carotenoids are one of the most abundant pigments found in nature. In addition to their role in light harvesting, carotenoids are involved in photoprotection by quenching the excited states of (bacterio)-chlorophylls in excess sunlight. This is an example of triplet-triplet energy transfer and is known as nonphotochemical quenching (NPQ).<sup>15</sup> Carotenoids also help scavenging harmful singlet oxygen<sup>15,16</sup> and other reactive species, and therefore provides protection mechanisms against deceases like cancer, arteriosclerosis and macular degeneration in human retina.<sup>17</sup> All these functional properties of are intimately related to the ultrafast photophysics of carotenoids.

Carotenoids belong to the class of conjugated polyenes and they are generally treated as a three electronic levels system. The electronic energy levels of carotenoids are shown in Figure 1.3. Since linear polyenes belong to the  $C_{2h}$  point group, the ground electronic state  $S_0$  of carotenoids has  $1^1A_g^-$  symmetry. The first excited state  $S_1$  has  $2^1A_g^-$  symmetry. Because of its symmetry property, one-photon optical transition from the ground state to the  $S_1$  state is dipole-forbidden. The  $S_1$  state is therefore formally referred to as an optically dark state. The lowest energy symmetry-allowed transition occurs from the  $S_0$  state to the  $S_2(1^1B_u^+)$  state and is responsible for the absorption band of carotenoids. The vibronic structure observed in the absorption spectrum (See Figure 1.3) of a carotenoid arises due to the stretching vibrational modes of the C–C (1150 cm<sup>-1</sup>) and C=C (1600 cm<sup>-1</sup>) bonds of its polyene backbone and combinations thereof. In accordance with the behavior of a particle in a box, both the  $S_0$ -S<sub>2</sub> and  $S_1$ -S<sub>0</sub> electronic energy gaps decrease with increasing conjugation length.<sup>18</sup>



**Figure 1.3** Nonradiative decay pathways in carotenoids. From reference 19. Copyright (2010) American Chemical Society, used with permission. Internal conversion processes are denoted by blue and red arrows; black arrows denote vibrational relaxation. Absorption spectra (top right) of LH2 (purple), PCP (orange), and LHCII (green). The range of energies of carotenoid  $S_2$  and  $S_1$  transitions are also shown by the orange and red bars beneath the spectra. Structure of different carotenoids are shown at the bottom.

Fluorescence up-conversion experiments determined that the lifetime of the S<sub>2</sub> state is less than 300 fs<sup>20,21</sup> and undergoes quick nonradiative decay to the S<sub>1</sub> state. Formation of the S<sub>1</sub> state can be identified using time-resolved transient absorption technique in which a prominent band arises in the 500–600 nm range due to the S<sub>1</sub> $\rightarrow$ S<sub>n</sub> excited state absorption.<sup>22</sup> The lifetime of the S<sub>1</sub> state varies from several picoseconds to couple of hundreds of picosecond depending on the conjugation length of the carotenoid. The decay of the S<sub>1</sub> state back to the ground state follows the energy-gap law.<sup>23–25</sup>

### 1.4.1 Dark Intermediate States in Carotenoids

The above-mentioned three-level description of carotenoid photophysics is probably oversimplified and cannot account for many of its spectroscopic observations. For example, the  $S_2 \rightarrow S_1$  nonradiative decay rate does not follow the energy gap law. With increasing conjugation length of carotenoids, the  $S_2 \rightarrow S_1$  energy gap decreases but the lifetime of the  $S_2$  state gets shorter.<sup>24,26</sup> This anomalous dependence on the conjugation length could be explained if an intermediate electronic state, denoted as  $S_x$ , mediates the  $S_2 \rightarrow S_1$  nonradiative decay. Similarly, hot vibrational spectral feature is observed in transient absorption experiments and is thought to be due to the formation of a dark S<sup>\*</sup> state. The presence of such dark states makes the carotenoid photophysics extremely difficult to understand and their assignment and role are still a topic of significant controversy.<sup>27</sup>

The presence of a dark electronic state with  $1^{1}B_{u}^{-}$  symmetry within the S<sub>2</sub>–S<sub>1</sub> gap was first predicted by Tavan and Schulten in 1987 based on electronic structure calculations on polyenes with N  $\geq$  9.<sup>28</sup> The first experimental support for this state came in 1999 from the resonance Raman experiments on all-*trans*-spheroidene by the Koyama group.<sup>29</sup> Subsequent transient absorption measurements on neurosporene from the same group assigned a near-IR band (800– 950 nm) as excited state absorption from the  $1^{1}B_{u}^{-}$  state. Later, transient absorption experiments on  $\beta$ -carotene and lutein performed with 15 fs pulses by Cerullo et al. showed that the S<sub>2</sub> state decays in 12 fs to produce a distinct electronic state S<sub>x</sub> with excited state absorption feature in the 800–900 range. Similar to the previous works, this was identified as the  $1^{1}B_{u}^{-}$  state.<sup>30</sup>

The presence of  $1^{1}B_{u}$  state was subsequently investigated employing a variety of four wave mixing techniques. Femtosecond Stimulated Raman spectroscopy experiments by McCamant et al. on  $\beta$ -carotene in cyclohexane<sup>31</sup> found that rise of the C=C stretch frequency of the S<sub>1</sub> state

follows the decay of the C–C stretch mode of the S<sub>2</sub> state implying that no intermediate state is needed to explain the observation. This was supported by the pump degenerate four wave mixing experiments which suggested that the transient Raman bands previously attributed to the intermediate state can be explained as a vibrational dynamics of the C=C stretching mode in the S<sub>1</sub> manifold. Christensson et al.<sup>32</sup> performed three-pulse photon echo peak shift (3PEPS) experiment on  $\beta$ -carotene and astaxanthin and found that the ground state bleach along with the excited state absorptions corresponding to S<sub>2</sub>→S<sub>n2</sub> and S<sub>1</sub>→S<sub>n1</sub> electronic transitions were suffice to model the experimental data. They didn't find evidence for the S<sub>x</sub> intermediate, but rather proposed that the S<sub>2</sub>→S<sub>1</sub> nonradiative decay might proceed through conical intersections.

Another dark intermediate, denoted as the S<sup>\*</sup> state, is identified as a shoulder on the shortwavelength side of the ESA spectrum from the S<sub>1</sub> state.<sup>33</sup> The shoulder becomes more pronounced when the carotenoid is bound to proteins than in solution. The S<sup>\*</sup> state can serve as acts as a precursor to the triplet state in carotenoids and can transfer energy to the BChl Q<sub>y</sub> state in the purple bacterial LH1 and LH2 complexes.<sup>34,35</sup> All these spectroscopic observation led to the suggestion that the S<sup>\*</sup> represents a separate electronic state positioned near the S<sub>1</sub> state and populated via nonradiative relaxation from the S<sub>2</sub> state. This assignment, however, was challenged based on the results from pump-dump-probe experiments.<sup>36,37</sup> Depletion of S<sub>2</sub> state population by the dump-pulse was found to selectively affect the transient absorption signal only from the S<sub>1</sub> state; the S<sup>\*</sup> feature remained unaffected. This led to the suggestion that the S<sup>\*</sup> state is hot ground state populated by impulsive stimulated Raman scattering from the S<sub>2</sub> state. In an alternative hypothesis, Frank et al. proposed that the S<sup>\*</sup> state is a twisted conformation of the S<sub>1</sub> state based on electronic structure calculations and temperature and solvent dependence of the S<sup>\*</sup>
signal.<sup>38–40</sup> In this model, the upon excitation the  $S_2$  state undergoes conformational changes prior to decaying to the  $S_1$  and  $S^*$  states.

#### 1.4.2 Intramolecular Charge Transfer in Carotenoids

In addition to the above-mentioned electronic states, carotenoids like peridinin and fucoxanthin are known to possess an additional intramolecular chare transfer (ICT) states. These carotenoids contain carbonyl groups in conjugation with the extended  $\pi$ -electron system of the polyene backbone and show unusual solvent dependence on excited state properties. This behavior is highly unusual for carotenoids; in general, the spectral properties and lifetimes of the S<sub>1</sub> state of carotenoids are independent of the solvent environment. The lifetime of the lowest excited singlet state of peridinin, however, changes from 7 ps in the highly polar solvent, trifluoroethanol, to 172 ps in two nonpolar solvents, cyclohexane and benzene.<sup>41,42</sup> The wavelengths of emission maxima, the quantum yields of fluorescence, and the transient absorption spectra were also affected by the solvent polarity. The solvent sensitivity of peridinin has been attributed by Frank and coworkers to the presence of an ICT state,<sup>41,42</sup> and this idea has been supported by both theoretical computations<sup>43</sup> and experiments on numerous other carbonyl-containing carotenoids and polyenals.<sup>44–53</sup>

Despite many studies, how the ICT character develops in the  $S_1$  state is not understood. Three hypotheses have been proposed. Two of the possible hypotheses (Figure1.4) suggest that the excited-state dynamics in the  $S_1$ -state manifold propagate along an intramolecular chargetransfer coordinate that links diabatic ( $S_1$  + ICT) states<sup>41,43,49,54</sup> or quantum-mechanically mixed ( $S_1$ /ICT) states.<sup>46,55</sup> A third hypothesis suggests that the  $S_1$  and ICT states are the same state; the ICT character in this case is derived from a quantum-mechanical mixing of the  $S_1$  and  $S_2$  states (S<sub>1</sub>(ICT)). This mechanism increases the dipole strength of the mixed state by borrowing intensity from the S<sub>2</sub> state.<sup>56</sup>



**Figure 1.4** Three different models for the nature of the ICT state in peridinin and other carbonylcontaining carotenoids dissolved in polar solvents. From reference 57. Copyright (2013) Biophysical Society, used with permission. (*left*)  $S_1$  and ICT strongly quantum mechanically mixed ( $S_1$ /ICT); (*middle*) ICT as a separate (diabatic) electronic state from  $S_1$  ( $S_1$  + ICT); and (*right*)  $S_1$  and the ICT state being one and the same state ( $S_1$ (ICT)). Solid lines represent radiative transitions. The dashed line represents internal conversion from  $S_2$  to  $S_1$ ; a is absorption; f is fluorescence.

From the above discussions, it is clear that the nonradiative decay pathways of carotenoids are extremely complicated due to the presence of possible dark electronic intermediate states. Detection and establishing the nature of these dark states is crucial to understand the light harvesting function of carotenoids. Understanding how the ICT character is developed in carbonyl-containing carotenoids is crucial to understand its role in the light-harvesting mechanisms of carotenoids.

# 1.5 Light Harvesting in the Peridinin–Chlorophyll *a* Protein

A perfect model system to study light harvesting strategy is the peridinin-chlorophyll a protein (PCP) from marine dinoflagellate Amphidinium carterae. The carotenoid peridinins serve as the main light harvester in PCP, unlike other light-harvesting complexes in purple bacteria and green plants where carotenoids serve as accessory light harvesting pigments. Peridinin captures mid-visible (470-570 nm) solar photons and delivers the excitation energy to Chl a acceptors very high (~90%) quantum efficiency.<sup>58,59</sup> The X-ray crystal structure (Figure 1.5) of trimeric PCP shows that in each subunit eight peridinin carotenoids and two Chl a chromophores are bound together in dense cluster by a basket of  $\alpha$  helices in a C<sub>2</sub>-symmetric, two-domain assembly. The distance between two Chl a molecules in the two domains are approximately 17 Å. In contrast, the four peridinins in each domain are effectively in van der Waals contact (3.3 to 3.8 Å separation) with a central Chl a acceptor.<sup>60</sup> Moreover, PCP has a well-characterized crystal structure<sup>60</sup> and amenable to single-point mutation and reconstitution with chlorophylls.<sup>61,62</sup> This feature offers us the unique opportunity to vary experimentally the singletstate energy gaps that control the various energy-transfer and nonradiative photophysical processes that arise from the structural interaction of Chls and carotenoids without significantly perturbing the structure.<sup>63–65</sup>



**Figure 1.5** Structure of the trimeric peridinin–chlorophyll *a* protein (PCP) from *Amphidinium carterae* (1PPR.pdb) as determined by Hofmann et al.<sup>60</sup>

Figure 1.6 shows the general scheme that applies to carotenoid–to–(B)Chl energy transfer in photosynthetic light harvesting proteins. Strong, electric-dipole-allowed transitions from the carotenoid ground state,  $S_0 (1^1A_g^-)$ , to the  $S_2 (1^1B_u^+)$  excited state are followed by downhill energy transfer to the  $Q_x$  or  $Q_y$  singlet states of chlorophylls (Chls) over short distances.<sup>27,66</sup> Because the carotenoid  $S_2$  state lies above that of the  $Q_x$  excited state of Chl or BChl,  $S_2 \rightarrow Q_x$  excitation energy transfer via the Förster resonant dipole–dipole mechanism<sup>7,67</sup> is energetically favored. This pathway competes with fast  $S_2 \rightarrow S_1$  nonradiative decay, however, so only a fraction of the total energy-transfer yield typically involves the  $S_2$  state. Given that there is very little dipole strength for the  $S_1 \rightarrow S_0$  transition, one might expect that the yield of Förster energy transfer to the Chl  $Q_y$  state would be very small. This expectation prompts the suggestion of a Dexter-type, orbital-overlap, electron exchange-mediated energy-transfer mechanism,<sup>68</sup>but

calculations suggest that energy transfer by the dipole–dipole mechanism can still be more favorable in light-harvesting proteins.<sup>59,69,70</sup>



**Figure 1.6** Energy levels and pathways for nonradiative decay in carotenoids and for excitation energy transfer from carotenoids to (B)Chls in photosynthetic light-harvesting proteins. After Beck et al. from 71.

In the LH2 complex from *Rhodopseudomonas acidophila*, it is known that that 85% of the energy-transfer yield between a carotenoid, rhodopin glucoside, and BChl *a* in the B800–B820 system from occurs in ~90 fs via the S<sub>2</sub>-state channel; only 15% of the yield goes through the S<sub>1</sub>-state channel using the dipole-dipole mechanism despite the short distance to the BChl *a* acceptor.<sup>69</sup> The total energy-transfer yield is only 70%. The S<sub>1</sub> state of rhodopin glucoside in this system has relatively low dipole strength and a short lifetime, so energy transfer competes unfavorably with nonradiative decay of the S<sub>1</sub> state. In contrast, the currently accepted picture in PCP is that nearly two thirds of the 90% total energy transfer yield involves coupling of the carotenoid S<sub>1</sub> state to the Chl Q<sub>y</sub> state via production of an ICT state, which lies below the S<sub>1</sub> state in polar solvents.<sup>41</sup> Despite having all of this structural information at hand, the mechanisms that mediate energy transfer from peridinin to Chl *a* via the S<sub>2</sub> and S<sub>1</sub> states are incompletely

understood. It remains to be established how the dark  $S_1$  state of the peridinins is coupled to that of the Chls so that energy transfer is efficient.

The enhanced light harvesting function in PCP is thought to be the result of two structural adaptations. The first is that peridinin adds an allene group and a lactone ring in conjugation with the extended  $\pi$ -electron system of the carotenoid polyene. These structures contribute to intramolecular charge-transfer (ICT) character that leads to significantly enhanced energy-transfer coupling to Chl *a*. Energy transfer from the S<sub>1</sub> state is also optimized in light-harvesting proteins by placing the carotenoid nearly in van der Waals contact with an adjacent Chl so that the Coulomb coupling between them is as large as possible.<sup>7,59,69,72</sup> The conclusion that the four peridinin carotenoids in a single domain of PCP interact in the strong electronic coupling limit is supported by circular dichroism spectroscopy<sup>73</sup> and by calculations,<sup>59</sup> but it remains an open question whether coherent energy transfer mechanisms are operative in PCP or not.

The intramolecular charge transfer and light harvesting function of PCP is further optimized by the imposition of conformational strain on peridinins by the surrounding binding sites. As shown in Figure 1.7, the average structure of peridinin found in the X-ray crystal structure is significantly distorted from the minimum energy, ground-state structure determined by density functional calculations. The allene end of the molecule is bent away from the mostly linear projection of the conjugated polyene backbone, but most importantly the length of the conjugated region is compressed by more than 0.2 Å and the pattern of bond-length alternation of the C–C and C=C bonds is partially inverted. These distortions are likely to enhance the energy transfer yield by mixing the dark S<sub>1</sub> state with the bright S<sub>2</sub> state.<sup>56,57</sup>



**Figure 1.7** Structure and conformation of the chromophores in a monomeric unit of PCP. From reference 56. Copyright (2003) American Chemical Society, used with permission. (a) arrangement of four distinct peridinins (Per) and two distinct chlorophylls (Chl)in each domain and numbered as in the X-ray crystal structure. (1PPR.pdb) (b) Comparison of the conformations of the peridinins showing the distinctive distortions of the symmetry in the PCP. Peridinins are oriented for maximal overlap in the polyene backbone (c) median structure of the peridinins. (d) lowest-energy vacuum structure of peridinin based on B3LYP/6-31G(d) density functional calculation.

### **1.6 Hypotheses and Proposed Work**

The observation that many carotenoids have distorted geometry in PCP and in other lightharvesting proteins including LH2<sup>5</sup> led us to hypothesize that the conformational motion of the conjugated polyene backbone of a carotenoid may play important roles in its excited state photophysics and energy transfer mechanism. The role of conformational dynamics in carotenoid photophysics has been described in detail in reference 71, in which Beck et al. proposed a new model for the mechanism of the nonradiative decay of carotenoids that explicitly involves largeamplitude out-of-plane motions. The central argument of the hypothesis is based on current theories<sup>74–79</sup> for nonradiative decay in polyenes and related systems.

# **1.6.1 Conformational Dynamics of Conjugated Polyenes**

A two-state-two-mode picture is generally employed to explain the photophysics of a conjugated polyene.<sup>74,75</sup> A particular feature of this model is that the nonradiative decay of a conjugated polyene proceeds along the two orthogonal coordinates; the bond length alternation and torsional modes. The stretching and compression motions which are directed from the Franck–Condon region, effectively reverses the bond order in the conjugated part of the molecule and initiates a nonradiative relaxation.<sup>33,80–82</sup> The initial momentum along the C=C and C–C bond stretching-compression coordinate is subsequently converted into motions of the torsional coordinates. Relaxation along the torsional gradient brings the molecules at 90° twisted minimum energy structure near the conical intersection with the ground state potential energy surface. Nonradiative relaxation through the conical intersection to the ground state then yields the original configuration or a photoisomerized configuration.



**Figure 1.8** Potential energy surfaces near the Franck–Condon structure for short cyanines, short PSBs, and for longer cyanines and PSBs. From reference 75. Copyright (2000) American Chemical Society, used with permission. The black lines indicate minimum-energy trajectory along the stretching and torsion coordinates towards a conical intersection region.

Figure 1.8 shows the two-state-two-mode model that is usually invoked to explain the photophysics of short cyanines and protonated Schiff bases (PSBs); the two orthogonal modes being the bond-length alternation and the torsional coordinates.<sup>74,75</sup> Depending on the energy barrier between the planar and twisted conformations of the molecule, the photoisomerization dynamics can involve a barrier or become barrierless. The height of the barrier and hence the gradients of the potential energy surface near the Franck–Condon geometry are controlled by the effective conjugation length.<sup>75</sup> For molecules with small conjugation lengths such as shorter cyanines, the photoisomerization dynamics is barrierless and the potential energy surface looks ridge-like. In such topology the system evolves from Franck–Condon region along the stretching coordinate and the strongly-coupled torsional motions at the same time. For short PSBs, the excited state topology looks valley-ridge like and few stretching vibrations takes place prior to the coupling of stretching-torsional motions. In contrast, the topology of the excited state surfaces for the longer cyanines and PSBs is valley-like and involves an energy barrier. The

Franck–Condon geometry lies at a local planar minimum with respect to the torsional coordinates and the initial evolution occurs considerably along the bond stretching coordinate. After several vibrations, a trajectory along the torsional coordinate is launched which overcomes the energy barrier and promotes a descent towards the conical intersection region.<sup>83</sup>

Unlike the cyanines and PSBs, the model that is used to describe the nonradiative decay of carotenoids invokes a barrierless picture. The  $S_2 \rightarrow S_1$  nonradiative decay of carotenoids has been explained in terms of progression along the C=C and C-C bond stretching-compression coordinate. The role of out-of-plane torsional coordinate has been hardly considered. As we will establish later in the dissertation, that the nonradiative decay of carotenoids can be explained in terms of conformational motions of its polyene backbone, including the presence of dark electronic states and formation of an intramolecular charge transfer which enhances the light harvesting function of carotenoids. The selection rules that govern the photophysics of linear polyene molecules are often relaxed for carotenoids with distorted conformations. As argued by Beck et al.,<sup>71</sup> owing to the properties of the conjugated polyene backbone, it is likely that the bent and twisted carotenoid structures further enhance the energy-transfer yield by increasing the transition dipole strength of formally dark electronic states and formation of an ICT state. The main questions that have been addressed in this dissertation are:

- 1. Whether the dark electronic state,  $S_x$  mediates the  $S_2 \rightarrow S_1$  nonradiative relaxation in carotenoids or not? If it does, what is the nature of the  $S_x$  state?
- 2. What is the mechanism of ICT formation in carotenoids containing carbonyl groups?
- 3. How does the ICT character enhance the light harvesting function of the peridinin– chlorophyll *a* protein?

In order to answer the above-mentioned questions, we have employed femtosecond transient grating technique with optical heterodyne detection.<sup>84–86</sup>

# 1.6.2 Approach: Heterodyne Detected Transient Grating Spectroscopy

The experimental implementation of heterodyne transient grating technique was first reported in the works of Miller and coworkers<sup>84,85</sup> and Fleming and coworkers.<sup>86–89</sup> The heterodyne detection allows complete characterization of the signal electric field emitted from the sample in terms of the two components in quadrature, real (absorption) and dispersion (imaginary) components.<sup>85,90–93</sup> The dispersion component is not detected in the conventional femtosecond pump–probe spectroscopy, which provides information only about the real, absorptive of the third-order signal; the absorption and dispersion components are combined as the sum of their squares in homodyne detected transient grating or photon echo experiments.

Figure 1.9 shows the contour representation of the absorption and dispersion components of the transient signal of rhodamine 6G in methanol obtained with 40 fs laser pulses centered at 520 nm wavelength. For a two-level system like rhodamine, both the absorption and dispersion components are dominated by the contributions from ground state bleach and stimulated emission signal. Figure 1.10 shows the time evolution of the integrated absorption and dispersion signals as function of delay between the pump and probe pulse. Both the components exhibit similar time profiles and contain no additional dynamics information than what is available from a pump-probe technique.



**Figure 1.9** Contour representations of the spectrally resolved components of the heterodyne transient grating signal from Rhodamine 6G in methanol at room temperature (22 °C). (a) absorption component; (b) dispersion component. For the absorption component, the yellow end of the color bar corresponds to net ground state bleaching and/or stimulated emission; the dark blue end indicates excited state absorption.



**Figure 1.10** Spectrally integrated absorption (red) and dispersion (blue) components of the transient grating signals from rhodamine 6G in methanol at room temperature (22 °C).

For Molecules which undergo excited state photoisomerization, such as cyanines and crystal violet, Xu et al. found that the absorption and dispersion components exhibit different dynamics.<sup>87–89</sup> They established that this behavior arises due to the formation of a hot ground state conformer which has shifted photoinduced absorption compared to the laser spectrum and hence contributes differently in the absorption and dispersion channel. This particular sensitivity towards spectral detuning makes heterodyne transient grating a suitable technique for investigating intermediate electronic states and conformational motions that accompanies a nonradiative decay processes. As we will show in subsequent chapters, when applied to carotenoids, this technique leads to considerable simplifications of their nonradiative decay channel and energy transfer pathways, which can be understood in terms of the conformational dynamics of their polyene backbone.

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# Chapter 2: Nonradiative Decay of the $S_2(1^1B_u^+)$ State of $\beta$ -Carotene: Contributions from Dark Intermediates and Double Quantum Coherences

Femtosecond transient grating spectroscopy with heterodyne detection was employed to characterize the nonradiative decay pathway in  $\beta$ -carotene from the  $S_2$   $(1^1B_u^+)$  state to the  $S_1$  $(2^{1}A_{g})$  state in benzonitrile solution. The results indicate definitively that the S<sub>2</sub> state populates an intermediate state, S<sub>x</sub>, on an ultrafast timescale prior to nonradiative decay to the S<sub>1</sub> state. The requirement for inclusion of the  $S_x$  state in the nonradiative decay pathway is the observed fast rise time of the dispersion component, which is predominantly controlled by the decay of the stimulated emission signal from the optically prepared S<sub>2</sub> state. The finding that the excited-state absorption spectrum from the  $S_{\boldsymbol{x}}$  state is significantly red shifted from that of  $S_2$  and  $S_1$  leads to a new assignment for the spectroscopic origin of the S<sub>x</sub> state. Rather than assigning S<sub>x</sub> to a discrete electronic state, such as the  ${}^{1}B_{u}^{-}$  state suggested in previous work, it is proposed that the  $S_{x}$  state corresponds to a transition state structure on the S<sub>2</sub> potential surface. In this hypothesis, the 12 fs time constant for the decay of the S2 state corresponds to a vibrational displacement of the C-C and C=C bond-length alternation coordinates of the conjugated polyene backbone from the optically prepared, Franck-Condon structure to a potential energy barrier on the S<sub>2</sub> surface that divides planar and torsionally displaced structures. The lifetime of the  $S_x$  state would be associated with a subsequent relaxation along torsional coordinates over a steep potential energy gradient towards a conical intersection with the S<sub>1</sub> state.

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# **2.1 Introduction**

The light-harvesting function of carotenoids in photosynthesis<sup>1-4</sup> is initiated by optical preparation of the second excited singlet state,  $S_2 (1^1B_u^-)$ , which decays nonradiatively to yield the first excited singlet state,  $S_1 (2^1A_g^-)$ . Both states can serve as excitation energy donors to the Q-band singlet states of (bacterio)chlorophyll ((B)Chl) molecules in light-harvesting complexes (Figure 2.1). The mechanism that enables the  $S_1$  state to function efficiently in energy transfer to (B)Chls is of considerable interest because only the  $S_2$  state can be directly populated by optical transitions; the  $S_1$  state is a dark state, lacking electric dipole-allowed one-photon transitions to or from the ground state,  $S_0 (1^1A_g^-)$ . The bulk of the quantum yield of energy transfer is nevertheless carried by the  $S_1$  state, however, because the lifetime of the  $S_2$  state is very short, typically 150 fs. The rate of excitation energy transfer in light-harvesting proteins is optimized structurally by placing the carotenoid donor nearly in van der Waals contact with the (B)Chl acceptor. Additionally, it is likely that an intramolecular charge-transfer (ICT) character develops in carbonyl-containing carotenoids, such as peridinin and fucoxanthin, during the nonradiative decay process from the  $S_2$  state to the  $S_1$  state.

Whether the S<sub>2</sub> state undergoes an ultrafast nonradiative decay process to populate one or more dark intermediates prior to populating the S<sub>1</sub> state remains indeterminate despite extensive debate in the literature. Polívka and Sundström<sup>4</sup> have reviewed the considerable body of evidence both for and against the existence of the state denoted S<sub>x</sub>, which is detected in femtosecond pump–probe experiments in terms of an excited-state absorption (ESA) band in the near-IR in <20 fs after optical preparation of the S<sub>2</sub> state.<sup>7–11</sup> Koyama and coworkers<sup>7</sup> assigned S<sub>x</sub> to the  $1^1B_u^-$  state, which was predicted by Tavan and Schulten<sup>12</sup> to lie below the S<sub>2</sub> state. More recently, Ikuta et al.<sup>13</sup> proposed that the S<sub>2</sub> state yields the  $3^1A_g^-$  state prior to populating the

 $1^{1}B_{u}^{-}$  state; it is notable that Shreve et al.<sup>14</sup> assigned the unusually large polarizability of the S<sub>2</sub> state to strong coupling to an A<sub>g</sub><sup>-</sup> state nearby.



**Figure 2.1** Pathways for nonradiative decay in carotenoids and for excitation energy transfer from carotenoids to (B)Chls in photosynthetic light-harvesting proteins. The one-photon transitions transitions between states of the same symmetry or pseudoparity are electric-dipole forbidden.<sup>5,6</sup> Nonradiative decay from the S<sub>2</sub> and S<sub>1</sub> ( $2^{1}A_{g}^{-}$ ) states is indicated with wavy arrows. Energy transfer pathways from the carotenoid S<sub>2</sub> and S<sub>1</sub> states to the (B)Chl Q<sub>x</sub> and Q<sub>y</sub> states, are indicated with filled arrows.

An additional dark intermediate state, X, was recently identified by Scholes and coworkers<sup>15,16</sup> in two-dimensional electronic spectra (2DES). An off-diagonal cross peak assigned to the X state was observed in solutions of sphaeroidene or rhodopin glucoside and in preparations of the LH2 complex at short delays after optical preparation of the S<sub>2</sub> state. The cross peak implicates the X state as an intermediate between the S<sub>2</sub> and S<sub>1</sub> states. In LH2, where BChl acceptors are present, cross peaks were also observed that connect X to the BChl Q<sub>x</sub> state. These results indicate directly that the X state can serve as an excitation energy donor to the BChl Q<sub>x</sub> state. Because the ESA spectrum from the S<sub>x</sub> state is significantly red shifted compared to that from the X state, it seems likely that S<sub>x</sub> and X states should be regarded as distinct states.<sup>17</sup> The 2DES spectra provide some relief from the overlapping ESA and stimulated emission (SE) signals that complicate analyses of the kinetic profiles of intermediates along the

 $S_2 \rightarrow S_1$  nonradiative decay pathway in conventional one-dimensional pump-probe spectra. At this point a definitive determination of the nature of the process that apparently yields the  $S_x$  and X states has not yet been made nor is there even a consensus that they exist.

Further complicating the interpretation of the nonradiative decay pathways of carotenoids is the presence of an upper singlet state,  $S_{n2}$ , which is populated directly from the  $S_2$  state by ESA transitions.<sup>18,19</sup> Because the energy gap between the  $S_2$  and  $S_{n2}$  states is almost the same as that between the  $S_0$  and  $S_2$  states, optical preparation of an  $S_{n2}$ – $S_0$  electronic coherence via a doublequantum pathway competes with pathways that yield population in the  $S_2$  state. In conventional two-beam pump–probe spectroscopy, the free-induction decay signals generated by the action of a delayed probe pulse on the  $S_{n2}$ – $S_0$  double-quantum coherence are radiated in the same direction and are superimposed spectrally and temporally on the population signals derived from population in the  $S_2$  state.<sup>20</sup> The dephasing time of the  $S_{n2}$ – $S_0$  double-quantum coherence would be expected to be very short, perhaps comparable to the pure dephasing time for the  $S_0$ – $S_2$ coherence, so its decay would be expected to accompany the decay of population from the  $S_2$ state into the  $S_x$  state.

In this contribution, we show for the first time that femtosecond transient grating spectroscopy with optical heterodyne detection provides crucial information on the nonradiative decay of the  $S_2$  state of carotenoids that is not available from conventional pump–probe spectroscopy. In comparison to pump–probe spectroscopy, heterodyne transient grating spectroscopy offers two distinct advantages in studies of carotenoids. First, the heterodyne detection scheme allows a full characterization of the complex third-order nonlinear optical signal by resolving its real (absorption) and imaginary (dispersion) components, the latter not being observed at all in pump–probe signals. These components interfere with each other in

transient-grating spectroscopy with homodyne detection, where only the total signal amplitude (the complex modulus) is detected directly by a square-law detector. Second, the doublequantum coherence and population signals occur in different time delay regions; the time orderings of the field-matter interactions that yield the two types of signals are restricted by detection of the signal in a particular phase-matched direction, as determined by the outgoing wavevectors of the three excitation pulses. As we demonstrate here, both advantages lead to a considerable simplification of the interpretation of the transient-grating signal from  $\beta$ -carotene.



Figure 2.1 Structure of  $\beta$ -carotene.

The results show that the dispersion component enables an unambiguous detection of the nonradiative decay of the S<sub>2</sub> state of  $\beta$ -carotene in benzonitile to a dark intermediate state in <20 fs. Numerical simulations of the absorption and dispersion components of the transient grating signal with an internally consistent model lead to an assignment of the intermediate to the S<sub>x</sub> state with models that incorporate lineshape parameters consistent with the near-IR ESA spectrum obtained by Cerullo et al.<sup>9</sup> This finding places some restrictions on the nature of the vibrational dynamics that follow optical preparation of the S<sub>2</sub> state. We suggest that results are consistent with the hypothesis<sup>17</sup> that S<sub>x</sub> state should be assigned to a transition state structure at the activation-energy barrier between planar and twisted structures on the S<sub>2</sub> potential energy surface rather than being assigned to a discrete electronic state of a planar conformation. This hypothesis leads to a natural explanation for some of the properties of carotenoids in photosynthetic light-harvesting proteins.

# **2.2 Experimental Section**

#### **2.2.1 Sample Preparation**

β-carotene was obtained from Sigma-Aldrich (catalog number C-9750) and was purified by high-performance liquid chromatography (HPLC) using a Waters 600E/600S multisolvent delivery system equipped with a 2996 photodiode array detector, as described previously.<sup>21</sup> The column was a Waters Atlantis Prep T3 OBD 5 µm column (19 x 100 mm). Acetonitrile was delivered isocratically at a flow rate of 7.0 mL/min. Samples collected from the HPLC were dried under nitrogen gas and stored at  $-20^{\circ}$ C until further use. For femtosecond spectroscopy, solutions of purified β-carotene were dissolved in benzonitrile and then spun in a desktop microcentrifuge to pellet any insoluble material or debris. The absorbance was then adjusted to 0.3 or less for a 1 mm path at the center of the laser spectrum, 520 nm, by adding neat benzonitrile. The samples were held in a 1 mm quartz cuvette at room temperature (295 K).

# 2.2.2 Linear Spectroscopy

Linear absorption spectra were acquired with a Hitachi U-4001 spectrophotometer.

#### 2.2.3 Nonlinear Spectroscopy

Femtosecond transient-grating signals were acquired at room temperature (295 K) with optical heterodyne detection using a passively phase-stabilized photon-echo spectrometer and Fourier-transform spectral interferometry. The experiments were performed with 520-nm excitation pulses obtained from an optical parametric amplifier (OPA, Coherent OPA 9400), which was pumped by a 250-kHz amplified Ti:sapphire laser (Coherent Mira-Seed oscillator and RegA 9050 regenerative amplifier). The pulse durations were estimated as having  $\geq$ 40 fs

durations from measurements of the typically 60 fs width of the third-order dispersion signal from neat methanol solvent, but the simulations that follow indicate that the effective pulse duration with group-velocity dispersion from the sample cell's contents included was 45 fs.

The photon-echo spectrometer built for use in these experiments follows aspects of the designs previously discussed by Brixner et al.<sup>22</sup> and Moran and Scherer.<sup>23</sup> Four laser pulses were obtained by splitting an incident pump–probe pulse pair with a transmission diffractive optic. A sequence of two spherical mirrors with 20 cm focal lengths was used to collimate and focus the four pulses onto the sample in the forward boxcars configuration. The pump–probe population delay was controlled using a time-of-flight delay driven by a Nanomover actuator (Melles Griot) prior to the diffractive optic; a second Nanomover actuator controlled the lateral translation of wedge-shaped prisms in the pump beams to control the coherence delay, which was set to zero for transient-grating experiments. The time delays were calibrated by spectral interferometry with a pinhole aperture at the sample position.<sup>22,24</sup> The three measurement pulses each carried <1 nJ/pulse; the local oscillator pulse was further attenuated by 3.5 O.D. using fused-silica neutral density filters.

The spatially overlapped third-order signal and local oscillator were spatially filtered after the sample using a series of iris diaphragms and then collimated and focused into a spectrograph (Spex 270m, 1200 gr/mm grating) by a pair of spherical lenses. The spectral interferogram from the signal and local oscillator was detected using a back-illuminated, liquid nitrogen-cooled CCD detector (Princeton Instruments VersArray 1300B, 700  $\times$  1300 pixels, 100-ms integration time/spectrum). Scattered light was removed using the beam-shuttering protocol outlined by Brixner et al.<sup>22</sup> The spectrometer was controlled by LabVIEW (National Instruments) programs.

# **2.3 Experimental Results**

# 2.3.1 Linear Spectroscopy and Laser Excitation Spectrum

Figure 2.3 shows the linear absorption spectrum corresponding to the  $S_0$   $(1^1A_g) \rightarrow S_2 (1^1B_u^+)$  transition of  $\beta$ -carotene in benzonitrile solvent at room temperature (295 K). For the transient-grating experiments discussed in this paper, the spectrum of the signal-beam output of the OPA was tuned to 520 nm. The pulse durations were estimated as 40 fs fwhm, Gaussian pulses assumed, from the 60 fs fwhm width of the dispersion component of the transient grating signal from neat methanol that was measured with these pulses. The OPA spectrum is centered on the red onset of the absorption spectrum and near to the wavelength of the 0–0 vibronic transition. This tuning photoselects the lowest energy conformers<sup>25</sup> from the ground-state ensemble and prepares the S<sub>2</sub> state with minimal excess vibrational excitation.



**Figure 2.3** Room-temperature absorption spectrum of  $\beta$ -carotene in benzonitrile solvent. Superimposed is the spectrum of the OPA signal-beam output (520 nm center wavelength), as tuned for the heterodyne transient-grating experiments.

#### 2.3.2 β-carotene transient grating signals

Transient-grating signals from  $\beta$ -carotene in benzonitrile were acquired with heterodyne detection and Fourier-transform spectral interferometry. The heterodyne detection scheme used in this work followed the general approach described by Jonas and coworkers<sup>26</sup> and several techniques implemented by Brixner et al.<sup>22</sup> The processing and interpretation of third-order nonlinear optical signals was previously outlined by Moran, Scherer, and coworkers,<sup>23,27</sup> and the following summary adopts their notation.

In the transient-grating experiment, two pump pulses arrive synchronously (with coherence time interval  $\tau = 0$ ) at the sample position and directed along wavevectors  $\mathbf{k}_1$  and  $\mathbf{k}_2$ . After a population (or waiting) interval  $T_{1,1}$  the probe pulse arrives along wavevector  $\mathbf{k}_{3}$ . The sample then emits a signal (frequency  $\omega_t$ , with emission time t > 0 with respect to the probe pulse). In our experimental configuration, the signal is detected along the  $\mathbf{k}_s = -\mathbf{k}_1 + \mathbf{k}_2 + \mathbf{k}_3$  direction by using the forward boxcars configuration for the pump and probe wavevectors; signals are also emitted along other combinations of the input wavevectors, but they are not detected in our experiment. When optical heterodyne detection is employed, a weak local oscillator pulse arrives at the sample prior to the probe pulse along the  $\mathbf{k}_s$  wavevector so as to interfere with the outgoing third-order signal; in our experiment the local oscillator and probe pulses are spaced by  $\sim$ 800 fs. The population-time dependence of the third-order signal is recorded by scanning the population time T to carry the probe pulse from negative to positive delays with respect to the pump pulses. The data acquisition and data processing procedures described by Brixner et al.<sup>22</sup> were implemented to acquire a spectral interferogram that carries the third-order signal and lacks background scattering by alternately shuttering beams 1 and 3 and taking differences.

The third-order signal field,  $E_s^{(3)}(t,T,\tau)$ , is the sum of the fields from the nonresonant solvent and resonant solute and is related to their third-order polarizations,  $P^{(3)}(t,T,\tau)$ , by

$$E_{\rm s}^{(3)}(t,T,\tau) = \frac{{\rm i} 2\pi l\omega_t}{n(\omega_t)c} \Big( P_{\rm solvent}^{(3)}(t,T,\tau) + P_{\rm solute}^{(3)}(t,T,\tau) \Big)$$
(2.1)

where *l* is the length of the sample cell,  $n(\omega_t)$  is the refractive index as a function of the signal frequency, *c* is the speed of light, and *T* and  $\tau$  are the interpulse delays that specify the threepulse program.  $P^{(3)}(t,T,\tau)$  is determined by the three successive actions of the electric fields from the three pulses,  $E_s^{(3)}(t,T,\tau)$  that define a particular Feynman pathway.<sup>23,28</sup> Optical heterodyne detection obtains the spectral interferogram  $I_{tot}(\omega_t,T,\tau,\overline{\tau}_{LO})$  as<sup>23</sup>

$$I_{\text{tot}}(\boldsymbol{\omega}_{t}, T, \tau, \overline{\tau}_{\text{LO}}) = \left| \int_{-\infty}^{\infty} \mathrm{d}t \left( E_{s}^{(3)}(t, T, \tau) + E_{\text{LO}}(t - \overline{\tau}_{\text{LO}}) \exp(\mathrm{i}\boldsymbol{\omega}_{t}t) \right) \right|^{2}$$
(2.2)

where  $E_{\rm LO}(t-\overline{\tau}_{\rm LO})$  is the electric field of the local oscillator and  $\overline{\tau}_{\rm LO}$  is the time delay between the local oscillator and probe pulses. The third-order signal is isolated by a Fourier-transform algorithm<sup>22,26</sup> as a complex cross term

$$I_{\rm het}(\omega_t, T, \tau, \overline{\tau}_{\rm LO}; \varphi_{\rm LO}(\omega_t)) = \sqrt{I_{\rm s}(\omega_t, T, \tau) I_{\rm LO}(\omega_t)} \exp\left[i(\varphi_{\rm s}(\omega_t) - \varphi_{\rm LO}(\omega_t) - \omega_t \overline{\tau}_{\rm LO})\right]$$
(2.3)

where  $I_s(\omega_t, T, \tau)$  is the signal intensity,  $I_{LO}(\omega_t)$  is the intensity of the local oscillator, and  $\varphi_s(\omega_t)$  and  $\varphi_{LO}(\omega_t)$  are the phases of the signal and local oscillator, respectively. The phase  $\varphi_{LO}(\omega_t)$  was determined experimentally in this work as described previously by Xu et al.<sup>29</sup> and by Moran et al<sup>23</sup> from the purely dispersive signal from neat solvent, which was measured in the same sample cuvette used for the solute  $\beta$ -carotene sample and with kinematic replacement in the

cuvette holder. The  $I_{LO}(\omega_t)$  term in equation 2.3 is canceled by dividing the signal with the square root of a separately acquired spectrum of the local oscillator,  $|I_{LO}(\omega_t)|^2$ , which isolates the signal field as

$$E_{s}^{(3)}(\boldsymbol{\omega}_{t},T,\tau) = \hat{\boldsymbol{\xi}}_{s}(\boldsymbol{\omega}_{t},T,\tau) \exp\left[-\mathrm{i}\boldsymbol{\varphi}_{s}(\boldsymbol{\omega}_{t})\right]$$
(2.4)

where  $\hat{\xi}_s(\omega_t, T, \tau)$  denoted the electric field amplitude. The real and imaginary parts of  $E_s^{(3)}(\omega_t, T, \tau)$  obtain the absorption and dispersion components, respectively. The sum of their squares obtains the modulus (or power spectrum),

$$\left|E_{s}^{(3)}(\boldsymbol{\omega}_{t},T,\tau)\right|^{2} \equiv \operatorname{Re}\left[E_{s}^{(3)}(\boldsymbol{\omega}_{t},T,\tau)\right]^{2} + \operatorname{Im}\left[E_{s}^{(3)}(\boldsymbol{\omega}_{t},T,\tau)\right]^{2}$$
(2.5)

which is proportional to the homodyne signal collected by a square-law detector along the  $\mathbf{k}_s$  wavevector in the absence of a local oscillator field.

Figures 2.4 and 2.5 show the absorption and dispersion components of the heterodyne transient grating signal from  $\beta$ -carotene in benzonitrile at room temperature over the 0–500 fs range for the population delay *T*. Both components were obtained with a consistent choice of signal phase,  $\varphi_s(\omega_t)$  that projects the ground state bleaching (GSB) and stimulated emission (SE) pathways for the resonant  $\beta$ -carotene solute in the absorption component as positive-going signals (increasing emission intensity). This choice of phase obtains negative-going signals for the dispersion component obtained from neat solvent.

The absorption component of the  $\beta$ -carotene signal exhibits a biphasic transient, with a prompt rise and an initial decay yielding a net excited-state absorption (ESA) signal in <100 fs. In contrast, the  $\beta$ -carotene dispersion signal is essentially monophasic; after a very short delay,

<20 fs, it exhibits a rising profile; as explained below, the rising signal has principal contributions from GSB and ESA. The negative-going peak observed near the zero of time comes from the dispersion signal from the benzonitrile solvent (Figure A2.1 in the Appendix). The weak, derivative shaped signal from benzonitrile in the absorption component arises from self-phase modulation due to the passage of the femtosecond excitation pulses. A  $\beta$ -carotene solute concentration series (Figure A2.2) shows that the contribution of benzonitrile to the modulus signal is small but not negligible at the 0.3 O.D. conditions used in Figures 2.4 and 2.5. At lower solute concentrations, the dispersion signal from the solvent is dominant near the zero of time; at higher solute concentrations, the signal/noise ratio decreases and distortions of the signal can be discerned, especially in 2DES signals.<sup>30</sup>



**Figure 2.4** Real (absorption) component of the heterodyne transient grating signal from  $\beta$ carotene in benzonitrile solvent at room temperature (22 °C). The yellow end of the color bar corresponds to net ground-state bleaching or stimulated emission; the dark blue end indicates excited state absorption.



Figure 2.5 Imaginary (dispersion) component of the heterodyne transient grating signal from  $\beta$ carotene in benzonitrile solvent at room temperature (22 °C). The phasing of the color bar is consistent with that used in Figure 2.4 for the absorption component. At 520 nm, the yellow contours correspond to the net ESA signal from the S<sub>1</sub> state.

In order to establish the principal time constants and lineshapes for the evolution of the transient grating spectrum, the absorption and dispersion transient grating data sets from the  $\beta$ -carotene transient grating signal over the -100 fs–12 ps range were subjected to a global target analysis.<sup>31–33</sup> The absorption and dispersion components are expressed in the global model as a set of spectral components and associated kinetic time constants with convolution with the instrument-response function, as defined by the electric field profiles of the pump and probe laser pulses. A preliminary singular value decomposition analysis indicated that the models for the absorption and dispersion components required at least three kinetic components. Figures 2.6 and 2.7 show the decay-associated spectra (DAS) and evolution-associated spectra (EAS) for the absorption and dispersion signals corresponding to a three component global model with common timescales. Figure 2.8 shows a slice through the data sets and fitted models at a probe wavelength of 520 nm, the center of the laser spectrum.



**Figure 2.6** Evolution-associated and decay-associated spectra (EAS and DAS, respectively) from a global analysis of the absorption component of the heterodyne transient-grating signal (Figure 2.4) from  $\beta$ -carotene in benzonitrile over the -100 fs–12 ps delay range. The DAS (bottom panel) characterize the spectral changes associated with a parallel decay of the three spectra; black: 12 fs decay; red: 142 fs decay; blue: 11.6 ps decay back to the ground state. The EAS (top panel) assume a sequential process: black: instantaneously formed initial species, 12 fs decay; red: first intermediate species, 12 fs rise, 142 fs decay; blue: second intermediate component, 142 fs rise, 11.6 ps decay back to the ground state. The inset shows the time evolution of the populations for the three components in the sequential model against a linear–logarithmic time axis split at 1 ps. The global model includes a convolution with a 60 fs Gaussian-shaped instrument response function centered at the zero of the probe delay axis.



**Figure 2.7** Evolution-associated and decay-associated spectra (EAS and DAS, respectively) from a global analysis of the dispersion component of the heterodyne transient-grating signal (Figure 2.5) from  $\beta$ -carotene in benzonitrile over the -100 fs–12 ps delay range. The DAS (bottom panel) characterize the spectral changes associated with a parallel decay of the three spectra; black: 12 fs decay; red: 142 fs decay; blue: 10.2 ps decay back to the ground state. The EAS (top panel) assume a sequential process: black: instantaneously formed initial species, 12 fs decay; red: first intermediate species, 12 fs rise, 142 fs decay; blue: second intermediate component, 142 fs rise, 10.2 ps decay back to the ground state. The inset shows the time evolution of the populations for the three components in the sequential model against a linear–logarithmic time axis split at 1 ps. The global model includes a convolution with a 60 fs Gaussian-shaped instrument response function centered at the zero of the probe delay axis.



**Figure 2.8** Time evolution of the absorption (red) and dispersion (blue) components of the heterodyne transient grating signals from  $\beta$ -carotene in benzonitrile solvent at 520 nm, as plotted against a linear–logarithmic time axis split at 1 ps. Both signals are superimposed with the fitted global models described in Figures 2.6 and 2.7, respectively.

The EAS describe the transient-grating signal in terms of a sequential model corresponding to the decay of the instantaneously formed S<sub>2</sub> state through two intermediates back to the ground state. The <500 fs parts of the absorption and dispersion components were well described with common decay time constants: 12 fs and 142 fs. Because the 12 fs decay involves kinetics that are shorter in timescale than the instrument-response width, as determined by the 40 fs pulses used in these experiments, this time constant is estimated with considerable indeterminacy. Both time constants are comparable to those determined for the decay of the S<sub>2</sub> and S<sub>x</sub> states determined in the Cerullo et al.<sup>9</sup> pump–probe experiments with  $\beta$ -carotene in cyclohexane solvent. The EAS spectrum of the first intermediate, presumably that corresponding to the S<sub>x</sub> state, is much weaker and is slightly red shifted compared to that of the instantaneously formed, S<sub>2</sub> state. Further, note that the dispersion spectrum of the S<sub>2</sub> state is biphasic, whereas the dispersion spectrum of the first intermediate is monophasic. As discussed in the simulations that
follow, these aspects provide some clues about the spectroscopic nature of the  $S_x$  state and the line-broadening dynamics that accompany optical excitation of the  $S_2$  state.

The global models for the absorption and dispersion signals, however, have significantly different time constants for the decay of the third spectrum, from the second intermediate state in the global model: 11.6 ps in the absorption signal and 10.2 ps in the dispersion signal. These decays correspond to nonradiative recovery back to the ground state,  $S_0$ , from the  $S_1$  state. The different time constants indicate that the ground-state recovery process from the  $S_1$  state yields initially a conformationally displaced ground-state intermediate prior to relaxation to the initial, photoselected ground-state conformational distribution. This aspect of the nonradiative decay process will be examined in a separate contribution, which will include numerical simulations of the transient spectra that accompany the relaxation process on the ground-state potential surface.

## 2.4 Numerical Simulation of Linear and Nonlinear Response

In order to understand the time evolution of the absorption and dispersion components of the heterodyne transient grating signal from  $\beta$ -carotene, we performed numerical simulations using the nonlinear optical response function formalism. This approach has been extensively used in combination with the multimode Brownian oscillator model (MBO),<sup>28</sup> which is used to treat the interactions of electronic chromophores with the surrounding solvent medium and thermodynamic bath.<sup>34–36</sup> The numerical simulations presented here follow the approach implemented by Brixner et al.<sup>22</sup> for a three-level system and as expanded by Christensson et al.<sup>19</sup> for simulations of carotenoids. The effects of the finite pulse durations and bandwidths of the excitation laser pulses are included so that the results can be explicitly compared with the experimental results in the frequency and time domains. Simulations of the homodyne transient grating signals of  $\beta$ -carotene in tetrahydrofuran were discussed previously by Hashimoto and

coworkers.<sup>37–40</sup> Christensson et al.<sup>18,41</sup> reported simulations of the photon echo and doublequantum coherence signals from  $\beta$ -carotene in benzonitrile. To our knowledge, the present work includes the first simulations of the heterodyne transient grating signal from a carotenoid system that employ simultaneous modeling of the absorption and dispersion components.



**Figure 2.9** Schematic energy-level diagram for  $\beta$ -carotene, as used in the simulations of the heterodyne transient grating signal from  $\beta$ -carotene. Solid green arrows indicate ground-state and excited-state absorption transitions. Blue wavy arrows mark internal conversion transitions. Double-headed arrows show the detuning ( $\Delta$ ) between the center of the laser spectrum and the vertical energy of an electronic transition. Note that in some of the simulations that follow, the S<sub>x</sub> manifold is omitted; in that case, the S<sub>2</sub> state decays nonradiatively directly to the S<sub>1</sub> state.

Figure 2.9 shows the energy level scheme of  $\beta$ -carotene that was employed in the simulations we performed. After optical preparation of the S<sub>2</sub> state, an intermediate electronic state, here labeled S<sub>x</sub>, mediates nonradiative decay to the S<sub>1</sub> state. Broadband pump-probe experiments have previously identified ESA transitions from S<sub>2</sub> and S<sub>x</sub>.<sup>9</sup> The S<sub>1</sub> state is known to exhibit a strong ESA signal in the visible region. Therefore, along with the S<sub>2</sub>  $\rightarrow$  S<sub>0</sub> stimulated emission (SE) and S<sub>0</sub>  $\rightarrow$  S<sub>2</sub> ground state bleaching (GSB) transitions, ESA transitions from S<sub>2</sub>, S<sub>x</sub>, and S<sub>1</sub> to higher lying singlet states with the appropriate symmetry need to be included in the simulation. Internal conversion pathways from the  $S_x$  state to the  $S_0$  state are not included in the simulation; further, no optical transitions from  $S_0$  to  $S_x$  have been observed, so the transition dipole moment for optical transitions between the  $S_0$  and  $S_x$  states is considered negligible.<sup>37</sup>

In order to determine whether the presence of the  $S_x$  state is indicated by the heterodyne transient grating signals we observed with  $\beta$ -carotene, in the following simulations we have compared the results obtained with two energy-level schemes, one with the  $S_x$  manifold included as shown in Figure 9 and the other without. The simulations performed by Christensson et al.<sup>41</sup> did not include an intermediate state between  $S_2$  and  $S_1$ . The homodyne transient grating signals from  $\beta$ -carotene and related carotenoids obtained by Hashimoto and coworkers<sup>37–40</sup> were best described with simulations that included an intermediate state. The goal of our simulations is to find a set of suitable parameters that fit the absorption and dispersion components of the transient grating signal at the same time. In each simulation, the parameters were varied first to find the best fit for the absorption component and then the same set of parameters were used to simulate the dispersion component.

In the MBO model, an energy-gap time-correlation function, M(t) is used to describe the fluctuations of the energy levels of a molecular system. It is constructed from the sum of terms associated with each type of Brownian mode: a Gaussian (G) inertial solvation term,<sup>42,43</sup> exponential (E) terms for diffusive solvation, and a series of cosinusoids for underdamped intramolecular vibrational modes  $(v)^{28,34}$ 

$$M(t) = \lambda_{\rm G} \exp\left[-(t / \tau_{\rm G})^2\right] + \sum_{i} \lambda_{\rm E,i} \exp(-t / \tau_{\rm E,i}) + \sum_{j} \lambda_{\nu,j} \exp(-\gamma_{\nu,j} t / 2) \left(\cos(\Omega_{\nu,j} t) + \left(\frac{\gamma_{\nu,j}}{2\Omega_{\nu,j}}\right) \sin(\Omega_{\nu,j} t)\right)$$
(2.6)

where  $\Omega_{\nu} = \left[ (\omega_{\nu})^2 - (\gamma_{\nu}/2)^2 \right]^{1/2}$ . Here,  $\lambda$  and  $\tau$  denote the reorganization energy and dephasing time of a specific Brownian mode. The inverse damping time of a vibrational mode with a natural frequency  $\omega_{\nu}$  is denoted by  $\gamma_{\nu} = 1/\tau_{\nu}$ .

The time evolution of the time-correlation function can then be represented by a linebroadening function, g(t)

$$g(t) = \Delta^2 \int_0^t dt_1 \int_0^{t_1} dt_2 M(t_2) + i\lambda \int_0^t dt_1 M(t_1)$$
(2.7)

where  $\Delta$  represents the total coupling strength, which tells how strongly the gap between energy levels is modulated by the Brownian modes, and  $\lambda$  stands for the total solvation reorganization energy.

M(t) can be directly estimated from a measurement of the three-pulse stimulated photonecho peak shift (3PEPS),<sup>44</sup> but a simulation of the ground-state absorption spectrum is usually performed to obtain several of the parameters in Equation 2.6. Once M(t) is determined and g(t) is calculated from it, all the linear and nonlinear optical responses of a system can be calculated with internal consistency using the Condon approximation and a second-order cumulant expansion.<sup>28</sup>

## 2.4.1 Numerical Simulation of Absorption Spectrum

In general, the ground-state absorption spectrum can be determined using the line-broadening function as<sup>28</sup>

$$\sigma_A(\omega) \propto \omega \operatorname{Re} \int_0^\infty dt \, \exp(\mathrm{i}(\omega - \omega_{eg})t) \, \exp(-g(t)) \tag{2.8}$$

where  $\omega_{eg} = \omega_{0-0} + \lambda$  is the vertical transition frequency,<sup>19</sup> which corresponds to the energy gap between the *e* and *g* potential surfaces at the ground-state structure;  $\omega_{0-0}$  denotes the frequency for the 0–0 vibronic transition. When applied to  $\beta$ -carotene, *e* and *g* correspond to the resonant S<sub>2</sub> and ground state S<sub>0</sub>, respectively.

Figure 2.10 compares the  $S_0 \rightarrow S_2$  absorption spectrum from  $\beta\text{-carotene}$  with a calculated spectrum determined using equations 2.6-2.8; the model parameters for different Brownian modes are listed in Table 2.1. The experimental and calculated spectra exhibit nearly identical profiles from the low-frequency onset to the peak absorbance and then the 0–2 vibronic satellite; while the vibrational structure is well modeled past that point to higher frequencies, the two spectra increasingly deviate on the blue side of the spectrum where twisted conformations (with somewhat different lineshape parameters) may make a contribution to the experimental profile. The optimized value for  $\omega_{eg}$  is 21550 cm<sup>-1</sup>. The width of the absorption spectrum is determined by the sum of the coupling strengths of the Brownian modes. Two high frequency underdamped vibrational modes are included in the calculation, 1150 cm<sup>-1</sup> and 1520 cm<sup>-1</sup>, which are the ground-state frequencies of the totally symmetric, resonance Raman active, C-C and C=C bonds in the conjugated polyene backbone of  $\beta$ -carotene.<sup>45</sup> These modes produce the vibronic progression that is visible in the absorption spectrum. The reorganization energies and vibrational dephasing times included in M(t) are similar to those employed by Christensson et al.<sup>41</sup> in their simulations. The exponential component in M(t) with a 1 ps time constant roughly corresponds to the line-broadening timescale observed in the time evolution of the pump-probe spectrum of  $\beta$ -carotene.<sup>46</sup>



**Figure 2.10** Comparison of the room temperature  $S_0 \rightarrow S_2$  absorption spectrum of  $\beta$ -carotene in benzonitrile (dark blue curve) with a simulation (red dash-dotted curve) obtained using the MBO model (Equations 2.6-2.8) and the parameters listed in Table 2.1.

Mode	$\lambda ~(\mathrm{cm}^{-1})$	au (fs)	$\boldsymbol{\omega}_{v} (\mathrm{cm}^{-1})$
Gaussian	100	10	
Exponential 1	300	500	
Exponential 2	450	1000	_
Vibration 1	520	2200	1150
Vibration 2	1120	2200	1520

**Table 2.1** MBO model parameters<sup>a</sup> for the simulation of the  $\beta$ -carotene  $S_0 \rightarrow S_2$  absorption spectrum in benzonitrile at 295 K.

<sup>a</sup>See Equations 2.6-2.8. For a given term in M(t),  $\lambda$  is the reorganization energy and  $\tau$  denotes the damping time constant;  $\omega_{\nu}$  is the frequency for a vibrational mode.

## 2.4.2 Numerical Simulation of Transient Grating Signal

In heterodyne transient grating spectroscopy, a sequential interaction with three laser pulses induces a third-order polarization in the sample. In the boxcar geometry, as implemented in our transient-grating spectrometer, the resulting signal field is detected in the phase-matched  $\mathbf{k}_s = -\mathbf{k}_1 + \mathbf{k}_2 + \mathbf{k}_3$  direction, where  $\mathbf{k}_i$  stands for the wavevector of the  $i^{\text{th}}$  pulse. The pulse sequence and the time variables are shown in Figure 2.11.



**Figure 2.11** Excitation pulse sequence for third-order nonlinear spectroscopies, including transient grating spectroscopy, after Brixner et al.<sup>22</sup> and Sugisaki et al.<sup>40</sup> Three excitation pulses with wavevectors  $\mathbf{k}_1$ ,  $\mathbf{k}_2$ , and  $\mathbf{k}_3$  are centered at times  $-T - \tau$ , -T, and 0, respectively. The time intervals  $t_1$ ,  $t_2$ , and  $t_3$  are between the interactions with the electric fields from the three pulses. The interactions can occur at any time inside the pulse envelope.  $P^{(3)}(t)$  denotes the resulting third-order nonlinear polarization at time t.

The third-order polarization,  $P^{(3)}(\tau,T,t)$ , can be written as a convolution of the system response and the electric fields of the three laser pulses as

$$P^{(3)}(\tau, T, t) = \int_{0}^{\infty} \int_{0}^{\infty} \int_{0}^{\infty} dt_1 dt_2 dt_3 S^{(3)}(t_1, t_2, t_3) E_3(t - t_3) E_2(t + T - t_3 - t_2)$$

$$\times E_1(t + \tau + T - t_3 - t_2 - t_1)$$
(2.9)

where  $S^{(3)}(t_1,t_2,t_3)$  is the system response determined by the sum of the relevant third-order Feynman pathways, as discussed below,  $E_i$  are the electric fields of the laser pulses, and  $t_i$  are the time intervals between the field-matter interactions. Assuming the weak-field limit and the and the rotating wave approximation, and assuming that only the terms that contribute in the phase-matched direction are included,  $P^{(3)}(\tau, T, t)$ , can be expressed as<sup>22</sup>

$$P^{(3)}(\tau, T, t) = \exp(-i\omega_{0}t + i\omega_{0}\tau)\int_{0}^{\infty}\int_{0}^{\infty}\int_{0}^{\infty}dt_{1} dt_{2} dt_{3}$$

$$\times \left\{ S_{R} \left[ A_{1}^{*}(t + \tau + T - t_{1} - t_{2} - t_{3}) A_{2}(t + T - t_{2} - t_{3}) A_{3}(t - t_{3}) + A_{1}^{*}(t + \tau + T - t_{1} - t_{2} - t_{3}) A_{3}(t - t_{2} - t_{3}) A_{2}(t + T - t_{3}) \right] + S_{NR} \left[ A_{2}(t + T - t_{1} - t_{2} - t_{3}) A_{1}^{*}(t + \tau + T - t_{2} - t_{3}) A_{3}(t - t_{3}) + A_{3}(t - t_{1} - t_{2} - t_{3}) A_{1}^{*}(t + \tau + T - t_{2} - t_{3}) A_{3}(t - t_{3}) \right] + S_{DQC} \left[ A_{2}(t + T - t_{1} - t_{2} - t_{3}) A_{3}(t - t_{2} - t_{3}) A_{1}^{*}(t + \tau + T - t_{3}) + A_{3}(t - t_{1} - t_{2} - t_{3}) A_{2}(t + T - t_{2} - t_{3}) A_{1}^{*}(t + \tau + T - t_{3}) \right] \right\}$$

$$(2.10)$$

Equation 2.10 is expressed in terms of the electric field envelope function for the *i*<sup>th</sup> pulse,  $A_i(t)$ , which scales the electromagnetic wave  $E_i(t) = A_i(t)\exp(-i\omega_0 t + i\mathbf{k}_i \cdot \mathbf{r})$ , where  $\mathbf{k}_i$  is the wave vector of the electric field and  $\omega_0$  is the carrier frequency. The times  $t_1$ ,  $t_2$ , and  $t_3$  indicate the time intervals between the electric field interactions,  $\tau$  is the time delay between the first and second pulses (the coherence delay), and *T* is the time delay between the second and third pulses (the population delay). Given that the laser pulses have finite pulse durations, all of the possible time orderings for the three field–matter interactions have to be taken into account. The system response terms  $S_R$ ,  $S_{NR}$ , and  $S_{DQC}$  represent the sums of the several responses contributing to the rephasing (R), nonrephasing (NR) and DQC Feynman pathways, respectively. The detailed expressions for these pathways depend on the particular model (Figure 2.9) under consideration. Once the polarization  $P^{(3)}(\tau, T, t)$  has been calculated, the signal field can be obtained using Equation 2.1, and then the absorption (real) and dispersion (imaginary) components of the transient grating signal can be calculated.



**Figure 2.12** Double-sided Feynman diagrams for pathways contributing to the third order nonlinear optical response of  $\beta$ -carotene based on the energy-level scheme shown in Figure 2.9 and the assumption of detection along the  $\mathbf{k}_s = -\mathbf{k}_1 + \mathbf{k}_2 + \mathbf{k}_3$  direction. The solid arrows indicate field-matter interactions on the ket (left) and bra (right) side of the density matrix. The curly arrow denotes the emission event in the phase-matched direction arising from the coherence created by the third field-matter interaction. The dashed lines indicate relaxation of population between states. Time increases as one goes from the bottom to the top in each diagram.

Figure 2.12 shows double-sided Feynman diagrams for all the pathways needed to calculate the transient grating signals for the case where the  $S_x$  state is included given the assumption of detection along the  $\mathbf{k}_s = -\mathbf{k}_1 + \mathbf{k}_2 + \mathbf{k}_3$  direction. For a simulation without inclusion of the  $S_x$ state, the Feynman diagrams associated with ESA transitions from the  $S_x$  state are omitted from the calculation, and nonradiative decay from  $S_2$  populates  $S_1$  directly. This effectively removes the  $S_x$  manifold from Figure 2.9. SE transitions from  $S_x$  are not included in the model because of the narrow probe bandwidth used in the present experiments.

The labels for the Feynman diagrams and the associated response functions shown in Figure 2.12 are as used in the previous work for a three-level system by Brixner et al.,<sup>22</sup> but we have added the diagrams required to handle the additional states included in Figure 2.9. The response functions  $R_{1,g} - R_{4,g}$  describe SE and GSB pathways for transitions between the ground state S<sub>0</sub> (*g*) and the resonant S<sub>2</sub> state, (*e*).  $R_{1,f}^*$  and  $R_{2,f}^*$  indicate the ESA pathways from the S<sub>2</sub> state to the S<sub>n2</sub> (*f*) state.  $R_{3,f}^*$  and  $R_{4,f}$  are the DQC pathways. The pathways labeled  $R_{S_x}$  and  $R_{S_1}$  are for the rephasing (R) and nonrephasing (NR) pathways involving ESA from the S<sub>x</sub> and S<sub>1</sub> states, respectively.

The DQC diagrams deserve additional explanation. The Feynman pathways that contribute to the DQC signals are shown in Figure 2.13 along with the corresponding wave-mixing energy level (WMEL) diagrams.<sup>47</sup> The first field–matter interaction creates a coherence between the ground state S<sub>0</sub> and the resonant excited state S<sub>2</sub>. Rather than creating a population in the S<sub>2</sub> or S<sub>0</sub> states, the second field–matter interaction results in a coherence between the ground state and the double-quantum excited state, S<sub>n2</sub>. This is called a DQC, and it emits light via a free-induction decay with a frequency corresponding to the S<sub>0</sub>–S<sub>n2</sub> energy gap, approximately twice the frequency of the S<sub>0</sub>–S<sub>2</sub> absorption that was initially pumped. The third field–matter interaction drives an ESA-like transition that prepares a S<sub>2</sub>– S<sub>n2</sub> coherence ( $R_{3,f}^*$ ), or it drives a SE-like transition yielding a S<sub>0</sub> – S<sub>2</sub> coherence ( $R_{4,f}$ ). Both of these coherences oscillate at frequencies near to the S<sub>0</sub>–S<sub>2</sub> absorption band so they can be detected experimentally in a one-color experiment. Depending on the relative transition dipole strengths for the third field–matter interaction in the two pathways, the total contribution of DQC pathways to the third-order signal can be positive (increasing light emission) or negative (decreasing light emission).

Note that the (2,3,1) time ordering shown in the DQC diagrams corresponds to a negative coherence time  $\tau$  (pulse 2 arrives prior to pulse 1) and to negative population delays *T* (pulse 3 arrives prior to pulse 1). This sequence of interactions is required if the signal is to be detected along the  $\mathbf{k}_s = -\mathbf{k}_1 + \mathbf{k}_2 + \mathbf{k}_3$  direction used in the present experiments. In pump–probe experiments, where the pump pulse accounts for the first two field–matter interactions ( $\mathbf{k}_1 = \mathbf{k}_2$ ) and the detected signal has to be emitted along the direction of the delayed probe pulse ( $\mathbf{k}_s = \mathbf{k}_3$ ), additional DQC pathways, corresponding to all possible time orderings for the three field–matter interactions, contribute to the signal.<sup>18,20</sup> Thus, the transient grating experiment ( $\tau = 0$ ) restricts the DQC pathways to a subset of the possible time orderings; it confines the DQC signal to the width of the excitation pulses symmetric with the zero of time (T = 0). Further, as shown below, the transient grating experiment can be conducted with different small and negative coherence delays ( $\tau < 0$ ) in order to project out the DQC contribution relative to the population-dependent pathways.

For use in nonlinear optical simulations, the third-order response functions associated with the Feynman pathways shown in Figure 2.12 and Figure 2.13 are expressed in terms of linebroadening functions, g(t). These functions have been listed elsewhere,<sup>19,22,28</sup> but they are reproduced in the Appendix for completeness and employ a consistent notation. In the following simulations, it is assumed that population relaxation between states results in vibrational decoherence; the phase for the underdamped vibrational terms in M(t) is randomized by the conversion from one state by the conversion from one state to another.<sup>48</sup> Accordingly, all the response functions that include nonradiative decay between states during the interval between the second and third field-matter interactions have to be scaled by Markovian population kinetics.<sup>48,49</sup> Note that the validity of this assumption will be revisited in the Discussion section. For now, consider that the use of 40 fs pulses in the present experiments limits us to an impulsive spectral bandwidth that is much narrower than required to observe coherent wavepacket motions in the high-frequency, bond-length alternation coordinates included in M(t).



**Figure 2.13** Wave-mixing energy level (WMEL) diagrams<sup>47</sup> and the corresponding double-sided Feynman diagrams for the double quantum coherence pathways in  $\beta$ -carotene given the assumption of detection along the  $\mathbf{k}_s = -\mathbf{k}_1 + \mathbf{k}_2 + \mathbf{k}_3$  direction. In the WMEL diagrams, time is ordered from left to right. The solid and dotted arrows in the WMEL diagrams indicate interactions from the ket and bra sides of the density matrix, respectively. The wavy line indicates the final emission of the signal.

The kinetic scheme is different for the two types of models used in the simulation that follow. For models omitting  $S_x$ , the expression for  $S_R$ ,  $S_{NR}$  and  $S_{DQC}$  in Equation 2.10 are:

$$S_{\rm R} = R_{3,g} \left[ \frac{-\Gamma_{\rm S_1}}{(\Gamma_{\rm S_2} - \Gamma_{\rm S_1})} \exp(-\Gamma_{\rm S_2} t_2) + \frac{\Gamma_{\rm S_2}}{(\Gamma_{\rm S_2} - \Gamma_{\rm S_1})} \exp(-\Gamma_{\rm S_1} t_2) \right] - R_{\rm S_1,R} \left[ \frac{-\Gamma_{\rm S_2}}{(\Gamma_{\rm S_2} - \Gamma_{\rm S_1})} \exp(-\Gamma_{\rm S_2} t_2) + \frac{\Gamma_{\rm S_2}}{(\Gamma_{\rm S_2} - \Gamma_{\rm S_1})} \exp(-\Gamma_{\rm S_1} t_2) \right] + (R_{2,g} - R_{1,f}^*) \exp(-\Gamma_{\rm S_2} t_2)$$
(2.11)

$$S_{\rm NR} = R_{4,g} \left[ \frac{-\Gamma_{\rm S_1}}{(\Gamma_{\rm S_2} - \Gamma_{\rm S_1})} \exp(-\Gamma_{\rm S_2} t_2) + \frac{\Gamma_{\rm S_2}}{(\Gamma_{\rm S_2} - \Gamma_{\rm S_1})} \exp(-\Gamma_{\rm S_1} t_2) \right] - R_{\rm S_1,NR} \left[ \frac{-\Gamma_{\rm S_2}}{(\Gamma_{\rm S_2} - \Gamma_{\rm S_1})} \exp(-\Gamma_{\rm S_2} t_2) + \frac{\Gamma_{\rm S_2}}{(\Gamma_{\rm S_2} - \Gamma_{\rm S_1})} \exp(-\Gamma_{\rm S_1} t_2) \right] + (R_{\rm I,g} - R_{2,f}^*) \exp(-\Gamma_{\rm S_2} t_2)$$
(2.12)

$$S_{\rm DQC} = R_{4,f} - R_{3,f}^* \tag{2.13}$$

Whereas for the model with the intermediate, the expressions for  $S_R$ ,  $S_{NR}$  and  $S_{DQC}$  in Equation (2.10) are determined as

$$S_{R} = R_{3,g} \left[ \frac{\Gamma_{S_{2}} \Gamma_{S_{x}}}{(\Gamma_{S_{2}} - \Gamma_{S_{1}})(\Gamma_{S_{x}} - \Gamma_{S_{1}})} \exp(-\Gamma_{S_{1}} t_{2}) - \frac{\Gamma_{S_{2}} \Gamma_{S_{x}}}{(\Gamma_{S_{2}} - \Gamma_{S_{x}})(\Gamma_{S_{x}} - \Gamma_{S_{1}})} \exp(-\Gamma_{S_{x}} t_{2}) + \frac{\Gamma_{S_{1}} \Gamma_{S_{x}}}{(\Gamma_{S_{2}} - \Gamma_{S_{x}})(\Gamma_{S_{2}} - \Gamma_{S_{1}})} \exp(-\Gamma_{S_{2}} t_{2}) \right] - R_{S_{x},R} \left[ \frac{\Gamma_{S_{2}}}{(\Gamma_{S_{2}} - \Gamma_{S_{x}})} \left\{ -\exp(-\Gamma_{S_{2}} t_{2}) + \exp(-\Gamma_{S_{x}} t_{2}) \right\} \right]$$

$$- R_{S_{1},R} \left[ \frac{\Gamma_{S_{2}} \Gamma_{S_{x}}}{(\Gamma_{S_{2}} - \Gamma_{S_{1}})(\Gamma_{S_{x}} - \Gamma_{S_{1}})} \exp(-\Gamma_{S_{x}} t_{2}) + \frac{\Gamma_{S_{2}} \Gamma_{S_{x}}}{(\Gamma_{S_{2}} - \Gamma_{S_{x}})(\Gamma_{S_{x}} - \Gamma_{S_{1}})} \exp(-\Gamma_{S_{x}} t_{2}) + \frac{\Gamma_{S_{2}} \Gamma_{S_{x}}}{(\Gamma_{S_{2}} - \Gamma_{S_{x}})(\Gamma_{S_{x}} - \Gamma_{S_{1}})} \exp(-\Gamma_{S_{x}} t_{2}) + \frac{\Gamma_{S_{2}} \Gamma_{S_{x}}}{(\Gamma_{S_{2}} - \Gamma_{S_{x}})(\Gamma_{S_{x}} - \Gamma_{S_{1}})} \exp(-\Gamma_{S_{x}} t_{2}) + \frac{\Gamma_{S_{2}} \Gamma_{S_{x}}}{(\Gamma_{S_{2}} - \Gamma_{S_{x}})(\Gamma_{S_{x}} - \Gamma_{S_{1}})} \exp(-\Gamma_{S_{x}} t_{2}) + \frac{\Gamma_{S_{2}} \Gamma_{S_{x}}}{(\Gamma_{S_{2}} - \Gamma_{S_{x}})(\Gamma_{S_{x}} - \Gamma_{S_{1}})} \exp(-\Gamma_{S_{x}} t_{2}) + \frac{\Gamma_{S_{2}} \Gamma_{S_{x}}}{(\Gamma_{S_{2}} - \Gamma_{S_{x}})(\Gamma_{S_{x}} - \Gamma_{S_{1}})} \exp(-\Gamma_{S_{x}} t_{2}) + \frac{\Gamma_{S_{x}} \Gamma_{S_{x}}}{(\Gamma_{S_{x}} - \Gamma_{S_{x}})(\Gamma_{S_{x}} - \Gamma_{S_{x}})} \exp(-\Gamma_{S_{x}} t_{2}) + \frac{\Gamma_{S_{x}} \Gamma_{S_{x}}}{(\Gamma_{S_{x}} - \Gamma_{S_{x}})(\Gamma_{S_{x}} - \Gamma_{S_{x}})} \exp(-\Gamma_{S_{x}} t_{2}) + \frac{\Gamma_{S_{x}} \Gamma_{S_{x}}}{(\Gamma_{S_{x}} - \Gamma_{S_{x}})(\Gamma_{S_{x}} - \Gamma_{S_{x}})} \exp(-\Gamma_{S_{x}} t_{2}) + \frac{\Gamma_{S_{x}} \Gamma_{S_{x}}}{(\Gamma_{S_{x}} - \Gamma_{S_{x}})(\Gamma_{S_{x}} - \Gamma_{S_{x}}})} \exp(-\Gamma_{S_{x}} t_{2}) + \frac{\Gamma_{S_{x}} \Gamma_{S_{x}}}{(\Gamma_{S_{x}} - \Gamma_{S_{x}})(\Gamma_{S_{x}} - \Gamma_{S_{x}})}} \exp(-\Gamma_{S_{x}} t_{2})$$

$$S_{NR} = R_{4,g} \left[ \frac{\Gamma_{S_{2}} \Gamma_{S_{x}}}{(\Gamma_{S_{2}} - \Gamma_{S_{1}})(\Gamma_{S_{x}} - \Gamma_{S_{1}})} \exp(-\Gamma_{S_{1}} t_{2}) - \frac{\Gamma_{S_{2}} \Gamma_{S_{x}}}{(\Gamma_{S_{2}} - \Gamma_{S_{x}})(\Gamma_{S_{x}} - \Gamma_{S_{1}})} \exp(-\Gamma_{S_{x}} t_{2}) + \frac{\Gamma_{S_{1}} \Gamma_{S_{x}}}{(\Gamma_{S_{2}} - \Gamma_{S_{x}})(\Gamma_{S_{2}} - \Gamma_{S_{1}})} \exp(-\Gamma_{S_{2}} t_{2}) \right] - R_{S_{x,NR}} \left[ \frac{\Gamma_{S_{2}}}{(\Gamma_{S_{2}} - \Gamma_{S_{x}})} \left\{ -\exp(-\Gamma_{S_{2}} t_{2}) + \exp(-\Gamma_{S_{x}} t_{2}) \right\} \right]$$
(2.15)  
$$- R_{S_{1,NR}} \left[ \frac{\Gamma_{S_{2}} \Gamma_{S_{x}}}{(\Gamma_{S_{2}} - \Gamma_{S_{1}})(\Gamma_{S_{x}} - \Gamma_{S_{1}})} \exp(-\Gamma_{S_{x}} t_{2}) + \frac{\Gamma_{S_{2}} \Gamma_{S_{x}}}{(\Gamma_{S_{2}} - \Gamma_{S_{x}})(\Gamma_{S_{2}} - \Gamma_{S_{1}})} \exp(-\Gamma_{S_{2}} t_{2}) \right] + \left( R_{1,g} - R_{2,f}^{*} \right) \exp(-\Gamma_{S_{2}} t_{2})$$
$$S_{DQC} = R_{4,f} - R_{3,f}^{*}$$
(2.16)

In equations 2.11–2.16,  $\Gamma_i$  denotes the inverse lifetime  $1/\tau_i$  for a given state *i*.

## 2.4.2.1 Simulation of Transient Grating Signal Without S<sub>x</sub>

Using the theory discussed above, we simulated the transient grating signal from  $\beta$ -carotene in benzonitrile assuming first that the S<sub>x</sub> manifold is omitted from Figure 2.9, so the radiationless decay proceeds directly from S<sub>2</sub> to S<sub>1</sub>. The absorption and dispersion components calculated with an optimized set of parameters are shown in Figures 2.14 and 2.15. The simulations employ a laser spectrum centered at 19230 cm<sup>-1</sup> and a pulse duration of 45 fs. The lifetime for the S<sub>2</sub> state is set here to 150 fs. The S<sub>1</sub> state is assigned an 11.6 ps lifetime, to match the decay time constant exhibited by the absorption component in the global model. These simulations are intended, however, to describe only the <500 fs population time range, so no attempt is made here to handle the spectroscopic origin of the different ground-state recovery time constants observed in the global model for the absorption and dispersion components. As we will discuss in a separate contribution with a more detailed model for that part of the response, the different kinetics observed in the two components arises from an initial production of a conformationally displaced structure on the  $S_0$  potential surface.



**Figure 2.14** Numerical simulation of the absorption component of the transient grating signal from  $\beta$ -carotene, with the S<sub>x</sub> state omitted from the energy level scheme shown in Figure 2.9. The parameters used in the calculation are listed in Table 2.1–2.3 and in the text. The yellow end of the color bar corresponds to net ground-state bleaching or stimulated emission; the dark blue end indicates excited state absorption.



**Figure 2.15**. Numerical simulation of the dispersion component of the transient grating signal from  $\beta$ -carotene, with the S<sub>x</sub> state omitted from the energy level scheme shown in Figure 9. The parameters used in the calculation are listed in Table 2.1–2.3 and in the text. At 520 nm, the yellow contours correspond to the net ESA signal from the S<sub>1</sub> state.

Table 2.2 gives the energy gaps and relative transition dipole moment strengths for the relevant spectroscopic transitions obtained after optimization of the model. These parameters are dependent on the reorganization energies used in the model and therefore they differ somewhat from the values employed by Christensson et al.<sup>41</sup> The lineshape parameters for the  $S_0 \rightarrow S_2$  transition were taken from the model for the absorption spectrum simulation, as discussed above and listed in Table 2.1. The ESA transition from  $S_2 \rightarrow S_{n2}$  was modeled with an identical set of line-broadening parameters. This choice results in an ESA lineshape for  $S_2$  that is similar to that observed by Cerullo et al,<sup>9</sup> but the simulations and DQC results discussed below require it to be shifted farther to the blue. The optimized model sets the  $S_2 \rightarrow S_{n2}$  energy gap to 17000 cm<sup>-1</sup>, as listed in Table 2.2. A similar value was obtained by Christensson et al.<sup>19</sup>. Also as specified by Christensson et al.,<sup>19</sup> the lineshape parameters for the off-diagonal functions<sup>22</sup> associated with the

 $S_2 \rightarrow S_{n2}$  transitions were assumed to have the same temporal dependence but half the reorganization energy of the ground state bleaching transition.

**Table 2.2** Transition frequencies ( $\omega_{ij}$ ) and relative transition dipole strengths ( $\mu_{ij}$ ) used in the simulation of the transient-grating signal from  $\beta$ -carotene without inclusion of the S<sub>x</sub> state (see Figure 2.9).

Transition	$\omega_{ij} (\mathrm{cm}^{-1})$	$\mu_{ij}$ (Debye)
$S_0 \rightarrow S_2$	21550	1
$S_2 \rightarrow S_{n2}$	17000	0.35
$S_1 \rightarrow S_{n1}$	17850	1.70

Lastly, the lineshape parameters for the  $S_1 \rightarrow S_{n1}$  ESA transition were chosen such that the simulated spectrum has the same shape as the pump-probe spectrum observed at long population delays.<sup>9</sup> The MBO parameters are listed in Table 2.3; as in previous work<sup>41</sup> an additional vibrational mode of 1820 cm<sup>-1</sup> is added to better simulate the vibronic structure observed in the ESA from the S<sub>1</sub> state. As determined in femtosecond stimulated Raman experiments,<sup>50,51</sup> this mode has a similar intensity as the 1520 cm<sup>-1</sup> mode in the S<sub>1</sub> state and therefore needs to be included in the simulation.

Mode	$\lambda (\text{cm}^{-1})$	au (fs)	$\boldsymbol{\omega}_{v}  (\mathrm{cm}^{-1})$
Gaussian	100	10	
Exponential 1	300	500	
Exponential 2	450	1000	
Vibration 1	200	200	1150
Vibration 2	250	200	1520
Vibration 3	300	200	1820

**Table 2.3** MBO model parameters<sup>*a*</sup> for the  $S_1 \rightarrow S_{n1}$  ESA transition of  $\beta$ -carotene in benzonitrile at 295 K.

<sup>a</sup>See Equations 2.7–2.10. For a given term in M(t),  $\lambda$  is the reorganization energy and  $\tau$  denotes the damping time constant;  $\omega_v$  is the frequency for a vibrational mode.

The simulated transient grating response shown in Figures 2.14 and 2.15 does not provide an acceptable description of both components of the experimentally observed transient grating signal even though a visual comparison with Figures 2.4 and 2.5, respectively, might initially suggest a reasonable concordance. In order to allow an easily visualized, quantitative comparison of the calculated and observed signals, Figure 2.16 and Figure 2.17 show plots of the absorption and dispersion components, respectively, as their integrals over the signal frequency axis with respect to the population delay *T*. These plots are comparable in appearance to the 520-nm experimental transients shown in Figure 2.8. The experimental and calculated signals are normalized at T = 500 fs, where the chief contribution to the signal is from ESA from the population in the S<sub>1</sub> state.



**Figure 2.16** Comparison of the experimental and calculated absorption components of the transient grating signal from  $\beta$ -carotene. The calculation omits the S<sub>x</sub> state from the energy level scheme shown in Figure 2.9. The experimental (Figure 2.4) and calculated signals (Figure 2.14) were integrated over the laser frequency axis and normalized at the 500 fs delay point. *Top Panel:* Absorption components from the experimental transient (data points) and from the simulation (solid line). *Bottom Panel:* Absorption components calculated from individual Feynman pathways: Orange, GSB; Red, SE from the S<sub>2</sub> state; Blue, S<sub>2</sub>  $\rightarrow$  S<sub>n2</sub> ESA; Green, DQC; Black, S<sub>1</sub>  $\rightarrow$  S<sub>n1</sub> ESA.



**Figure 2.17** Comparison of the experimental and calculated dispersion components of the transient grating signal from  $\beta$ -carotene. The calculation omits the S<sub>x</sub> state from the energy level scheme shown in Figure 2.9. The experimental (Figure 5) and calculated signals (Figure 2.15) were integrated over the laser frequency axis and normalized at the 500 fs delay point. *Top Panel:* Dispersion components from the experimental transient (data points) and from the simulation (solid line). *Bottom Panel:* Dispersion components calculated from individual Feynman pathways: Orange, GSB; Red, SE from the S<sub>2</sub> state; Blue, S<sub>2</sub>  $\rightarrow$  S<sub>n2</sub> ESA; Green, DQC; Black, S<sub>1</sub>  $\rightarrow$  S<sub>n1</sub> ESA.

With the parameters chosen, the rise and decay of the experimental and simulated absorption transients match reasonably well (Figure 2.16), but keep in mind that the absorption component was used as the target for the simulation. The rapid initial decay, which exhibits a 12 fs time constant in the global model, mostly arises from line broadening of the GSB. The decay of the SE from the  $S_2$  state with a 150 fs time constant is accompanied by a concurrent rise of negative going ESA from the  $S_1$  state. Because the  $S_2$  and  $S_1$  states share the same ground state, the GSB is essentially nondecaying after the initial line broadening. The DQC pathways contribute a relatively small contribution to the overall absorption signal with a Gaussian shape coinciding

with the zero of the delay T axis. A larger contribution arises from the ESA signal from the S<sub>2</sub> state, which decays with a 150 fs time constant; owing to detuning of the laser spectrum from its energy gap, the S<sub>2</sub> ESA signal is smaller that one might have expected just considering the relative transition dipole strengths of the ESA and GSB contributions.

Using the same model parameters, however, the simulated dispersion component very poorly matches the experimental signal because it rises too slowly (Figure 2.17). Note that the dispersion signal has a significant negative going contribution near the zero of time from the benzonitrile solvent (Figure A2.1), which is not included in the calculated response. Nevertheless, over the 50–500-fs range, the calculated dispersion signal is well below that of the experiment. The calculated dispersion signal catches up with the experimental signal only at the 500 fs delay point, where the two transients are normalized.

The origin of the problem with the rise of the calculated dispersion signal is made clear upon examination of the time dependence of the contributions from the different Feynman pathways. Recall that the dispersion and absorption components are related by the Kramers–Kronig relationship,<sup>29,52,53</sup> so the signs of the different contributions to the dispersion signal depend sensitively on the lineshape for each contribution and on the detuning of the laser spectrum. Owing to spectral diffusion, the probe laser spectrum used in the present experiments is effectively detuned to the red side of the GSB signal that is present at long delay times *T*. In contrast, the probe spectrum is on the blue side of the ESA signal from the S<sub>1</sub> state. Accordingly, both the GSB and ESA signals contribute positive going signals to the dispersion component. The GSB contributes a prompt rising signal that is synchronized with the arrival of the pump pulses, but the rise of the S<sub>1</sub> ESA signal is slower, as it is determined in this model by the rate at which S<sub>2</sub> decays. The DQC and S<sub>2</sub> ESA signals also contribute prompt positive signals to the dispersion, so they contribute to the rising character of the dispersion component, but as in the absorption component their contributions are relatively weak. Thus, the problem is with the SE signal from the S<sub>2</sub> state. Because it is shifted to the red of the laser spectrum, as was observed in the work by de Weerd et al.,<sup>54</sup> it contributes a negative-going signal to the dispersion component. The overall rise of the calculated dispersion signal is significantly slower than that for the experimental transient because the SE signal decays with a 150 fs time constant. This comparison indicates directly that the SE signal is actually decaying much more rapidly than calculated with the model lacking the S<sub>x</sub> state.

#### 2.4.2.2 Simulation of Transient Grating Signal Without S<sub>x</sub>

We obtained much better results in simulations that included the  $S_x$  state as an intermediate along the radiationless decay pathway between  $S_2$  and  $S_1$  state, as indicated in Figure 2.9. The absorption and dispersion components calculated with an optimized set of parameters are shown in Figures 2.18 and 2.19. Figures 2.20 and 2.21 compare the experimental and calculated frequency integrated absorption and dispersion transients, respectively. In these simulations, the lifetimes of  $S_2$  and  $S_x$  states were set to be 12 fs and 142 fs respectively, as determined from the global model (Figures 2.6–2.8).



Figure 2.18 Numerical simulation of the absorption component of the transient grating signal from  $\beta$ -carotene, with the S<sub>x</sub> state included in the energy level scheme shown in Figure 2.9. The parameters used in the calculation are listed in Table 2.1, 2.2, and 2.4 and in the text. The yellow end of the color bar corresponds to net ground-state bleaching or stimulated emission; the dark blue end indicates excited state absorption.



**Figure 2.19** Numerical simulation of the dispersion component of the transient grating signal from  $\beta$ -carotene, with the S<sub>x</sub> state included in the energy level scheme shown in Figure 2.9. The parameters used in the calculation are listed in Table 2.1, 2.2, and 2.4 and in the text. The phasing of the color bar is consistent with that used in Figure 2.18 for the absorption component. At 520 nm, the yellow contours correspond to the net ESA signal from the S<sub>1</sub> state.

The transition dipole moment for optical transitions between the S<sub>0</sub> and S<sub>x</sub> states is set to zero,<sup>37</sup> so in this model S<sub>x</sub> contributes only an S<sub>x</sub>  $\rightarrow$  S<sub>nx</sub> ESA pathway, as shown in Figure 2.9, to the system response. The model parameters for the ESA transition are not well defined owing to the probe detuning in the present experiments. A guess for the energy gap (11200 cm<sup>-1</sup>, Table 4) was obtained by fitting the ESA lineshape assigned to S<sub>x</sub> that Cerullo et al.<sup>9</sup> observed in their broadband pump–probe experiments. The lineshape parameters and relative transition dipole strength applied for the S<sub>x</sub> ESA transition are the same as used for the GSB. The lineshape parameters used for the S<sub>1</sub> state are the same as used above for the model omitting the S<sub>x</sub> state. Note that the transition dipole strengths of the S<sub>2</sub>  $\rightarrow$  S<sub>n2</sub> ESA and S<sub>1</sub>  $\rightarrow$  S<sub>n1</sub> ESA transitions had to be altered from those used above (Table 2.2) to obtain a better agreement between the experimental and simulated absorption transient; in particular, the transition dipole strength for the S<sub>2</sub> state was strengthened by more than a factor of 2.

**Table 2.4** Transition frequencies ( $\omega_{ij}$ ) and relative transition dipole strengths ( $\mu_{ij}$ ) used in the simulation of the transient-grating signal from  $\beta$ -carotene with inclusion of the S<sub>x</sub> state (see Figure 2.9).

Transition	$\omega_{ij}$ (cm <sup>-1</sup> )	$\mu_{ij}$ (Debye)
$S_0 \rightarrow S_2$	21550	1
$S_2 \rightarrow S_{n2}$	17000	0.75
$S_x \rightarrow S_{nx}$	11200	1
$S_1 \rightarrow S_{n1}$	17850	1.54



**Figure 2.20** Comparison of the experimental and calculated absorption components of the transient grating signal from  $\beta$ -carotene. The calculation includes the S<sub>x</sub> state in the energy level scheme shown in Figure 2.9. The experimental (Figure 2.4) and calculated signals (Figure 2.18) were integrated over the laser frequency axis and normalized at the 500 fs delay point. *Top Panel:* Absorption components from the experimental transient (data points) and from the simulation (solid line). *Bottom Panel:* Absorption components calculated from individual Feynman pathways: Orange, GSB; Red, SE from the S<sub>2</sub> state; Blue, S<sub>2</sub>  $\rightarrow$  S<sub>n2</sub> ESA; Green, DQC; Black, S<sub>1</sub>  $\rightarrow$  S<sub>n1</sub> ESA.



**Figure 2.21** Comparison of the experimental and calculated dispersion components of the transient grating signal from  $\beta$ -carotene. The calculation includes the S<sub>x</sub> state in the energy level scheme shown in Figure 2.9. The experimental (Figure 2.5) and calculated signals (Figure 2.19) were integrated over the laser frequency axis and normalized at the 500 fs delay point. *Top Panel:* Dispersion components from the experimental transient (data points) and from the simulation (solid line). *Bottom Panel:* Dispersion components calculated from individual Feynman pathways: Orange, GSB; Red, SE from the S<sub>2</sub> state; Blue, S<sub>2</sub>  $\rightarrow$  S<sub>n2</sub> ESA; Green, DQC; Black, S<sub>1</sub>  $\rightarrow$  S<sub>n1</sub> ESA.

The optimized parameters in this model again result in a good match of the simulated absorption component with experiment. The ability to model the absorption component equally well with or without  $S_x$  included in the energy level scheme shows that detuning of the probe bandwidth is large compared to the absorption bandwidth. The observed 12 fs decay is accordingly assigned again predominantly to line broadening of the GSB contribution. Again, the GSB does not recover after the initial line broadening because  $S_x$  shares the same ground state with  $S_2$  and  $S_1$ . The 142 fs decay observed in the absorption component mainly reflects the rise of the  $S_1$  ESA. The contributions of the SE and ESA from the  $S_2$  are comparable in this model

owing to the strengthening of the transition dipole moment for the  $S_2$  ESA transition, which also strengthens the DQC contribution.

A comparison of the experimental and calculated dispersion components, both as contour figures (Figures 2.5 and 2.19) and as frequency integrated transients (Figure 2.21), however, shows that inclusion of the  $S_x$  state in the model provides a much better description of the experimental results. The model follows the experimental dispersion signal very well at delays >100 fs. The difference between the experimental and simulated dispersion transient now mainly arises from the negative going contribution from the benzonitrile solvent (Figure A2.1), especially near the zero of time. As in the absorption component, the ESA signal of the  $S_x$  state makes only a very small contribution to the overall signal because the detuning of the probe bandwidth is large compared to the width of the ESA lineshape. This means that the  $S_x$  state behaves kinetically as a "dark" state overall, with no strong contributions on resonance with the probe bandwidth, that effectively quenches the signals from the optically prepared S<sub>2</sub> state. The rising trend over the 100-500-fs period is now much better simulated because the negative going SE contribution from the S<sub>2</sub> state decays much more rapidly. In fact, this is the most important conclusion from the simulations: the fast rise of the dispersion component is associated with the decay of the SE component from the resonant  $S_2$  state as the  $S_x$  state is populated. The dispersion component makes it possible to discern separately the contributions of spectral diffusion from the relaxation of population from  $S_2$ .

A similar conclusion was made by Sugisaki et al.,<sup>37</sup> who showed that no recurrent excitedstate wavepacket motions contribute to the homodyne transient grating signal, at least as probed in the region of the  $S_0$ - $S_2$  absorption band. Their results require that nonradiative decay of the  $S_2$ state to the  $S_x$  state occurs with a time constant that is shorter than the period of the high-

frequency, C-C and C=C stretching frequencies. The model used by Sugisaki et al., however, includes an ESA lineshape from the  $S_x$  state that overlaps with the  $S_0 \rightarrow S_2$  ground state absorption region of the spectrum. Figure 2.22 shows, however, that use of the Sugisaki et al.  $S_x$ -S<sub>nx</sub> energy gap in the model results in a poorer description of the present experimental results. The S<sub>x</sub> ESA now makes a stronger contribution to the absorption component, so the calculated signal decays much more rapidly than we observe experimentally. The dispersion component is not as strongly affected because its lineshape has a zero crossing in the probed region, but the detuning to the blue results in an overall negative contribution to the dispersion that results in a poorer fit to the experimental signal. As the Sx-Snx energy gap is scanned, the simulations indicate differential effects on the absorption and dispersion components that are inconsistent with the observed results unless it is tuned far enough to the red of the probed energy gap with respect to the width of the ESA lineshape. These results show that despite the "dark" character of the  $S_x$  state, we can reasonably conclude that the ESA spectrum from the  $S_x$  state has parameters like that observed in the Cerullo et al.<sup>9</sup> pump-probe experiments. Note also that this conclusion argues against the assignment by Kosumi et al.<sup>55</sup> of the S<sub>x</sub> ESA spectrum to a nonresonant, twophoton absorption pathway.



**Figure 2.22** Comparison of the experimental and calculated dispersion components of the transient grating signal from  $\beta$ -carotene. The calculation includes the S<sub>x</sub> state in the energy level shown in Figure 2.9 but employs the S<sub>x</sub>–S<sub>nx</sub> energy gap in the visible region (~22000 cm<sup>-1</sup>) used in the calculations by Sugisaki et al.<sup>37</sup> The experimental (Figure 2.5) and calculated signals were integrated over the laser frequency axis and normalized at the 500 fs delay point. *Top Panel:* Absorption components from the experimental transient (data points) and from the simulation (solid line). *Bottom Panel:* Dispersion components from the experimental transient (data points) and from the simulation (solid line).

## 2.4.2.3 Contributions of Double-Quantum Coherence Pathways

In both simulations, with and without inclusion of  $S_x$  in the nonradiative decay pathway, the DQC contribution to the transient grating signal is relatively small compared to the contributions from population-dependent pathways. The heterodyne transient grating experiment makes the number of DQC pathways that contribute to the signal smaller than in pump–probe spectroscopy because, as explained above, only certain time orderings for the field–matter interactions are detected. With  $S_x$  included in the model, however, the DQC signals are more intense because the

transition dipole strength for the ESA from the  $S_2$  had to be increased from that used in the model without  $S_x$ .

In order to experimentally test the parameters that affect the DQC pathways, we performed a series of heterodyne transient grating experiments with the coherence delay  $\tau$  scanned to negative values, as described in the work by Joo and coworkers.<sup>56,57</sup> The analysis of the field-matter time orderings that follows from the DQC Feynman diagrams (Figure 2.13) indicates that when  $\tau < 0$ , the DQC pathways are enhanced in probability but that the DQC signal itself is limited to population delays T < 0. When  $\tau = 0$ , as in the conventional transient grating experiments discussed above, the DQC signal decays at population delays T > 0 following the intensity profile of the laser pulses.

Figure 23 shows the absorption component from a series of transient grating experiments in which  $\tau$  was scanned from 0 fs to -15 fs. The transient grating signals are normalized at the population decay T = 500 fs, where only population signals contribute, in order to project out the DQC-dependent parts of the signal. The rise of the signal moves to increasingly negative T values, as expected, as  $\tau$  is scanned to negative values. The peak intensity is largest for  $\tau = 10$  fs, and the signal is about 20% larger than with  $\tau = 0$ . Qualitatively, these results indicate that the effective dephasing time for the S<sub>n2</sub>S<sub>0</sub> DQC is ~10 fs; as  $\tau$  is scanned to more negative delays, the peak signal intensity decays because the S<sub>n2</sub>S<sub>0</sub> DQC has to wait longer for the last pulse in the (2,3,1) sequence to arrive. Further, the relative strength of the DQC signals estimated in the simulations above including S<sub>x</sub> (Figures 2.18–2.21) is approximately correct; DQC pathways are contributing a minor fraction compared to the  $\tau$ -invariant population signals measured at T = 0 in the  $\tau = 0$  experiments.



**Figure 2.23** Absorption component of the heterodyne transient grating signal from  $\beta$ -carotene in benzonitrile measured with a range of negative coherence delays,  $\tau < 0$ . The signals are normalized at the population delay T = 500 fs, where only population signals contribute.

These findings allow a clear assessment of the contributions of DQC pathways to the observed signals under our conditions. Owing to being confined to the T < 0 region in the heterodyne transient grating experiments, the impact of DQCs where  $S_x$  contributes to the time evolution of the signal is non-zero but minimal, especially at T > 20 fs, as controlled by the 40 fs pulses used in the present experiments. Because they are included in both simulations, the DQC pathways do not have an impact on the decision about whether  $S_x$  needs to be included in the nonradiative decay pathway from  $S_2$ .

# **2.5 Discussion**

The absorption and dispersion components of the heterodyne transient-grating signal from  $\beta$ carotene in benzonitrile, Figures 2.4 and 2.5, respectively, exhibit large changes in intensity and lineshape during the 12 fs kinetic component of the global model (Figures 2.6–2.8). To determine what causes these changes, we performed a set of numerical simulations employing the nonlinear optical response function formalism and the MBO model. Pathways for the populations and DQCs that follow excitation of the  $S_0$  to  $S_2$  transition were included in the models we tested.

The results of the simulations show that the time evolution of the transient-grating signal includes significant contributions from three processes with similar effective timescales: spectral diffusion and dynamic solvation, DQCs, and nonradiative decay from the S<sub>2</sub> state to the S<sub>x</sub> intermediate state. The distinct temporal profiles exhibited by the absorption and dispersion components, however, are principally determined by the S<sub>2</sub> to S<sub>x</sub> nonradiative decay process. The simulations indicate that good simultaneous fits of the absorption and dispersion components of the transient grating signal of  $\beta$ -carotene cannot be obtained unless the S<sub>x</sub> state is included in the model as an intermediate state between S<sub>2</sub> and S<sub>1</sub>. The key observation is an ultrafast rise of the dispersion component, which is controlled primarily by the decay of the SE from S<sub>2</sub>. If S<sub>x</sub> is not included in the model, the dispersion exhibits a much slower rise that is controlled by the rise of the ESA signal from the S<sub>1</sub> state. As we will explain in the following, it is significant that the S<sub>x</sub> ESA spectrum is well red shifted from the S<sub>0</sub>–S<sub>2</sub> energy gap because it impacts the choice of hypotheses for the nature of the S<sub>x</sub> intermediate and for the mechanism for its formation after optical preparation of the S<sub>2</sub> state.

As observed in the work by Xu and Fleming,<sup>29,53,58</sup> with excitation pulses on resonance with a strong electronic transition, the absorption and dispersion components of a nonlinear optical signal exhibit distinct time profiles when an intermediate state is populated on the nonradiative decay pathway from the initially prepared state to the ground state. This feature of heterodyne transient grating spectroscopy allows us to determine several of the properties of the  $S_x$  state despite being limited by the probe bandwidth to a narrow spectral range. Owing to the Kramers– Kronig relation between the absorption and dispersion components for any response pathway, the shape of the temporal response for each component depends quite sensitively on how far the laser spectrum is tuned to the red or blue of the energy gap between the levels being driven in a particular pathway.

The energy gap and lineshape parameters we obtained for the  $S_x$  ESA signal from the simulations of the transient grating signals are actually quite consistent with the near-IR ESA spectrum observed by Cerullo et al.<sup>9</sup> for  $\beta$ -carotene at a pump–probe delay of 50 fs, which maximally populates the  $S_x$  state. This finding is inconsistent with the conclusion by Kosumi et al.<sup>55,59</sup> that the near-IR ESA signal from  $S_x$  is predominantly due to excitation of nonresonant, two-photon absorption pathways. The direct contribution of the  $S_x$  state to the absorption and dispersion signals with 520 nm probing is very weak due to the detuning to the blue, but the flow of population through  $S_x$  has a big impact on the temporal response because the decay from  $S_2$  to  $S_x$  effectively quenches the SE and ESA signals of the  $S_2$  state.

The conclusion that the  $S_2$  state is rapidly depopulated by nonradiative decay, much more rapidly than is consistent with the 150 fs rise time of the  $S_1$  state, is consistent with the finding by Sugisaki et al.<sup>37</sup> that the vibrational coherence observed in  $\beta$ -carotene solutions with probe light tuned into the  $S_2$  state absorption region is due to stimulated Raman transitions to the ground state manifold. If the  $S_2$  state lived as long as 150 fs, then there would be enough time prior to a nonradiative crossing to the  $S_1$  state potential surface for several vibrations of the highfrequency, C–C and C=C bond-length alternation coordinates of the conjugated polyene backbone.<sup>60,61</sup> That these motions are not observed in the Sugisaki et al. experiments means that wavepacket motion is not recurrent to the Franck–Condon region of the  $S_2$  state potential surface. This conclusion is consistent with an ultrafast surface-crossing event to the  $S_x$  state. With a broadband continuum reaching into the near-IR, however, Liebel et al.<sup>62</sup> observed excited state wavepacket motions with a damping time of 140 fs when probed at energy gaps well to the red of that for the  $S_0$ - $S_2$  transition. Their observations are consistent, then, with an assignment to coherent wavepacket motions after formation of the  $S_x$  state. If this assignment is correct, the vibrational phase is retained during the ultrafast surface-crossing event from the  $S_2$  state to the  $S_x$  state. The present simulations, which employed a simple Markov population transfer model, assume that vibrational decoherence accompanies the conversion of  $S_2$  to  $S_x$ . Owing to our use of 40 fs pulses, however, it would not have been possible to observe excited-state vibrational coherence in modes with frequencies above about 400 cm<sup>-1</sup>.

Simulations employing an ESA spectrum for  $S_x$  set to the blue of that from  $S_2$ , as in the work by Sugisaki et al.,<sup>37</sup> result in simulated absorption and dispersion transients that do not match the observed signal very well (Figure 2.22). A blue shifted ESA spectrum for  $S_x$  would be more consistent with a conventional hypothesis for the vibrational dynamics that accompany radiationless decay from  $S_2$  to  $S_1$ . As shown in Figure 24, displacements along the bond-length alternation coordinates would promote a crossing of the  $S_2$  and  $S_x$  potential surfaces, the latter at slightly lower energy. The  $S_x$  surface would then serve as a bridge to the  $S_1$  state. Such a scheme would in principle account for a deviation from the energy-gap law of the  $S_2$  to  $S_1$  nonradiative decay rate.<sup>63–65</sup> It would also account for the observations of Maiuri et al.,<sup>11</sup> who observed that a crossing from the  $S_2$  state to the  $S_x$  state does not occur for sphaeroidene dissolved in the highly polarizable solvent  $CS_2$ . The observation of electronic quantum beats with excitation near the peak of the  $S_0$ – $S_2$  absorption spectrum by Ostroumov et al.,<sup>66</sup> however, indicates that the  $S_2$  state interacts fairly strongly with an adjacent state at higher energies than the Franck–Condon energy gap, not at lower energies as proposed in the figure. This idea is compatible with the aforementioned work by Shreve et al.,<sup>14</sup> who detected a strong coupling of the  $S_2$  state to a nearby state with *g* symmetry.



Bond Length Alternation

**Figure 2.24** Diabatic energy curves for all-*trans* polyenes with respect to a generalized bond length alternation coordinate. The diagram includes a dark intermediate state ( $S_x$ ) in the transfer of population from the bright  $S_2$  (1<sup>1</sup> $B_u^+$ ) state to the dark  $S_1$  (2<sup>1</sup> $A_g^-$ ) state.

As an alternative hypothesis, we suggest that a red-shifted ESA spectrum from  $S_x$  can be better explained by vibrational dynamics involving a strong coupling of displaced bond-length alternation coordinates with torsional coordinates of the conjugated polyene backbone. This idea is based on the scheme shown in Figure 2.25, which describes the general nature of the potential energy surface for conjugated polyenes, cyanines, and protonated Schiff bases of intermediate lengths;<sup>67</sup> it has several features in common with that suggested previously for carotenoids by de Weerd et al.<sup>54</sup> We previously discussed in detail the theoretical background for this scheme and outlined some of its implications for the nature of the dark intermediate states that are encountered in the nonradiative decay of carotenoids in solution and in photosynthetic lightharvesting proteins.<sup>17</sup>



**Figure 2.25** Proposed scheme for radiationless decay of carotenoids after optical preparation of the S<sub>2</sub> state; after similar diagrams in Sanchez-Galvez et al.<sup>67</sup> and Beck et al.<sup>17</sup> The states that apply to planar structures are indicated by symmetry labels. Key points along the radiationless path from the Franck–Condon S<sub>2</sub> state structure are labeled with ethylenic structures, which depict the S<sub>x</sub> and X dark states as structures near the S<sub>2</sub> transition state and along the torsional gradient, respectively.

Figure 2.25 schematically represents the potential energy surfaces of carotenoids in terms of a reaction coordinate principally composed of sequential motions along two generalized vibrational coordinates, the bond-length alternation coordinates and torsional coordinates with respect to one of the C=C bonds of the conjugated polyene backbone. Based on the calculations discussed by Olivucci et al. for molecules with intermediate to long conjugation lengths,<sup>67</sup> the forces that act on the S<sub>2</sub> state Franck–Condon structure are predominantly along the bond-length
alternation coordinates. A coupling to torsional modes, however, occurs with displacement from the Franck–Condon structure and leads to the formation of a local activation barrier, which divides planar and twisted conformations. The transition state structure at the barrier would be expected to exhibit a red-shifted ESA spectrum; the energy gap between the S<sub>2</sub> state and the S<sub>n2</sub> state narrows there because the barrier height will decrease as the  $\pi^*$  character increases and the two states are displaced with respect to each other along the bond-length-alternation coordinates.

The scheme shown in Figure 2.25 identifies  $S_x$  as a transition-state structure on the  $S_2$  surface rather than as a discrete electronic state. The ultrashort time constant for the formation of  $S_x$ corresponds to the time it takes for the bond-length-alternation modes to undergo nearly a full excursion from the Franck–Condon structure, about one-half of a vibrational period. The molecule is acted upon then by a steep torsional gradient that directs the structure towards a conical intersection, where the  $S_2$ ,  $S_1$ , and  $S_0$  surfaces would be expected to converge. The torsional motions are not resonance Raman active, so the key event in the nonradiative decay process amounts to an intramolecular conversion of the initial momentum of the Franck-Condon structure from the bond-length alternation coordinates into the torsional coordinates. This idea has been discussed previously in theoretical and experimental work on PSBs in rhodopsins<sup>68,69</sup> and in cyanines in solution.<sup>70</sup>

The 142 fs lifetime of the  $S_x$  state determined in the present results, then, would correspond to the time it takes for a wavepacket to propagate down the  $S_2$  torsional gradient until it crosses through a seam with the  $S_1$  state potential surface.<sup>71</sup> It follows that the X state observed in 2DES by Ostroumov et al.<sup>15,16</sup> in terms of cross peaks should be assigned to a twisted  $S_2$  structure. This assignment accounts for the longer timescale they associate with formation of the X state compared to that observed here for the  $S_x$  state. The simple model used in our simulations treats  $S_x$  just as an intermediate state between  $S_2$  and  $S_1$ ; the kinetics are determined only by the loss of the  $S_2$  SE signal and the appearance of the  $S_1$  ESA signal. The intervening dynamics after the formation of  $S_x$  are hidden from us due to the narrow probe bandwidth we used in the present experiments.

Once the torsional motions begin as the  $S_x$  transition state structure is reached, the increasing displacement that follows along torsional coordinates of the conjugated polyene will naturally lead to an ICT character and a resultant frictional coupling to the surrounding solvent.<sup>72,73</sup> An interpretation of the aforementioned results of Liebel et al.<sup>62</sup> is that underdamped coherent wavepacket motions occur beyond the transition state on the  $S_2$  surface at least with respect to the high frequency, bond-length alternation coordinates. The minimum-energy path on the  $S_2$  potential surface would not necessarily be expected to exhibit strong oscillations with respect to the torsional or other out-of-plane normal coordinates, however, because these motions are likely to be increasingly diffusive in character as the displacement past the transition state increases if the solvent friction is significant. The ICT character may be of considerable importance in the energy-transfer function of carotenoids in photosynthetic light-harvesting proteins because it would increase the Coulomb coupling to the (B)Chl acceptor.

Given that the *trans–cis* photoisomerization quantum yield for longer polyenes is much less than unity, it is likely that an irreversible crossing through a seam to the  $S_1$  state occurs far from the 90° torsional geometry of the conical intersection.<sup>74</sup> The  $S_1$  state is produced with a considerable excess vibrational excitation, which leads to the observed blue shifting and sharpening of the ESA bandwidth at delays >500 fs as this population cools owing to intramolecular vibrational redistribution and vibrational relaxation.<sup>75</sup>

This hypothesis naturally accounts for the behavior of the broad ESA spectrum observed by Cerullo et al. for the  $S_2$ ,  $S_x$ , and  $S_1$  states;<sup>9</sup> it should evolve initially due to displacement with respect to the bond-length alternation coordinates and then along the torsional coordinates, where spreading of the wavepacket would reflect the action of solvent friction. The observed ESA spectrum moves initially very rapidly to the red after optical preparation of the  $S_2$  state as the transition state ( $S_x$ ) is reached, and then the ESA spectrum moves to the blue, which is consistent with relaxation down the torsional gradient. The blue-shifting ESA spectrum would be expected to be inhomogeneous and be strongly overlapped with a red-shifting SE signal, so parts of this response are likely to be obscured in conventional, one-dimensional spectra.<sup>17</sup>

We will treat the time evolution of the heterodyne transient grating signal from  $\beta$ -carotene over the >500 fs range in a separate contribution, but an important point to make now is that Figure 25 anticipates that a conformationally displaced ground state structure is expected to be the initial product of the S<sub>1</sub> to S<sub>0</sub> nonradiative decay event. Simulations can be used to show how the conformational relaxation process on the S<sub>0</sub> surface back to the originally photoselected ground state structure accounts for the distinct time constants that we observed for the recovery of the absorption and dispersion components of the transient grating signal. Further, because the twisted structures would have an ICT character, as noted above, the S<sub>1</sub> to S<sub>0</sub> decay would be anticipated to have a reverse charge transfer character and an associated strong solvent coupling.<sup>17</sup> This feature would cause the conformational relaxation to be slower in some cases than a conventional vibrational cooling process. In carbonyl-substituted carotenoids, such as peridinin<sup>76–79</sup> and fucoxanthin,<sup>80</sup> the time constants for the S<sub>1</sub> to S<sub>0</sub> and ground-state conformational relaxation for this process would be expected to have an unusual and distinctive dependence on the solvent polarity and polarizability.

#### 2.6 Conclusions

We report in this contribution the first femtosecond transient grating experiments on  $\beta$ carotene that employ optical heterodyne detection with spectral interferometry. In addition to providing the information required to assess several important controversies in the carotenoid literature, this work supports a structural hypothesis that accounts for the timescales and dynamics that depopulate the resonant S<sub>2</sub> state. The detection scheme we employed is critically important because it limits the order with respect to time with which the three field–matter interactions produce coherences and populations and because it provides information on the linebroadening dynamics that is ordinarily hidden from conventional pump–probe experiments, especially when narrow band probe spectra are employed.

A global target analysis establishes the key time constants and principal spectra that describe the time evolution of the absorption and dispersion components of the transient grating signal. But as in previous work in the ultrafast regime where coherences contribute to the signal, a proper interpretation of the system response requires the use of numerical simulations of the detected signals. The simulations establish how the relevant third-order Feynman pathways involving populations and double quantum coherences contribute to the response. For the first time to our knowledge for a carotenoid, these simulations simultaneously describe the absorption and dispersion components of the signal using a consistent set of parameters that effectively fit the observed response.

The results of this work establish clearly that the  $S_2$  state of  $\beta$ -carotene undergoes a radiationless decay pathway involving an ultrafast (12 fs) depopulation of the initial optically prepared state. The simulations show that the SE contribution to the signal decays due to population of a spectroscopic intermediate state,  $S_x$ , that is effectively dark with respect to the

probe bandwidth used in the present experiments. This is an important conclusion because it argues against previous suggestions<sup>55,59</sup> that a nonresonant two-photon absorption process rather than an intermediate state accounts for the ESA signals from the  $S_x$  state. Further, the contribution of DQCs to the transient grating signal were demonstrated experimentally and in the simulations not to be a significant determining factor in making the conclusion that the  $S_2$  state decays rapidly to  $S_x$  rather than going directly to  $S_1$ .

The model parameters that fit the experimental signals best are consistent with the previous observation by Cerullo et al.<sup>9</sup> of an near-IR ESA spectrum for the  $S_x$  state. Rather than supporting assignment of the  $S_x$  state to the  ${}^1B_u{}^-$  state of Tavan and Schulten, as previously suggested, the parameters favor a structural hypothesis for the deactivation of the  $S_2$  state involving a coupling between displaced high-frequency, bond-length alternation coordinates and torsional coordinates of the conjugated polyene backbone. In this hypothesis, the  $S_x$  state is a transition state structure, at the peak of the activation energy barrier that divides planar and twisted structures on the  $S_2$  potential surface. It is produced by the prompt excursion from the Franck–Condon structure along the bond-length alternation coordinates; the timescale for the formation of the transition state corresponds to less than a vibrational period for a C=C stretching mode.

Once activated, the conjugated backbone would then undergo a torsional relaxation from the  $S_x$  structure. This establishes the lifetime of the  $S_x$  state, 142 fs, as the time it takes a wavepacket to diffuse down the steep torsional gradient on the  $S_2$  surface and cross through a seam to the  $S_1$  surface, and it further suggests that the X state detected previously in 2DES experiments<sup>15,16</sup> arises from a twisted structure along the way. The proposed mechanism naturally suggests that an ICT character develops as the twisting occurs and that solvent friction retards the decay to the

 $S_1$  state. An important additional suggestion is that the structures that are operative in excitation energy transfer to carotenoids in photosynthetic light-harvesting proteins involve twisted structures with ICT character, which will be expected to exhibit improved donor-acceptor couplings and transition dipole strengths for energy transfer to adjacent (B)Chls. APPENDIX

## APPENDIX

# **A2.1 Supporting Results**



**Figure A2.1** Frequency integrated transient grating signal from neat benzonitrile at room temperature under the same measurement conditions used for  $\beta$ -carotene in Figures 2.4 and 2.5: (a) absorption component; (b) dispersion component; (c) complex modulus.



**Figure A2.2** Complex modulus of the heterodyne transient-grating signals from  $\beta$ -carotene in benzonitrile at three solute optical densities: (a) A = 0.26, as in Figures 4 and 5; (b) A = 0.13; and (c) A = 0.07.

For use in simulations of nonlinear optical signals, the third-order response functions associated with the Feynman pathways shown in Figures 2.12 and 2.13 are usually expressed in terms of line broadening functions, g(t). These functions have been listed elsewhere<sup>19,22,28</sup> but they are reproduced in the following for completeness and employ a consistent notation.

For a three level system, the ground state and the first and second excited states are denoted as g, e and f, respectively. The line broadening function that is used to simulate the absorption spectrum is represented by  $g_{ee}(t)$ . The line broadening function for the ESA transition from the S<sub>2</sub> state is given by  $g_{ff}(t)$ . The off-diagonal line broadening function  $g_{ef}(t)$  and  $g_{fe}(t)$  depend on the cross-correlation between the GSB and ESA transitions from the S<sub>2</sub> state and they are assumed to be the same. The line broadening functions  $g_{S_xS_x}(t)$  and  $g_{S_yS_1}(t)$  are related to the fluctuations of the ESA transitions from the S<sub>x</sub> and S<sub>1</sub> states, respectively. In the expressions that follow, the detunings (Figure 2.9) between the laser and the vertical transition energies are determined as

$$\Delta_{S_0} = \omega_0 - \omega_{S_0 \to S_2}$$
$$\Delta_{S_2} = \omega_0 + \omega_{s_2 \to s_{n_2}}$$
$$\Delta_{S_x} = \omega_0 - \omega_{s_x \to s_{n_x}}$$
$$\Delta_{S_1} = \omega_0 - \omega_{s_1 \to s_{n_1}}$$

Here  $\omega_0$  is the center frequency of the laser spectrum and  $\omega_{i \rightarrow j}$  indicates the frequency of a particular transition.

Using the notations introduced above, and letting  $\mu_{ij}$  stand for the transition dipole moment for a given transition, the expression for the response functions reads

$$R_{1,g}(t_1,t_2,t_3) = |\mu_{eg}|^2 |\mu_{eg}|^2 \exp(i\Delta_{s_0}t_1 + i\Delta_{s_0}t_3)$$
  
  $\times \exp\left[-g_{ee}^*(t_3) - g_{ee}(t_1) - g_{ee}^*(t_2) + g_{ee}^*(t_2 + t_3) + g_{ee}(t_1 + t_2) - g_{ee}(t_1 + t_2 + t_3)\right]$ 

$$R_{2,g}(t_1,t_2,t_3) = |\mu_{eg}|^2 |\mu_{eg}|^2 \exp(-i\Delta_{S_0}t_1 + i\Delta_{S_0}t_3)$$
  
 
$$\times \exp\left[-g_{ee}^*(t_3) - g_{ee}^*(t_1) + g_{ee}(t_2) - g_{ee}(t_2 + t_3) - g_{ee}^*(t_1 + t_2) + g_{ee}^*(t_1 + t_2 + t_3)\right]$$

$$R_{3,g}(t_1, t_2, t_3) = \left| \mu_{eg} \right|^2 \left| \mu_{eg} \right|^2 \exp(-i\Delta_{s_0} t_1 + i\Delta_{s_0} t_3) \\ \times \exp\left[ -g_{ee}(t_3) - g_{ee}^*(t_1) + g_{ee}^*(t_2) - g_{ee}^*(t_2 + t_3) - g_{ee}^*(t_1 + t_2) + g_{ee}^*(t_1 + t_2 + t_3) \right]$$

$$R_{4,g}(t_1, t_2, t_3) = \left| \mu_{eg} \right|^2 \left| \mu_{eg} \right|^2 \exp(i\Delta_{S_0}t_1 + i\Delta_{S_0}t_3)$$
  
 
$$\times \exp\left[ -g_{ee}(t_3) - g_{ee}(t_1) - g_{ee}(t_2) + g_{ee}(t_2 + t_3) + g_{ee}(t_1 + t_2) - g_{ee}(t_1 + t_2 + t_3) \right]$$

$$R_{1,f}^{*}(t_{1},t_{2},t_{3}) = \left| \mu_{eg} \right|^{2} \left| \mu_{ef} \right|^{2} \exp(-i\Delta_{S_{0}}t_{1} + i\Delta_{S_{2}}t_{3})$$

$$\times \exp\left[ -g_{ee}^{*}(t_{1}) - g_{ee}(t_{2}) + g_{ef}(t_{2}) - g_{ee}(t_{3}) + 2g_{ef}(t_{3}) - g_{ff}(t_{3}) + g_{ee}^{*}(t_{1} + t_{2}) \right]$$

$$-g_{ef}^{*}(t_{1} + t_{2}) + g_{ee}(t_{2} + t_{3}) - g_{ef}(t_{2} + t_{3}) - g_{ee}^{*}(t_{1} + t_{2} + t_{3}) + g_{ef}^{*}(t_{1} + t_{2} + t_{3}) \right]$$

$$R_{2,f}^{*}(t_{1},t_{2},t_{3}) = |\mu_{eg}|^{2} |\mu_{ef}|^{2} \exp(i\Delta_{S_{0}}t_{1} + i\Delta_{S_{2}}t_{3})$$

$$\times \exp\left[-g_{ee}(t_{1}) + g_{ee}^{*}(t_{2}) - g_{ef}^{*}(t_{2}) - g_{ee}(t_{3}) + 2g_{ef}(t_{3}) - g_{ff}(t_{3}) - g_{ee}(t_{1} + t_{2})\right]$$

$$+ g_{ef}(t_{1} + t_{2}) - g_{ee}^{*}(t_{2} + t_{3}) + g_{ef}^{*}(t_{2} + t_{3}) + g_{ee}(t_{1} + t_{2} + t_{3}) - g_{ef}(t_{1} + t_{2} + t_{3})\right]$$

$$R_{3,f}^{*}(t_{1},t_{2},t_{3}) = \left| \mu_{eg} \right|^{2} \left| \mu_{ef} \right|^{2} \exp \left[ i\Delta_{S_{0}}t_{1} + i(\Delta_{S_{0}} + \Delta_{S_{2}})t_{2} + i\Delta_{S_{2}}t_{3} \right] \\ \times \exp \left[ -g_{ee}(t_{1}) + g_{ef}(t_{1}) + g_{ee}(t_{2}) - g_{ef}(t_{2}) - g_{ee}^{*}(t_{3}) + g_{ef}^{*}(t_{3}) - g_{ee}(t_{1} + t_{2}) \right] \\ -g_{ee}(t_{2} + t_{3}) + 2g_{ef}(t_{2} + t_{3}) - g_{ff}(t_{2} + t_{3}) + g_{ee}(t_{1} + t_{2} + t_{3}) - g_{ef}(t_{1} + t_{2} + t_{3}) \right]$$

$$R_{4,f}(t_1,t_2,t_3) = \left| \mu_{eg} \right|^2 \left| \mu_{ef} \right|^2 \exp \left[ i\Delta_{S_0} t_1 + i(\Delta_{S_0} + \Delta_{S_2}) t_2 + i\Delta_{S_2} t_3 \right] \\ \times \exp \left[ -g_{ee}(t_1) + g_{ef}(t_1) - g_{ee}(t_2) + 2g_{ef}(t_2) - g_{ff}(t_2) - g_{ee}(t_3) + g_{ef}(t_3) \right. \\ \left. + g_{ee}(t_1 + t_2) - g_{ef}(t_1 + t_2) + g_{ee}(t_2 + t_3) - g_{ef}(t_2 + t_3) - g_{ee}(t_1 + t_2 + t_3) \right]$$

$$R_{S_{x},R}(t_{1},t_{2},t_{3}) = \left|\mu_{eg}\right|^{2} \left|\mu_{S_{x}}\right|^{2} \exp\left[-i\Delta_{S_{0}}t_{1} + i\Delta_{S_{x}}t_{3}\right] \exp\left[-g_{ee}^{*}(t_{1}) - g_{S_{x}S_{x}}(t_{3})\right]$$

$$R_{S_{x,NR}}(t_{1},t_{2},t_{3}) = |\mu_{eg}|^{2} |\mu_{S_{x}}|^{2} \exp\left[i\Delta_{S_{0}}t_{1} + i\Delta_{S_{x}}t_{3}\right] \exp\left[-g_{ee}(t_{1}) - g_{S_{x}S_{x}}(t_{3})\right]$$

$$R_{S_{1,R}}(t_{1},t_{2},t_{3}) = |\mu_{eg}|^{2} |\mu_{S_{1}}|^{2} \exp\left[-i\Delta_{S_{0}}t_{1} + i\Delta_{S_{1}}t_{3}\right] \exp\left[-g_{ee}^{*}(t_{1}) - g_{S_{1}S_{1}}(t_{3})\right]$$

$$R_{S_{1,NR}}(t_{1},t_{2},t_{3}) = \left|\mu_{eg}\right|^{2} \left|\mu_{S_{1}}\right|^{2} \exp\left[i\Delta_{S_{0}}t_{1} + i\Delta_{S_{1}}t_{3}\right] \exp\left[-g_{ee}(t_{1}) - g_{S_{1}S_{1}}(t_{3})\right]$$

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# Chapter 3: Nonradiative Deactivation of the $S_2$ $(1^1B_u^+)$ State of Peridinin: Detection and Spectroscopic Assignment of an Intermediate in the Decay Pathway

Femtosecond heterodyne transient grating spectroscopy was employed to investigate the nonradiative decay pathway from the  $S_2$   $(1^1B_u^+)$  state to the  $S_1$   $(2^1A_g^-)$  state of peridinin in methanol solution. Just as previously observed by this laboratory for  $\beta$ -carotene in benzonitrile, the real (absorption) and imaginary (dispersion) components of peridinin exhibit ultrafast responses indicating that S<sub>2</sub> state decays in 12 fs to produce an intermediate state, S<sub>x</sub>. The excited state absorption spectrum from the S<sub>x</sub> state of peridinin, however, is found to be markedly blue shifted from that of  $\beta$ -carotene because it makes a substantial contribution to the signal observed with 40 fs, 520 nm pulses. The results of a global target analysis and numerical simulations using nonlinear response functions and the multimode Brownian oscillator model support the assignment of  $S_x$  to a displaced conformation of the  $S_2$  state rather than to a vibrationally excited (or hot) S<sub>1</sub> state. The S<sub>x</sub> state in peridinin is assigned to a structure with a distorted conjugated polyene backbone moving past an activation-energy barrier between planar and twisted structures on the S<sub>2</sub> potential surface. The lengthened lifetime of the S<sub>x</sub> state of peridinin in methanol,  $900 \pm 100$  fs, much longer than that typically observed for carotenoids lacking carbonyl substituents, ~150 fs, can be attributed to the slowing of torsional motions by solvent friction. In peridinin, the system–bath coupling is significantly enhanced over that in  $\beta$ -carotene solution most likely due to the intrinsic intramolecular charge transfer character it derives from the electron withdrawing nature of the carbonyl substituent.

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### **3.1 Introduction**

Carotenoids perform several essential functions in photosynthetic organisms,<sup>1,2</sup> including photoprotection<sup>3</sup> and light harvesting in the blue-green (450-550 nm) part of the solar spectrum where (bacterio)chlorophyll ((B)Chl) absorbs inefficiently.<sup>3,4</sup> The mechanisms that allow efficient excitation energy transfer between carotenoids and (B)Chl in light-harvesting proteins have yet to be fully elucidated owing to the complexity of the nonradiative decay pathways and the production of dark intermediate states<sup>5</sup> following optical excitation of carotenoids in solution and in proteins.

This contribution deals with the earliest dynamics that follow optical preparation of the S<sub>2</sub> (1<sup>1</sup>B<sub>u</sub><sup>+</sup>) state of peridinin, the principal light-harvesting chromophore in the peridininchlorophyll *a* protein (PCP).<sup>6-8</sup> This work is motivated by our recent proposal<sup>9</sup> that the S<sub>2</sub> state in carotenoids naturally obtains an intramolecular charge-transfer (ICT) character by undergoing activated torsional motions of the conjugated polyene backbone. In recent work on  $\beta$ -carotene,<sup>10</sup> we suggested that structures near an activation energy barrier on the S<sub>2</sub> potential surface dividing planar and twisted structures account for the formation in <20 fs of an intermediate state generally denoted as S<sub>x</sub>, which has been the focus of considerable controversy.<sup>5</sup> A similar nonradiative decay intermediate has not yet been detected for peridinin, but as discussed below, knowledge of its presence and of the mechanism for its formation would aid an understanding of how peridinin can function efficiently as a light-harvesting chromophore. The idea that excitedstate twisting motions contribute to the ICT character of peridinin was considered early on by Bautista et al.,<sup>11</sup> and the possibility that the especially large transition dipole moment for the  $S_1$ state of peridinin in the peridinin-chlorophyll a protein arises from twisted structures was raised previously by Krueger et al.<sup>7</sup>

Because of the nominal  $C_{2h}$  symmetry of carotenoids, strong one-photon optical transitions occur from the ground state  $S_0$  (1<sup>1</sup>A<sub>g</sub><sup>-</sup>) to the  $S_2$  (1<sup>1</sup>B<sub>u</sub><sup>+</sup>) state, whereas transitions to the  $S_1$ (2<sup>1</sup>A<sub>g</sub><sup>-</sup>) state are symmetry forbidden. Some carotenoids, particularly those with fewer than ten carbon–carbon bonds, exhibit a very weak fluorescence from the  $S_1$  state, so the dipole strength of the  $S_1$  state in these cases is very small.<sup>12,13</sup> Because the carotenoid  $S_2$  state lies above that of the  $Q_x$  excited state of Chl or BChl,  $S_2 \rightarrow Q_x$  excitation energy transfer via the Förster resonant mechanism<sup>14,15</sup> would be generally favored. This pathway competes with ultrafast  $S_2 \rightarrow S_1$ nonradiative decay,<sup>16</sup> however, so only a fraction of the total energy-transfer yield from peridinin involves the  $S_2$  state. In PCP, two-thirds of the total energy transfer yield (~90%) is currently thought to occur via energy transfer from the carotenoid  $S_1$  state to the Chl  $Q_y$  state.<sup>6,8,17</sup>

Given that the  $S_1 \rightarrow S_0$  transition has very little oscillator strength, one might expect that the yield of energy transfer from peridinin to the Chl Q<sub>y</sub> state would be very small. But as exemplified in the X-ray crystal structures of carotenoid-containing light-harvesting proteins,<sup>18,19</sup> including that of PCP,<sup>20,21</sup> the efficiency of excitation energy transfer from a carotenoid to a B(Chl) is apparently enhanced by placing the donor and acceptor molecules nearly in van der Waals contact.<sup>17</sup> This observation initially prompts the suggestion of a Dexter-type, orbital-overlap, electron exchange-mediated energy-transfer mechanism,<sup>22</sup> but calculations suggest that energy transfer by the Förster mechanism are more favorable in light-harvesting proteins because of a relatively strong Coulomb coupling of the transition dipoles of the donor carotenoid and acceptor (B)Chl.<sup>17,23-25</sup>

It is evident also that the efficiency of excitation energy transfer from carotenoids to (B)Chls can be further improved by chemical substituents that alter the distribution of  $\pi$  electrons along the conjugated polyene backbone. Peridinin (Figure 3.1) is an example of a carotenoid with a

carbonyl group in conjugation with the extended conjugated polyene backbone; its electron withdrawing character combined with the electron releasing properties of the allene group on the other end of the molecule contributes to a very large change in permanent dipole moment accompanying the vertical  $S_0$  to  $S_2$  transition.<sup>26,27</sup>



Figure 3.1 Structure of peridinin

An excited-state intramolecular redistribution of  $\pi$ -electron density in peridinin is further indicated by the strong solvent dependence of the S<sub>1</sub> state lifetime, as shown by Frank and coworkers,<sup>11,28</sup> and this idea has been supported by both theoretical computations<sup>25</sup> and experiments on numerous other carbonyl-containing carotenoids and polyenals.<sup>8,16,29–36</sup> The lifetime of the S<sub>1</sub> state of peridinin directly correlates with solvent polarity, ranging from 7 ps in a highly polar solvent, trifluoroethanol, to 172 ps in two nonpolar solvents, cyclohexane and benzene. The fluorescence emission maximum, the quantum yield of fluorescence, and the excited-state absorption (ESA) line shape from the S<sub>1</sub> state are also affected by the choice of solvent. This behavior is considered highly unusual for carotenoids; in general, the spectral properties and lifetimes of the S<sub>1</sub> state of carotenoids are independent of the solvent environment. Frank and coworkers attributed the solvent sensitivity of the S<sub>1</sub> state of peridinin to the nonradiative formation of an ICT state, and subsequent work in several laboratories has indicated that other carbonyl-substituted carotenoids exhibit similar properties.<sup>37,38</sup> Owing to the ICT character, the energy transfer pathways in PCP would be expected to be much more sensitive to the reaction field arising from the polarity and polarizability of the surrounding protein and chromophore medium.<sup>26,27,39</sup>

Despite the experimental and theoretical studies reviewed above, how the ICT character develops in the S1 state of peridinin remains unclear. Four hypotheses have been proposed in previous work.<sup>40</sup> Two of the possible hypotheses suggest that a redistribution of charge follows formation of the S<sub>1</sub> state due to propagation along an intramolecular charge-transfer coordinate that connects locally excited and ICT states, either in the weak coupling or nonadiabatic regime<sup>11,25,32,41</sup> or in the strong coupling or adiabatic regime.<sup>16,42</sup> A third hypothesis suggests that the S1 state obtains oscillator strength and an intrinsic ICT character from a strong quantummechanical mixing of the  $S_1$  and  $S_2$  states.<sup>43</sup> A more recent hypothesis, based on electronic structure calculations and a consideration of the role of the solvent, suggests that the ICT state is formed upon optical preparation of the S2 state via bond-order reversal and configurational mixing of the  $S_1$  and  $S_2$  states,<sup>40,43</sup> which accompanies the large vertical change in permanent dipole moment.<sup>26,27</sup> The  $S_1$  and  $S_2$  states would be further mixed by torsional motions of the conjugated polyene backbone. In our hypothesis,<sup>9</sup> an instantaneous displacement along the resonance Raman-active C–C and C=C coordinates that contribute to the bond-length alternation of the conjugated polyene backbone<sup>44</sup> promotes formation in <20 fs of a transition-state structure at a barrier dividing planar and twisted structures on the S2 state potential surface. The torsional motions that accompany relaxation from the transition state promote the formation of a biradicaloid electronic structure and the development of additional ICT character.<sup>45-48</sup> The ICT character would be considerably enhanced by out-of-plane (pyramidal) distortions of the conjugated polyene backbone accompanying the torsional displacement.<sup>45,46</sup>

In this contribution, we report the first observations and numerical modeling of the femtosecond transient grating signal from peridinin obtained with heterodyne detection. The results represent a full characterization of the evolution of the third-order nonlinear optical signal after optical preparation of the  $S_2$  state in terms of its real (absorption) and imaginary (dispersion) signal components. As shown in our recent work on  $\beta$ -carotene,<sup>10</sup> the dispersion component provides information that is crucial in the detection of dark intermediate states. For a given pair of states, the absorption and dispersion line shapes are defined by the Kramers–Kronig relation. Because the dispersion line shape is bipolar, however, the sign of the detected dispersion signal is sensitive to the detuning of the probe laser spectrum. Accordingly, when nonradiative decay yields intermediate structures along the pathway back to the ground state, the absorption and dispersion components exhibit distinct time profiles that turn out to be extremely selective for the model parameters for a particular multilevel scheme. This approach had been previously used by Xu et al. to study the excited-state conformational dynamics of crystal violet and Rhodamine 640.<sup>49,50</sup>

The results show that the nonradiative decay of the  $S_2$  state of peridinin in methanol follows a pathway very much like that of  $\beta$ -carotene. Global target analyses<sup>51</sup> and numerical simulations using nonlinear response function theory<sup>52</sup> show that the  $S_2$  state of peridinin decays in 12 fs to form an intermediate state well prior to the formation of the  $S_1$  state. Just as in  $\beta$ -carotene, we attribute the intermediate,  $S_x$ , to a twisted form of the  $S_2$  state. The ESA spectrum from the  $S_x$  state in peridinin, however, is found to be markedly blue shifted from that of  $\beta$ -carotene because it makes a substantial contribution to the signal observed with 40 fs, 520 nm pulses. These results are best interpreted in terms of a substantial stabilization of the twisted region of the  $S_2$  potential surface of peridinin due to the presence of a substantial ICT character. The relatively long

lifetime of the  $S_x$  state, 900 fs, suggests that it could serve as the principal excitation energy transfer donor to Chl *a* in PCP.

#### **3.2 Experimental Section**

#### **3.2.1 Sample Preparation**

Peridinin was isolated<sup>53</sup> from laboratory-grown Amphidinium carterae (National Center for Marine Alga and Microbiota, strain CCMP121). Cells were disrupted by adding 20 mL of acetone to an equal volume of loosely packed cell pellet and gently shaking. Two hundred mL of a 1:1 mixture (v/v) of hexane and methyl tert-butyl ether were added and the mixture was transferred to a 250 mL volumetric flask. The lower phase containing cellular debris, Chl, and residual growth media was separated and discarded. The pigmented hyperphase was washed with an additional 9 mL of water, and collected, combined, and dried using a rotary evaporator. The sample was then redissolved in 10 mL of acetonitrile. In order to remove Chl, ~2 mL aliquots were filtered through a Waters Sep-Pak 12cc C18 cartridge (Waters Corp., WAT036915). Samples were additionally purified by high-performance liquid chromatography (HPLC) which removed other carotenoid pigments and the remaining Chl. This was performed using a Waters 600E/600S multi-solvent delivery system equipped with a 2996 photodiode array detector and a Waters Atlantis Prep T3 OBD 5 µm column (19 x 100 mm).<sup>54</sup> Acetonitrile was delivered isocratically at a flow rate of 7.0 mL/ min. Samples collected from the HPLC were dried using nitrogen gas and stored at -20°C. All solvents were purchased from Fisher Scientific (Waltham, MA). For femtosecond spectroscopic experiments, peridinin was dissolved in methanol to obtain an optical density of 0.3 for at the center of the laser spectrum (520 nm) in a cuvette with a 1 mm optical path length.

#### **3.2.2 Linear and Nonlinear Spectroscopy**

Linear absorption spectra were recorded at room temperature with a Hitachi U-4001 spectrophotometer. The experimental setup and techniques used for heterodyne transient grating spectroscopy were described in detail in the previous chapter.

#### **3.3 Experimental Results**

#### 3.3.1 Linear Spectroscopy and Laser Excitation Spectrum.

The continuous-wave absorption spectrum of peridinin in methanol at room temperature (295 K) is shown in Figure 3.2. The broad, structureless band spanning the 350–550-nm region arises from the electric-dipole-allowed  $S_0 \rightarrow S_2$  transition. The laser spectrum was tuned to the red onset near the 0–0 vibronic transition to prepare the  $S_2$  state with minimal excess vibrational energy. This tuning also selects the lowest energy ground-state conformers.<sup>37</sup>



**Figure 3.2** Room-temperature absorption spectrum of peridinin in methanol solvent. Superimposed is the spectrum of the OPA signal-beam output (520 nm center wavelength), as tuned for the heterodyne transient-grating experiments.

#### 3.3.2 Heterodyne Transient Grating Signal of Peridinin

Figures 3.3 and 3.4 shows the experimental real (absorption) and imaginary (dispersion) components of the heterodyne transient grating signals of peridinin in methanol at room temperature. As plotted, the purely dispersive nonresonant signal from neat solvent is a negatively going signal. In the absorption component for the resonant peridinin solute, the ground-state photobleaching (GSB) and stimulated emission (SE) contributions to the absorption component are positive signals, and excited state absorption (ESA) contributes a negative signal.<sup>10</sup>

The absorption component of the peridinin transient grating signal in methanol is a biphasic transient. Near time zero a strong positive (yellow in the figure) signal centered around 515 nm is observed due to an instrument-limited rise of the GSB and SE contributions. The signal decays very rapidly, in <50 fs, to yield a negative going signal (blue in the figure) centered around 525 nm mainly due to the loss of SE and formation of strong ESA. In contrast, the dispersion component is monophasic and positive going throughout the experimental delay time. At 520 nm, where the dispersion signal is strongest, the signal corresponds predominantly to the rise of an ESA contribution. The pattern of intensity changes exhibited by both components of the transient grating signal from peridinin is very similar to that observed previously in our laboratory for  $\beta$ -carotene in benzonitrile solution.<sup>10</sup>



**Figure 3.3** Real (absorption) component of the heterodyne transient grating signal from peridinin in methanol solvent at room temperature (22 °C). The yellow end of the color bar corresponds to net ground-state bleaching or stimulated emission; the dark blue end indicates excited state absorption.



**Figure 3.4** Imaginary (dispersion) component of the heterodyne transient grating signal from peridinin in methanol solvent at room temperature (22 °C). The phasing of the color bar is consistent with that used in Figure 3.3 for the absorption component. At 520 nm, the yellow contours correspond to a net ESA signal.

In order to estimate the lineshapes and timescales that contribute to the absorption and dispersion components of the heterodyne transient grating signal from peridinin, a global target analysis<sup>51</sup> using the Glotaran/TIMP package<sup>55,56</sup> was performed over the -100 fs-12 ps delay range. This analysis obtains a set of principal spectral components and their associated kinetic timescales; a 60 fs Gaussian instrument response function was included in the model. The global model reported in Figures 3.5-3.8 corresponds to a sequential decay of three species, the minimum number indicated by a preliminary singular value decomposition (SVD) analysis. Figure 3.5 shows the time profile of the populations for the three species, the instantaneously formed S<sub>2</sub> state and two sequentially populated intermediates. The lifetimes and confidence intervals for the three species, as listed in the caption to Figure 3.5 and discussed in the following, were obtained from the global analysis. The confidence intervals were determined to be consistent with the fit parameters obtained from analyses of additional data sets. Figures 3.6 and 3.7 show the evolution associated spectra for each species, as obtained for the global models of the absorption and dispersion components, respectively. Figure 3.8 shows a slice across the data set at a central probe wavelength of 520 nm that compares the time evolution of the experimental absorption and dispersion signals with the fitted model.



**Figure 3.5** Time evolution of the populations for the three species in a global analysis of the heterodyne transient grating signal (Figures 3.3 and 3.4) from peridinin in methanol over the -100 fs-12 ps delay range, as plotted against a linear–logarithmic time axis split at 1 ps: *black*: instantaneously formed species,  $12 \pm 4$  fs decay; *red*: first intermediate species,  $12 \pm 4$  fs rise,  $900 \pm 100$  fs decay; *blue*: second intermediate species in the absorption component,  $900 \pm 100$  fs rise,  $9.9 \pm 0.5$  ps decay back to the ground state; *dashed blue*: second intermediate species in the dispersion component,  $900 \pm 100$  fs rise ,  $7.5 \pm 0.5$  ps decay.



**Figure 3.6** EAS from a global analysis of the absorption component of the heterodyne transient grating signal (Figure 3.3) from peridinin in methanol over the -100 fs-12 ps delay range.



**Figure 3.7** Evolution associated spectra (EAS) from a global analysis of the dispersion component of the heterodyne transient-grating signal (Figure 3.4) from peridinin in methanol over the -100 fs-12 ps delay range. The EAS assume a sequential pathway from the optically prepared S<sub>2</sub> state (black) through two intermediates (red, then blue) back to the ground state.



**Figure 3.8** Time evolution of the absorption (red) and dispersion (blue) components of the heterodyne transient grating signals from peridinin in methanol solvent at 520 nm, as plotted against a linear–logarithmic time axis split at 1 ps. Both signals are superimposed with the fitted global models described in Figures 3.5–3.7, respectively.

Over the <1 ps delay range, the absorption and dispersion components of the heterodyne transient signal from peridinin in methanol are satisfactorily modeled with the same time constants for the decay of the first two kinetic components:  $12 \pm 4$  fs and  $900 \pm 100$  fs. The indeterminacy for the 12 fs component is proportionally large because of the larger width of the instrument-response function. As discussed below, an additional determination of the lifetime of the S<sub>2</sub> state and the associated confidence interval is obtained from numerical simulations. The lifetime of the intermediate species,  $900 \pm 100$  fs, is considerably longer than the  $142 \pm 20$  fs lifetime of the intermediate in  $\beta$ -carotene, as determined using the same measurement conditions and with the same analysis techniques. The confidence interval for the S<sub>2</sub> state.

The instantaneously formed spectrum from the  $S_2$  state of peridinin exhibits an absorption spectrum with net GSB/SE character (Figure 3.6) over the entire probe bandwidth; the first intermediate yielded by its decay exhibits a spectrum dominated by ESA, with only a narrow region in the blue, <515 nm range showing net GSB character. The dispersion spectrum (Figure 3.7) for the initial,  $S_2$  component is bipolar, with a central positive going region flanked by two negative regions. The dispersion lineshapes for the subsequent spectra are positive over the entire probe bandwidth, which is consistent with the ultrafast formation of a red-shifted and linebroadened spectrum from an initially narrow, hole-burned spectrum. As in  $\beta$ -carotene, the time evolution of the transient grating spectrum from peridinin is dominated at short delays by spectral line-broadening and solvation processes.

The first intermediate then decays to yield a second intermediate exhibiting in the absorption component a spectrum with predominantly ESA character (Figure 3.6). This spectrum is assigned to the  $S_1$  state.<sup>11,29</sup> It is apparent that the narrow probe spectrum used in these

experiments limits detection of the ESA signals especially on the red side of the plotted range, so the band shape should not be overinterpreted; it is known that the S<sub>1</sub> and ICT states of peridinin exhibit overlapping ESA lineshapes, with the broad ICT band extending at least to 725 nm in methanol from a maximum near 590 nm.<sup>11</sup> The net GSB character observed to the blue of 515 nm in the spectrum of the first intermediate has decayed and the apparent ESA minimum has shifted only 2 nm to the blue. In the dispersion component, the spectrum of the second intermediate (Figure 3.7) exhibits a similar small shift to the blue but mainly on the red edge. Following the Kramers–Kronig relationship, because the dispersion line shape is significantly broader than that of the absorption component, spectral shifts are accordingly less obvious in the dispersion component.

The Kramers–Kronig relationship also comes into play in the interpretation of the groundstate recovery time constants in the absorption and dispersion components of the peridinin transient grating signal,  $9.9 \pm 0.5$  ps and  $7.5 \pm 0.5$  ps, respectively (Figure 3.5). A somewhat smaller difference in time constant for the ground-state recovery components was observed in  $\beta$ carotene.<sup>10</sup> These time constants should be regarded as effective, tail limited estimates. The ground-state recovery portions of the absorption and dispersion signals are multiexponential waveforms due to the initial production of conformationally displaced structures upon nonradiative decay from S<sub>1</sub> to S<sub>0</sub>.<sup>10,50</sup> The longer time constant estimated for the decay of the absorption component includes the time required for the originally photoselected ground-state distribution of conformers to be recovered. Because its line shape is broader than that of the absorption component, the dispersion component predominantly senses the time constant for nonradiative decay from S<sub>1</sub> to S<sub>0</sub>; it is less sensitive than the absorption component to the blue shift and narrowing of the photoinduced absorption spectrum from the displaced  $S_0$  conformers that accompanies their relaxation.

#### **3.4 Numerical Simulations**

In order to assign the ultrafast intermediate observed in peridinin in 12 fs after optical preparation of the  $S_2$  state, we have performed numerical simulations of the transient grating signal using the nonlinear response function formalism and the multimode Brownian oscillator model (MBO).<sup>52</sup> The theory used in these simulations is exactly as described in detail in our previous work on  $\beta$ -carotene, which extends the treatments provided by Brixner et al.<sup>57</sup> and of Christensson et al.<sup>58</sup>

Figure 3.9 shows the energy level scheme for peridinin that was applied in the simulations. After optical preparation of the  $S_2$  state, population decays nonradiatively to the  $S_1$  state by passing through an intermediate state, here labeled  $S_x$  as in the model for  $\beta$ -carotene, but the nature of this state in peridinin remains to be determined from the results of the simulations. As noted above, the narrow probe spectral bandwidth used in the present experiments precludes detection of the red-shifted ESA band from the ICT state,<sup>6</sup> so the scheme shown in Figure 3.8 only includes a generic  $S_1$  state serving as the kinetics compartment that receives population from the  $S_x$  intermediate. As discussed previously,<sup>10</sup> the calculations assume that population transfers are handled with Markovian population kinetics.<sup>59,60</sup> This assumption impacts primarily whether or not coherent wavepacket motions modulate the SE and ESA contributions to the transient grating signal, and here our use of nominally 40 fs pulses precludes detection of high-frequency modes, especially those of the C–C and C=C bond-length alternation coordinates of the conjugated

polyene. Along with the GSB and SE transitions associated with the  $S_0$  and  $S_2$  states, ESA transitions from the  $S_2$ ,  $S_x$  and  $S_1$  states to higher lying  $S_n$  states are included in the simulation. SE transitions from the  $S_x$  and  $S_1$  states are not included in the model, again due to the narrow probe spectral bandwidth used in the experiments; SE from the ICT state is anticipated in the near-IR, >900 nm region of the spectrum.<sup>8,29,61</sup> Since we are mainly interested here in the population dynamics on the <1 ps timescale, the separate ground-state recovery time constants for the absorption and dispersion components are not treated explicitly in the model; modeling of this aspect of the results would require the addition of a conformationally displaced  $S_0$  species. The lifetimes of the  $S_2$ ,  $S_x$  and  $S_1$  states are taken to be 12 fs, 900 fs, and 9.9 ps, as obtained from the global analysis of the absorption component of the transient grating signal.



**Figure 3.9** Schematic energy-level diagram for peridinin, as used in the simulations of the heterodyne transient grating signal from peridinin and as used previously with  $\beta$ -carotene.<sup>10</sup> Solid green arrows indicate ground state and excited-state absorption transitions. Blue wavy arrows mark internal conversion transitions. Double-headed arrows show the detuning ( $\Delta$ ) between the center of the laser spectrum and the vertical energy of an electronic transition. The laser tuning for the present experiments is at the red onset of the S<sub>0</sub>  $\rightarrow$  S<sub>2</sub> absorption spectrum, so a negative detuning from the absorption maximum is indicated in the diagram.

#### **3.4.2 Simulation of the Absorption Spectrum.**

Figure 3.10 compares the experimentally observed ground-state absorption spectrum for peridinin in methanol with a simulated spectrum obtained using the MBO model. The line broadening parameters used in the simulations are listed in Table 3.1. The optimized value of the vertical transition energy for the peridinin  $S_0$  to  $S_2$  transition in methanol is 21670 cm<sup>-1</sup>. Two high frequency vibrational modes, corresponding to the totally symmetric C-C and C=C stretching modes of the conjugated polyene backbone,<sup>62</sup> are included in the simulation. Most of the model parameters are quite similar to those used for  $\beta$ -carotene in benzonitrile, but the total reorganization energy,  $\lambda$ , for the Gaussian and exponential Brownian modes is much larger for peridinin. The reorganization energies and dephasing times of the vibrational modes used here in the model for the absorption spectrum are similar to those employed by Christensson et al.<sup>63</sup> These parameters indicate that the system–bath coupling is stronger for peridinin than  $\beta$ carotene. The origin of the stronger interaction with the solvent in peridinin is the presence of hydrogen-bonding interactions of solvent molecules with the carbonyl substituent. The increased system-bath coupling causes the underlying vibrational structure from the two underdamped high-frequency modes in the model to be unresolved in the absorption spectrum. As was also observed with  $\beta$ -carotene,<sup>10</sup> the simulation describes the absorption spectrum very well from the red onset to just past the maximum, but the observed spectrum exhibits additional intensity beyond the maximum that may have contributions from higher-energy conformers.



**Figure 3.10** Comparison of the room temperature  $S_0 \rightarrow S_2$  absorption spectrum of peridinin in methanol (dark blue curve) with a simulation (red dash-dotted curve) obtained using the MBO model and the parameters listed in Table 3.1.

**Table 3.1** MBO model parameters<sup>a</sup> for the simulation of the peridinin  $S_0 \rightarrow S_2$  absorption spectrum in methanol at 295 K (Figure 3.1).

Mode	$\lambda (cm^{-1})$	au (fs)	$\omega_v (\mathrm{cm}^{-1})$
Gaussian	300	10	
Exponential 1	700	150	
Exponential 2	1500	1000	
Vibration 1	460	2200	1150
Vibration 2	1060	2200	1520

<sup>a</sup>For each mode,  $\lambda$  is the reorganization energy and  $\tau$  denotes the damping time constant;  $\omega_v$  is the frequency for a vibrational mode.

#### 3.4.3 Simulation of the Heterodyne Transient Grating Signals

Table 3.2 gives the energy gaps and relative dipole strengths of the electronic transitions for the scheme shown in Figure 3.9, as obtained after optimization of the numerical simulation. The energy gap and the MBO lineshape parameters for the  $S_0 \rightarrow S_2$  GSB and  $S_0 \rightarrow S_2$  SE transitions are obtained from those used to fit the absorption spectrum. The energy gaps for the  $S_2 \rightarrow S_{n2}$  and
$S_1 \rightarrow S_{n1}$  ESA transitions are set to 14800 cm<sup>-1</sup> and 17390 cm<sup>-1</sup>, respectively, as determined from published pump-continuum-probe spectra from peridinin in methanol.<sup>11,29</sup> The lineshape parameters for the ESA transitions are assumed to be the same as for the  $S_0 \rightarrow S_2$  transition. This choice results in a  $S_1 \rightarrow S_{n1}$  ESA lineshape that is similar to that observed in the pump-probe spectrum from peridinin in methanol.<sup>11,29</sup> The transition dipole strength for the  $S_1$  ESA transition was adjusted to describe the transient grating signal at delays T > 5 ps, where only the  $S_1$  state contributes significantly. The energy gap and dipole strength of the  $S_x \rightarrow S_{nx}$  transition were optimized to fit both the absorption and dispersion components simultaneously.

**Table 3.2** Transition frequencies ( $\omega_{ij}$ ) and relative transition dipole strengths ( $\mu_{ij}$ ) used in the simulation of the transient-grating signal from peridinin shown in Figures 3.11–3.13.

Transition	$\boldsymbol{\omega}_{ij} (\mathrm{cm}^{-1})$	$\mu_{ij}$ (Debye)	
$S_0 \rightarrow S_2$	21670	1	
$S_2 \rightarrow S_{n2}$	14800	0.35 <sup>a</sup>	
$S_x \rightarrow S_{nx}$	17300	1.58	
$S_1 \rightarrow S_{n1}$	17390	1.65	

<sup>a</sup> The ESA transition for the  $S_2$  state is well to the red of the probe bandwidth, so it makes no contribution to the signals calculated for the bandwidth probed with 520 nm pulses. The value indicated for the transition dipole strength for this ESA transition is the same as that used previously for  $\beta$ -carotene in benzonitrile,<sup>10</sup> and it is not optimized for peridinin in methanol.

The absorption and dispersion components of the transient grating signal calculated with these parameters are shown in Figures 3.11 and 3.12 with the assumption of excitation pulses centered at 520 nm and 45 fs pulse durations. In order to permit an easily visualized and quantitative comparison of the experimental results with the simulations, Figures 3.13 and 3.14 compare the calculated models with the experimental absorption and dispersion signals, as integrated over the signal emission frequency axis. As in our previous simulations of the

transient grating signal from  $\beta$ -carotene in benzonitrile,<sup>10</sup> there are discrepancies at short probe delays particularly in the rise of the absorption component. The simulations employed 45 fs pulse durations, which are somewhat broader than estimated from the width of the dispersion component in neat methanol solvent. This choice was made to obtain a better simulation of the shape of the rise of the dispersion component. A 45 fs effective pulse width was also required in the previously reported simulations of the transient grating signal from  $\beta$ -carotene, which were obtained under the same measurement conditions.<sup>10</sup> The rise of the absorption component is also affected by the lack of inclusion of low- to mid-frequency vibrational components in the MBO model, which make negative-going contributions at short delays.<sup>64</sup>



**Figure 3.11** Numerical simulation of the absorption component of the transient grating signal from peridinin calculated according to the energy level scheme shown in Figure 3.9. The parameters used in the calculation are listed in Tables 3.1 and 3.2 and in the text. The yellow end of the color bar corresponds to net ground–state bleaching or stimulated emission; the dark blue end indicates excited state absorption.



**Figure 3.12** Numerical simulation of the dispersion component of the transient grating signal from peridinin calculated according to the energy level scheme shown in Figure 3.9. The parameters used in the calculation are listed in Tables 3.1 and 3.2 and in the text. The phasing of the color bar is consistent with that used in Figure 3.11 for the absorption component. At 520 nm, the yellow contours correspond to the net ESA signal from the S<sub>1</sub> state.

As was also observed for  $\beta$ -carotene in benzonitrile, the ultrafast rise of the dispersion component of the transient grating signal provides the strongest evidence that the S<sub>2</sub> state has a very short lifetime. Owing to the inner filter effect,<sup>65,66</sup> the dispersion signal from the methanol solvent is effectively negligible under the 0.3 OD resonant solute conditions used in this experiment. This is quite different from the situation for  $\beta$ -carotene in benzonitrile, where the solvent makes a significant dispersion signal owing to its much higher polarizability. The fast rise in the dispersion signal from peridinin is predominantly controlled by the decay of the SE signal from the S<sub>2</sub> state, but the rise in ESA from the S<sub>x</sub> signal also makes a significant contribution to the response. The confidence interval for the lifetime of the S<sub>2</sub> state was tested in a series of calculations in which the lifetime of the S<sub>2</sub> state was varied from 6 fs to 56 fs (Figure A3.1 in the Appendix). The rise of the dispersion component is evidently consistent with the  $\pm 4$  fs interval obtained from the global modeling, and it is clear that the lifetime cannot be as long as 20 fs because of significant negative deviations from the experiment at probe delays >25 fs. Also, as also observed and demonstrated explicitly in the previous work on  $\beta$ -carotene,<sup>10</sup> simulations (not shown) were performed using models that omit the S<sub>x</sub> manifold of states shown in Figure 3.9. The calculated transient grating signal exhibits a much slower rise in the dispersion owing to the slow decay of the SE from the S<sub>2</sub> state and the concomitant slow rise of the contribution by ESA from the S<sub>1</sub> state.



**Figure 3.13** Comparison of the experimental and calculated absorption components of the transient grating signal from peridinin. The experimental (Figure 3.3) and calculated signals (Figure 3.11) were integrated over the laser frequency axis and normalized at the 1 ps delay point. *Top Panel:* Absorption component from the experimental transient grating signal (data points) superimposed with those from the numerical simulation (solid line). *Bottom Panel:* Absorption components calculated from individual Feynman pathways: Orange, GSB; Red, SE from the S<sub>2</sub> state; Blue, S<sub>2</sub>  $\rightarrow$  S<sub>n2</sub> ESA; Green, DQC; Violet, S<sub>x</sub>  $\rightarrow$  S<sub>nx</sub> ESA, Black, S<sub>1</sub>  $\rightarrow$  S<sub>n1</sub> ESA.



**Figure 3.14** Comparison of the experimental and calculated dispersion components of the transient grating signal from peridinin. *Top Panel:* Dispersion component from the experimental transient grating signal (data points) and superimposed with those from the numerical simulation (solid line). *Bottom Panel:* Dispersion components calculated from individual Feynman pathways: Orange, GSB; Red, SE from the S<sub>2</sub> state; Blue, S<sub>2</sub>  $\rightarrow$  S<sub>n2</sub> ESA; Green, DQC; Violet, S<sub>x</sub>  $\rightarrow$  S<sub>nx</sub> ESA, Black, S<sub>1</sub>  $\rightarrow$  S<sub>n1</sub> ESA.

# **3.5 Discussion**

The global target analysis shown in Figures 3.3 and 3.4 indicate that the heterodyne transient grating signal from peridinin in methanol undergoes very rapid changes in intensity and lineshape after optical preparation of the S<sub>2</sub> state with 40 fs pulses at 520 nm. In order to understand better the dynamics underlying these changes, we performed an extensive series of numerical simulations using nonlinear optical response functions and the MBO model.<sup>67,68</sup> The simple nonradiative decay scheme used in the simulations (Figure 3.9) is the same as that used previously for  $\beta$ -carotene,<sup>10</sup> where it was determined that the intermediate state called S<sub>x</sub> is formed in only 12 fs after optical preparation of the S<sub>2</sub> state. The experimental results and

simulations reported in this paper indicate that a similar nonradiative decay intermediate is also produced in peridinin. The global model and numerical simulations provide strong evidence for the conclusion that the decay of the S<sub>2</sub> state of peridinin occurs with a  $12 \pm 4$  fs time constant, which is much shorter than the previous estimate of 56 fs<sup>16</sup> and indistinguishable from the values we determined for  $\beta$ -carotene in benzonitrile<sup>10</sup> or by Cerullo et al. in hexane.<sup>69</sup> The spectroscopic assignment of the intermediate state produced by the decay of S<sub>2</sub> and the nature of the relaxation process that produces it in peridinin, however, require further discussion. The case for not assigning the intermediate to a vibrationally excited S<sub>1</sub> state needs to be outlined first.

The 900 fs lifetime of the intermediate state determined in the global model and used in the numerical simulations of the transient grating signal from peridinin in methanol is in the regime generally associated with vibrational cooling in carotenoids,<sup>70,71</sup> so it is reasonable to suggest that the intermediate we detect upon decay of the S<sub>2</sub> state is a vibrationally excited S<sub>1</sub> state. This was the assignment proposed in a number of previous studies using femtosecond pump-probe spectroscopy.<sup>8,16,29,32,39</sup> But as indicated by the studies of radiationless decay in crystal violet and malachite green by Xu et al.,<sup>50</sup> vibrational cooling would be accompanied by a significant blue– shifting and narrowing of a photoinduced absorption or ESA line shape. The very small change in lineshape retrieved by the global model for the  $S_x$  to  $S_1$  decay over the narrow probe bandwidth of the 520 nm pulses used in the present experiments is not obviously consistent with this expectation. Further, the finding that the same time constant can be used to fit the decay of the intermediate satisfactorily in both the absorption and dispersion component is inconsistent with a significant shift or narrowing of the spectrum; vibrational cooling would have been expected to contribute a longer time constant in the absorption component. Lastly, the strongest argument against a conclusion that the intermediate is a vibrationally excited  $S_1$  state is

indicated by simulations of the transient grating signal. Figures A3.2–A3.5 in the Appendix present a simulation showing the impact of replacing the  $S_x$  state in Figure 3.9 with an excited vibrational level in the  $S_1$  manifold. The decay of the  $S_2$  state in this simulation results in a much more pronounced biexponentiality in the decay of the absorption component or in the rise of the dispersion component than is observed experimentally.

Instead, we suggest that the decay of the S<sub>2</sub> state detected in the transient grating experiment is better explained in terms of a prompt vibronic displacement on the S<sub>2</sub> potential surface that results in a distorted conformation. We recently proposed a general nonradiative decay model<sup>9</sup> for carotenoids that involves vibrational displacements in the C-C and C=C bond-length alternation coordinates followed by torsional and out-of-plane coordinates of the conjugated polyene backbone. This scheme was constructed from a consideration of the potential energy surfaces of conjugated polyenes, cyanines, and protonated Schiff bases of intermediate lengths.47,72 The extent of coupling between the stretching and torsional motions of the conjugated polyene backbone depends on several factors, including the conjugation length and the nature of the surrounding medium (solvent and/or protein derived). For molecules with intermediate to long conjugation lengths, such as β-carotene and peridinin, the Franck-Condon S<sub>2</sub> geometry would be expected to lie at a potential minimum with respect to the torsional coordinates. This results in a local activation barrier that separates planar and twisted regions of the  $S_2$  potential energy surface (Figure 3.15). The forces that act on the Franck–Condon  $S_2$ structure are chiefly along the bond-length alternation coordinates, which accordingly exhibit strong resonance Raman activity. In this picture, the 12 fs lifetime of the S<sub>2</sub> state in β-carotene and peridinin corresponds approximately to a half period of the bond-length alternation coordinates. The S<sub>x</sub> state would be produced by a coherent displacement from the FranckCondon geometry to the activation barrier region, where a torsional gradient on the  $S_2$  potential surface would be encountered for the first time. In this model, the  $S_x$  state corresponds to transition-state-like structures near the barrier that have just begun to twist with respect to one or more of the C=C bonds. The system would then start a decent along a steep torsional gradient leading towards a conical intersection, where the  $S_2$ ,  $S_1$ , and  $S_0$  surfaces would converge.



**Figure 3.15** Proposed scheme for radiationless decay of peridinin after optical preparation of the  $S_2$  state; after similar diagrams in Sanchez-Galvez et al.<sup>72</sup> and Beck et al.<sup>9</sup> The states that apply to planar structures are indicated by symmetry labels. Key points along the radiationless path from the Franck–Condon  $S_2$  state structure are labeled with ethylenic structures, which depict the  $S_x$  and  $X^{76}$  dark states as structures near the  $S_2$  transition state and along the torsional gradient, respectively.

The formation of the twisted structures we assign to the  $S_x$  state accounts for the rapid and irreversible loss of SE from the  $S_2$  state in the region of the spectrum probed near the Franck– Condon energy gap, as in the present experiments. This is the main origin of the ultrafast rise component in the dispersion channel observed in  $\beta$ -carotene and peridinin. Nonradiative decay from the twisted part of the S<sub>2</sub> surface to the S<sub>1</sub> state requires propagation along the torsional gradient from the barrier region to reach a seam between the two surfaces,<sup>47</sup> most likely well before a  $\pi/2$  twist occurs.<sup>73,74</sup> In peridinin, given the restraint to out-of-plane motions of the conjugated polyene backbone by the  $\gamma$ -lactone ring, the twisted region of the molecule would be anticipated on the opposite end of the molecule, producing a twist or pyramidalization<sup>45,46</sup> (or kink<sup>75</sup>) in the backbone<sup>9</sup> before before the allene moiety.

As torsional motions and out-of-plane motions are initiated in the S<sub>x</sub> state near the barrier on the twisted part of the  $S_2$  potential energy surface of a carotenoid, an ICT character  $% S_2$  and a stronger frictional coupling to the surrounding solvent would be expected to arise naturally as the conjugated polyene backbone is distorted.9,45,46,48 In general, because the carotenoids exhibit singlet excited states with extensive delocalization of the  $\pi^*$  character, the separation of charge would be expected to be distributed over much of the length of the conjugated polyene backbone on either side of the point where the twisting distortion begins. In the case of peridinin, the enhanced system-bath coupling and the large change in permanent dipole moment that accompanies optical preparation of the  $S_2$  state<sup>26,27</sup> will dramatically enhance the additional ICT character obtained from torsional displacements over that for carotenoids lacking carbonyl substitution. This idea accounts both for the blue shifted Sx ESA spectrum we detect in the simulations and for the dramatically enhanced S<sub>x</sub> state lifetime of peridinin (900 fs) in methanol compared to that for  $\beta$ -carotene in benzonitrile (140 fs). Given the near coincidence of the S<sub>0</sub>-S<sub>2</sub> energy gaps of  $\beta$ -carotene in benzonitrile and for peridinin in methanol, which effectively normalizes the solvent shifts for the S2 planar structures, one can infer from the blue-shifted ESA spectrum that the potential energy region for the S<sub>x</sub> intermediate for peridinin in methanol near the barrier is substantially stabilized. The longer lifetime of the S<sub>x</sub> state in peridinin would then

be associated with slowing by solvent friction of the torsional decent to the seam leading to the  $S_1$  state. Vibrational equilibration in the  $S_1$  vibrational manifold would only then be expected to follow on the 0.5–1 ps timescale, as detected in previous work with broader probe spectral coverage.<sup>70,71,77–81</sup> Note that this model anticipates that in some solvents the decay of the  $S_x$  state will be so slowed by solvent friction that the rate of vibrational energy dissipation will be much faster than the torsional relaxation; the decay of  $S_x$  will be rate limited by the polar solvation timescale due to the coupling to the ICT character and/or the viscosity of the solvent medium due to the involvement of large-amplitude torsional motions.

# **3.6 Conclusion**

This contribution discusses new details of the nonradiative decay pathway from the  $S_2 (1^1B_u^+)$  state to the  $S_1 (2^1A_g^-)$  state of peridinin in methanol solution, obtained for the first time with heterodyne transient grating spectroscopy. As was also observed in the previous study from this laboratory on  $\beta$ -carotene in benzonitrile, detection of the real (absorption) and imaginary (dispersion) components of the transient grating signal from peridinin provides a more precise measurement of the lifetime of the  $S_2$  state compared to previous work using pump-probe spectroscopy employing longer excitation pulses. The results provide some new insight into the nature of the dynamics that are associated with the decay of the  $S_2$  state. The time evolution of the transient grating signal establishes that the  $S_2$  state decays in only 12 fs to produce an intermediate state,  $S_x$ . Numerical modeling using the response function formalism and the MBO model establishes that a simultaneous description of the ultrafast decay and rise of the absorption and dispersion components of the transient grating signal, respectively, requires a nonradiative process that quenches the SE contribution from the  $S_2$  state. The modeling further

indicates that the intermediate state cannot be satisfactorily assigned to a vibrationally excited  $S_1$  state.

The observation that the excited state absorption spectrum from the  $S_x$  state of peridinin is significantly blue shifted from that of β-carotene supports the radiationless decay model we proposed recently for carotenoids; the  $S_x$  state in peridinin is assigned to a transition-state-like structure near an activation-energy barrier between planar and twisted structures on the S<sub>2</sub> potential surface. The short lifetime for the S<sub>2</sub> state is associated with a nonrecurrent vibrational displacement from the Franck-Condon geometry along the C-C and C=C bond-length alternation coordinates of the conjugated polyene backbone to the region of the barrier, where a gradient along torsional coordinates is encountered. Due to the intrinsic redistribution of  $\pi$ electron density obtained from the electron withdrawing character of the carbonyl substituent of peridinin, the barrier and the twisted region of the  $S_2$  potential surface are substantially stabilized compared to  $\beta$ -carotene and other carotenoids lacking carbonyl substitution. The ICT character that results upon further relaxation on the torsional gradient on the S2 potential surface after the barrier is passed accounts for the lengthening of the lifetime of the  $S_x$  state of peridinin over that observed in  $\beta$ -carotene; the resulting increase in the solvent friction would slow the passage from the  $S_2$  surface through a seam to the  $S_1$  surface.  $^{47}$ 

The finding that the lifetime of the  $S_2$  state is much shorter than previously estimated and the suggestion that the decay of the resulting  $S_x$  state is significantly slowed by solvent friction has significant implications in understanding the mechanism of excitation energy transfer from peridinin to Chl *a* in PCP. Based on fluorescence excitation experiments,<sup>11</sup> the overall energy transfer efficiency from peridinin to Chl *a* after optical preparation of the  $S_2$  state is found to be ~90%, of which 25% is thought to occur nearly instantaneously between the peridinin  $S_2$  state to

the Chl a Q<sub>x</sub> state; the remaining 75% of the energy transfer yield is currently thought to involve energy transfer from the  $S_1/ICT$  state to  $Q_y$  with a 3 ps time constant.<sup>8,23</sup> The present results suggest, in contrast, that it may actually be the  $S_x$  state that serves as the excitation energy donor to either of the Chl a excited states. The structure of peridinin in PCP is somewhat compressed along the conjugated polyene and bent near the allene moiety at the opposite end of the conjugated polyene in a manner that promotes a partial inversion of the bond-length alternation pattern compared to that in the planar, all-trans ground state obtained as a energy minimum in electronic structure calculations. It is proposed that this distortion results in a strong mixing of the characters of the S<sub>2</sub> and S<sub>1</sub> states and a large enhancement of the energy transfer yield to the Chl a acceptor.<sup>40,43</sup> We suggest that the ground-state conformation favored in PCP results in an  $S_x\mbox{-like}$  conformation immediately upon optical preparation of the  $S_2$  state. This conformation would be expected to relax further along torsional or out-of-plane coordinates in the protein to assume an even larger ICT character and a larger Förster transition-dipole-transition-dipole coupling to the Chl a acceptor. The result is a distorted donor state that should have some correspondence to that of the X intermediate (Figure 3.15) reported by Ostroumov et al.<sup>76,82</sup> in two-dimensional electronic spectra recorded from the LH2 complex from purple bacteria.

APPENDIX

### APPENDIX

# A3.1 Lifetime of the S<sub>2</sub> State

As discussed in the main text, the 12 fs lifetime of the  $S_2$  state is determined with considerable indeterminacy due to the relatively longer, 40 fs pulses used in the experiment. To place some additional restrictions on the lifetime of the  $S_2$  state, we performed additional numerical simulations of the heterodyne transient grating signal in which the lifetime of the  $S_2$  state was varied from 6 to 56 fs. All of the other parameters in the simulation were fixed to the optimum values used with the 12 fs model; as in the main simulation shown in the text, the effective pulse duration was set to 45 fs. The results of these simulations clearly indicate that the previously estimated  $S_2$  lifetime of 56 fs<sup>16</sup> is much too long because it makes the rise of the dispersion signal poorly match that of the experimentally determined signal. The results indicate that  $S_2$  lifetime is definitely shorter than 20 fs. The confidence interval range indicated by the global model,  $12 \pm 4$  fs, evidently provides a reasonable estimate of the actual lifetime of the  $S_2$  state.



**Figure A3.1** Comparison of the experimental and simulated dispersion rise of peridinin in methanol for models employing  $S_2$  lifetime ranging from 6–56 fs. The dotted curve indicates the experimental data. The black curve, for 12 fs, is from the model reported in the main text.

### A3.2 Assignment of S<sub>x</sub> to a Vibrationally Excited S<sub>1</sub> State

A range of simulations was performed in which the  $S_x$  manifold shown in Figure 3.9 represents an excited vibrational state of  $S_1$ . The energy level scheme for these calculations is the same as shown in Figure 3.9, but now the manifold of states for the  $S_x$  intermediate corresponds to that for the  $S_1$  state. Due to the initial vibrational excitation in the  $S_1$  manifold obtained from the nonradiative transfer from the  $S_2$  state, the energy gap to the  $S_{nx}$  (=  $S_{n1}$ ) state is narrower than for the relaxed  $S_1$  state manifold. Further, because by hypothesis the vibrationally excited  $S_1$  intermediate and the relaxed  $S_1$  state have the same electronic configuration, the transition dipole strengths for their ESA transitions are set to the same values (Table A3.1). All other parameters are the same as the those used in the calculation presented in Figures 3.11–3.14.

Table A3.1 gives the energy gaps and relative dipole strengths of the electronic transitions. The absorption and dispersion signals determined with the above parameter sets are shown in Figure A3.2 and A3.3 with the assumption of excitation pulses at 520 nm and 45 fs durations. Figures A3.4 and A3.5 compare the experimental absorption and dispersion signals with models as integrated over the emission frequency axis.

The red shift for the ESA spectrum arising from the assumption of the vibrational excitation of the intermediate has a large effect on the calculated absorption component. Figure A3.2–A3.6 show the impact of a 2590 cm<sup>-1</sup> excitation, which corresponds to about two quanta for a C–C or C=C mode of the conjugated polyene backbone. If the initial vibrational excitation is even 400 cm<sup>-1</sup> above the 0–0 level of the S<sub>1</sub> state, the ESA transition from the intermediate is shifted completely out of the probe's bandwidth, and the calculated absorption component then exhibits a more pronounced biexponential decay to net ESA character. A large deviation from the experimental trace is observed chiefly in the amplitude of the slower, 900 fs portion. The corresponding defect in the calculated dispersion component is in the slower, 900 fs part of the rising transient. To get a good fit to the experimental results, one has to set the initial vibrational excitational excitation above the 0–0 energy of the S<sub>1</sub> state to a much smaller value.

**Table A3.1** Transition frequencies ( $\omega_{ij}$ ) and relative transition dipole strengths ( $\mu_{ij}$ ) used in the simulation of the transient-grating signal from peridinin in which the identity of the state  $S_x$  is equated with the  $S_1$  state but with an initially higher energy due to vibrational excitation, here about two quanta for C–C or C=C modes of the conjugate polyene backbone (+2590 cm<sup>-1</sup>).

Transition	$\omega_{ij}  (\mathrm{cm}^{-1})$	$\mu_{ij}$ (Debye)	
$S_0 \rightarrow S_2$	21670	1	
$\mathbf{S}_2 \rightarrow \mathbf{S}_{n2}$	14800	0.35 <sup>a</sup>	
$S_x \rightarrow S_{nx}$	14800	1.65	
$\mathbf{S}_1 \rightarrow \mathbf{S}_{n1}$	17390	1.65	

<sup>a</sup> The ESA transition for the  $S_2$  state is well to the red of the probe bandwidth, so it makes no contribution to the signals calculated for 45 fs, 520 nm pulses. The value indicated for the transition dipole strength here is the same as that used previously for  $\beta$ -carotene in benzonitrile<sup>10</sup> and is not optimized for peridinin.



**Figure A3.2** Numerical simulation of the absorption component of the transient grating signal from peridinin calculated by treating  $S_x$  as vibrationally excited  $S_1$  state. The parameters used in the calculation are listed in Tables 3.1 and A3.1 and in the text. The yellow end of the color bar corresponds to net ground–state bleaching or stimulated emission; the blue end indicates excited state absorption.



**Figure A3.3**. Numerical simulation of the dispersion component of the transient grating signal from peridinin calculated by treating  $S_x$  as a vibrationally excited  $S_1$  state. The parameters used in the calculation are listed in Tables 3.1 and A3.1 and in the text. At 520 nm, the yellow contours correspond to the net ESA signal from the  $S_1$  state.



**Figure A3.4** Comparison of the experimental and calculated absorption components of the transient grating signal from peridinin calculated by treating  $S_x$  as vibrationally excited  $S_1$  state. The parameters used in the calculation are listed in Tables 3.1 and A3.1 and in the text. The experimental (Figure 3.3) and calculated signals (Figure 3.11) were integrated over the laser frequency axis and normalized at the 1 ps delay point. *Top Panel:* Absorption components from the experimental transient (data points) and from the simulation (solid line). *Bottom Panel:* Absorption components calculated from individual Feynman pathways: Orange, GSB; Red, SE from the S<sub>2</sub> state; Blue, S<sub>2</sub>  $\rightarrow$  S<sub>n2</sub> ESA; Green, DQC; Violet, S<sub>x</sub>  $\rightarrow$  S<sub>nx</sub> ESA, Black, S<sub>1</sub>  $\rightarrow$  S<sub>n1</sub> ESA.



**Figure A3.5** Comparison of the experimental and calculated dispersion components of the transient grating signal from peridinin calculated by treating  $S_x$  as vibrationally excited  $S_1$  state. The parameters used in the calculation are listed in Tables 3.1 and A3.1 and in the text. The experimental (Figure 3.4) and calculated signals (Figure 3.12) were integrated over the laser frequency axis and normalized at the 1 ps delay point. *Top Panel:* Dispersion components from the experimental transient (data points) and from the simulation (solid line). *Bottom Panel:* Dispersion components calculated from individual Feynman pathways: Orange, GSB; Red, SE from the S<sub>2</sub> state; Blue, S<sub>2</sub>  $\rightarrow$  S<sub>n2</sub> ESA; Green, DQC; Violet, S<sub>x</sub>  $\rightarrow$  S<sub>nx</sub> ESA, Black, S<sub>1</sub>  $\rightarrow$  S<sub>n1</sub> ESA.

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# Chapter 4: Torsional Dynamics and Intramolecular Charge Transfer in the $S_2(1^1B_u^+)$ Excited State of Peridinin: A Mechanism for Enhanced Mid-Visible Light Harvesting

Of the carotenoids known in photosynthetic organisms, peridinin exhibits one of the highest quantum efficiencies for excitation energy transfer to chlorophyll (Chl) *a* acceptors. The mechanism for this enhanced performance involves an order-of-magnitude slowing of the  $S_2 (1^1B_u^+) \rightarrow S_1 (2^1A_g^-)$  nonradiative decay pathway compared to carotenoids lacking carbonyl substitution. Using femtosecond transient grating spectroscopy with optical heterodyne detection, we have obtained the first evidence that the nonradiative decay of the  $S_2$  state of peridinin is promoted by large-amplitude torsional motions. The decay of an intermediate state termed  $S_x$ , which we assign to a twisted form of the  $S_2$  state, is substantially slowed by solvent friction in peridinin due to its intramolecular charge transfer (ICT) character.

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# 4.1 Introduction

Obtaining photochemically robust chromophores with strong absorption in the mid-visible portion of the spectrum and efficient pathways of excitation energy transfer for use in light-harvesting materials is a particularly challenging problem that photosynthetic organisms have solved by inclusion of carotenoids in light-harvesting complexes.<sup>1–3</sup> Peridinin (1), the principal light absorber in the peridinin–chlorophyll *a* protein (PCP) in dinoflagellates,<sup>4</sup> exhibits one of the highest known efficiencies (~90%) for excitation energy transfer from a carotenoid to Chl acceptors.<sup>5,6</sup> The structural mechanism that enhances this energy transfer function involves the presence of an unusual carbonyl substituted  $\gamma$ -lactone ring in conjugation with the polyene backbone. The electron-withdrawing tendency of the carbonyl group contributes to a redistribution of  $\pi$ -electron density and an ICT character.<sup>7</sup>



**Figure 4.1** Structures of peridinin (1) and  $\beta$ -carotene (2)

The light-harvesting function of carotenoids employs strong absorption bands in the midvisible part of the spectrum arising from the electric-dipole allowed  $S_0(1^1A_g^-) \rightarrow S_2(1^1B_u^+)$ transition.<sup>8</sup> Energy transfer between the S<sub>2</sub> state and a Chl acceptor is accordingly favorable through the Förster mechanism,<sup>9</sup> which relies on the strong oscillator strength of the donor carotenoid for the downward S<sub>2</sub>  $\rightarrow$  S<sub>0</sub> transition that accompanies excitation of the Chl Q<sub>x</sub> or Q<sub>y</sub> transitions. The energy-transfer yield, however, is usually constrained by the very short (<150 fs) lifetime of the S<sub>2</sub> state due to nonradiative decay to the S<sub>1</sub> ( $2^1A_g^-$ ) state. From a Förster perspective, energy transfer from the S<sub>1</sub> state would be expected to be less efficient than from the S<sub>2</sub> state because the S<sub>1</sub>  $\rightarrow$  S<sub>0</sub> transition is effectively forbidden.<sup>10,11</sup> Birge, Frank, and coworkers have proposed that peridinin performs better than non-carbonyl substituted carotenoids in energy transfer to Chls because the S<sub>2</sub> and S<sub>1</sub> states are mixed as a result of the partial C–C/C=C excited-state bond-order reversal that accompanies the carbonyl-induced ICT character.<sup>12</sup> The results of the present contribution, however, indicate that the carbonyl substitution in peridinin also markedly improves the ability of the S<sub>2</sub> state to serve as an efficient energy transfer donor by substantially lengthening its lifetime. We can now show that decay from the S<sub>2</sub> state to the S<sub>1</sub> state is promoted by large-amplitude torsional motions, which are strongly retarded by solvent friction because of the enhanced ICT character peridinin obtains as it twists.

# **4.2 Experimental Methods**

### 4.2.1 Sample Preparation

Peridinin from laboratory-grown *Amphidinium carterae* (strain CCMP121) was isolated as described in detail previously and purified by high-performance liquid chromatography.<sup>13</sup> For the femtosecond spectroscopic experiments, peridinin was dissolved in a given solvent to obtain an optical density of 0.3 at the center of the laser spectrum in a fused silica cuvette with a 1 mm optical path length.

### 4.2.2 Femtosecond Spectroscopy

Transient grating signals were obtained at 22 °C as described in detail previously.<sup>13,14</sup> 40 fs excitation pulses were obtained from an optical parametric amplifier (OPA, Coherent OPA

9450), which was pumped by a 250 kHz amplified Ti:sapphire laser (Coherent Mira Seed oscillator and RegA 9050 amplifier). The transient grating signals were recorded with a diffractive-optic based, passively phase stabilized, photon echo spectrometer.<sup>15,16</sup>

# 4.3 Results and Discussions

We studied the nonradiative decay of the  $S_2$  state of peridinin in four polar solvents with femtosecond transient grating spectroscopy and optical heterodyne detection<sup>17</sup> using a passively phase-stabilized, diffractive optic based spectrometer.<sup>15,16</sup> The  $S_0 \rightarrow S_2$  transition was excited with 40 fs laser pulses tuned to the red edge of the absorption spectrum of peridinin near the wavenumber of the 0–0 vibronic transition in a given solvent. The absorption spectrum and the tuning of the laser in each solvent are shown in the Appendix.

Figure 4.2 shows contour representations of the spectrally resolved absorption component of the transient grating spectrum of peridinin in four polar solvents over the 0–500 fs probe delay range. The time evolution of both components of the transient grating spectrum is well described by a sequential pathway from the instantaneously formed (Franck–Condon) S<sub>2</sub> state through two intermediate states,  $S_x$  and  $S_1$ , prior to recovery to the ground state,  $S_0$ . The evolution-associated spectra (EAS) and the formation and decay time constants (Table A4.1) obtained for each state by fitting the absorption and dispersion components of the transient grating signal in each solvent using a sequential global target model<sup>18</sup> are presented in the Appendix. Assignments for the nonlinear optical pathways that contribute to the EAS from each state were made using the previously published numerical simulations of the transient grating signal from peridinin in methanol, which were performed using the response function formalism and the multimode Brownian oscillator model.<sup>13</sup>



**Figure 4.2** Contour representations of the spectrally resolved absorption component of the heterodyne transient grating signal from peridinin at room temperature (22 °C) in: (a) acetonitrile; (b) ethyl acetate; (c) methanol; and (d) 2-propanol. The corresponding dispersion components are shown in the Appendix. The yellow end of the color bar corresponds to net ground state bleaching or stimulated emission; the dark blue end indicates excited state absorption. The black dotted lines indicate the wavelengths for the single-wavelength slices plotted in Figure 4.3.

Figure 4.3 compares examples of single-wavelength slices of the absorption component of the transient grating signal that show how the populations of the three states in the nonradiative decay pathway control the shape of the response in the four solvents. The Franck–Condon  $S_2$  state species decays almost instantaneously (<16 fs) in each case. The spectrum from  $S_2$  consists mainly of ground-state depletion and stimulated emission (SE) signals over the probed bandwidth; a net excited-state absorption (ESA) spectrum appears upon formation of the first intermediate state,  $S_x$ , which is apparently comparable<sup>13</sup> to the intermediate state observed with similar kinetics after optical preparation of the  $S_2$  state in other carotenoids.<sup>2,14</sup> The numerical

simulations show that the ultrafast kinetics for the formation of  $S_x$  in  $\beta$ -carotene and peridinin can be clearly discerned especially in the rising ESA kinetics of the dispersion component.<sup>13,14</sup> Nonradiative decay of  $S_x$  then yields a net ESA spectrum from the  $S_1$  state, which is somewhat more intense and only slightly blue shifted from that of  $S_x$  as probed here. It should be noted that the limited probe bandwidth used in these experiments precludes detection of the red shifted, solvent dependent ESA band in the 600 nm region of the spectrum that is usually assigned to an ICT (or  $S_{1/ICT}$ ) state in polar solutions of peridinin,<sup>7,19,20</sup> so the use of an generic compartment for the  $S_1$  state is appropriate in the present global models.



**Figure 4.3** Single-wavelength slices of the absorption component of the heterodyne transient grating signal from peridinin (see Figure 4.2), as fitted by a global target model: in (a) acetonitrile, at 507 nm; (b) ethyl acetate, at 507 nm; (c) methanol, at 520 nm; and (d) 2-propanol, at 520 nm. The lower panels for each solvent show the relative population in the three states in the fitted model: black, Franck–Condon S<sub>2</sub> state; red, S<sub>x</sub> intermediate state; blue, S<sub>1</sub> state.

Solvent	S <sub>x</sub> lifetime (ps)	$\eta(cP)^a$	$ au_{avg}(ps)^b$
Acetonitrile	$0.39\pm0.14$	0.345	0.26
Ethyl acetate	$0.64\pm0.14$	0.45	0.86
Methanol	$0.90\pm0.10$	0.597	5
2-propanol	$1.70 \pm 0.13$	2.4	26

**Table 4.1** Lifetime of the  $S_x$  state of peridinin, as obtained from the global target models of the absorption and dispersion components of the transient grating signal in four solvents.

<sup>a</sup>Solvent viscosity, from reference 23. <sup>b</sup>Average polar solvation time, from Table 3 of reference 24.

While the conversion of the S<sub>2</sub> state to the S<sub>x</sub> intermediate occurs at the same ultrafast rate in all of the solvents (see Table A4.1), the lifetime of  $S_x$  is more than quadrupled by moving from acetonitrile to 2-propanol. Table 4.1 reports the lifetimes for the S<sub>x</sub> state in comparison with two properties of the solvent, the viscosity and the average polar solvation time. Figure 4.4 shows that the lifetime of S<sub>x</sub> increases monotonically with respect to both properties and tends towards saturation in the most viscous or slow solvent, 2-propanol. In contrast, Figure A4.29 shows that the lifetime of the S<sub>x</sub> state is not monotonically dependent on the solvent polarity. This behavior makes it clear that the Sx state has a distinct electronic configuration from the S1 state, which exhibits a strong, essentially linear dependence on the solvent polarity (Table A4.1 and Figure A4.31), as was observed previously by Frank and coworkers.<sup>7</sup> It was proposed previously<sup>20,21</sup> that a vibrationally excited level of the S<sub>1</sub> state is formed in peridinin directly upon nonradiative decay of the  $S_2$  state on the ~50 fs time scale, but as discussed in detail in the previous work on the transient grating signal from peridinin in methanol,<sup>13</sup> numerical simulations of the vibrational cooling of such a "hot" S1 intermediate yield multiexponential absorption and dispersion decays that are markedly different from those observed experimentally. Further, the wide range of lifetimes observed for Sx over the range of solvents studied here is inconsistent with the weak

dependence on the structure of the solvent that would be expected for a vibrational cooling process.<sup>22</sup>



**Figure 4.4** Power-law dependencies of  $S_x$  lifetime,  $\tau_{Sx}$  on solvent properties: (a)  $\tau_{Sx} = a + b\eta^{\alpha}$  with respect to the solvent viscosity,  $\eta$ , with a = -0.57, b = 1.7 and  $\alpha = 0.4$ . (b)  $\tau_{Sx} = a + b\tau^{\alpha}{}_{avg}$  with respect to the average polar solvation time,  $\tau_{avg}$ , with a = 0.18, b = 0.41 and  $\alpha = 0.4$ .

Instead, as proposed previously,<sup>13,14,25</sup> the  $S_x$  state is best assigned to a twisted form of the  $S_2$  state. The time evolution of the high-frequency, C=C bond stretching bands recently observed using transient IR and stimulated Raman spectroscopy by Di Donato et al.<sup>26</sup> supports the previous conclusion from pump–probe and pump–dump–probe spectroscopy by Zigmantas et al.<sup>20</sup> and Papagiannakis et al.,<sup>27</sup> respectively, that distortions of the conjugated polyene backbone of peridinin occur after optical preparation of the  $S_2$  state. The viscosity dependence reported here for the lifetime of the  $S_x$  state, however, establishes for the first time that large-amplitude torsional motions promote nonradiative decay from  $S_x$  to  $S_1$ . As is also observed for the decay of excited states in triphenylmethane (TPM) dyes<sup>28</sup> and cyanines, <sup>29</sup> the lifetime of the  $S_x$  state

follows a power law function with respect to the solvent viscosity (Figure 4.4a), which indicates that solvent friction retards progress along torsional coordinates towards a position-sensitive sink<sup>30</sup> (or seam<sup>13,14,31</sup>) leading to the S<sub>1</sub> state. But a better correlation of the lifetime of S<sub>x</sub> is obtained with respect to the average polar solvation time (Figure 4.4b), which suggests that the friction that slows the decay from S<sub>x</sub> to S<sub>1</sub> includes a solvent reorganization <sup>32</sup>in response to the net transfer of charge that accompanies the twisting and pyramidalization of a C=C bond.<sup>33–35</sup> A comparable lifetime dependence was observed in the dye LDS–821, which exhibits a twisted intramolecular charge-transfer (TICT) excited state.<sup>36</sup>

The present results strongly support our previous hypothesis that the structure of peridinin in the S<sub>2</sub> state undergoes a two-step structural evolution prior to decaying to the S<sub>1</sub> state. This hypothesis is framed by the potential energy surfaces depicted schematically in Figure 4.5, which are based on aspects of the potential energy surfaces of conjugated polyenes, cyanines, and protonated Schiff bases (PSBs) of intermediate conjugation lengths.<sup>31,37</sup> The literature background and the implications of this scheme, with application to the nonradiative decay and nonlinear spectroscopic properties of carotenoids in solution and in light-harvesting proteins, was discussed in detail previously.<sup>13,14,25</sup> The key feature of Figure 4.5 is that the  $\pi^*$  excited state potential surfaces are divided by a low transition state barrier between planar and twisted conformations of the conjugated polyene backbone.<sup>25,37</sup> After optical preparation of the S<sub>2</sub> state from the planar minimum of the ground state, the first step in the nonradiative decay process is a solvent-independent displacement from the planar Franck–Condon structure along the resonance Raman-active C-C and C=C stretching coordinates of the conjugated polyene. In no more than half a vibration of these high-frequency coordinates, the system passes the transition-state barrier to form the S<sub>x</sub> state, which is assigned to S<sub>2</sub> structures in which the conjugated polyene backbone has just begun distortions with respect to torsional coordinates. Because the  $\gamma$ -lactone ring inhibits out-of-plane motions, the distortion of the conjugated polyene backbone of peridinin would be expected to occur along the intervening length prior to the allene moiety. Vertical excitations of conformationally distorted ground state structures directly to the twisted part of the S<sub>2</sub> potential surface might contribute to the increased excited-state ICT character of peridinin detected in previous work with excitation in the red tail of the S<sub>0</sub>  $\rightarrow$  S<sub>2</sub> absorption spectrum.<sup>20,26</sup>



**Figure 4.5** Proposed scheme for the radiationless decay of the S<sub>2</sub> state of carotenoids.<sup>13,25</sup> The ethylenic structures shown depict the S<sub>x</sub> and X<sup>3</sup> states as torsionally distorted S<sub>2</sub> structures near the transition state barrier ( $\ddagger$ ) and further along the torsional gradient, respectively.

The second step in the nonradiative decay of the  $S_2$  state involves further torsional distortions of the conjugated polyene backbone accompanying a descent of the steep potential energy gradient from the transition state barrier towards a convergence of the energies of the  $S_2$ ,  $S_1$  and  $S_0$  states. The rate at which evolution occurs along the torsional coordinates controls the

lifetime of the  $S_x$  state, and here friction from the surrounding solvent makes a strong contribution to the dynamics. Nonradiative decay from  $S_x$  would occur by passage through a seam to the  $S_1$  state, probably well before a 90° twist, given that photoisomerization to the *cis* conformation is not observed.

The torsional and/or pyramidal distortions that peridinin undergoes on the  $\geq 20$  fs timescale during relaxation of the S<sub>x</sub> state and subsequently upon nonradiative decay to the S<sub>1</sub> state in polar solution result in an enhanced ICT character and an unusual sensitivity to the polarity of the solvent medium, as observed previously in extensive studies of the characteristic ICT ESA band in the 600 nm region of the spectrum.<sup>7</sup> The net SE signal observed in the near-IR (950 nm) region of the spectrum<sup>19,38,39</sup> may include contributions from torsionally displaced structures on the  $S_2$  potential surface near the seam to the  $S_1$  state and/or from the vibrationally excited structures produced after passage to the S<sub>1</sub> state; the rise time of the SE signal is comparable to that associated with the decay of  $S_x$  measured in the present work but there is an additional sensitivity to the solvent polarity.<sup>19</sup> A similar net SE signal has been observed in distorted PSBs.<sup>40</sup> As discussed previously,<sup>13,25</sup> an additional redistribution of  $\pi$ -electron density along the distorted conjugated polyene backbone and further out-of-plane distortion would be expected to accompany a search of the  $S_1$  potential surface as vibrational cooling occurs on the >0.5 ps timescale. This cooling process is not detected in the present experiments due to the limited probe bandwidth, but experiments with broadband continuum probing have detected it in peridinin<sup>39</sup> and in other carotenoids.<sup>41,42</sup>

The mechanism shown in Figure 4.5 should also apply to carotenoids lacking carbonyl substitution. In general, an ICT character and a frictional coupling to the surrounding solvent would be expected to develop as a carotenoid relaxes along torsional coordinates in the  $S_2$  state.<sup>25</sup>
Owing to extensive delocalization of the  $\pi^*$  character, the separation of charge in the S<sub>2</sub> state would be distributed over much of the length of the conjugated polyene backbone on either side of the twisting distortion.<sup>13</sup> For peridinin, the ICT character is especially large because of the contribution of the carbonyl substituent, which results in a very large permanent dipole moment increase upon photoexcitation to the S<sub>2</sub> state.<sup>43,44</sup> The ICT character is then further enhanced in peridinin by distortion of the conjugated polyene backbone.<sup>26,45</sup> As a result, the solvent friction in the S<sub>2</sub> state of peridinin is much larger than for  $\beta$ -carotene and retinal, where the excited state lifetimes are essentially independent of the solvent.<sup>46,47</sup> The lifetime detected previously for the S<sub>x</sub> state of  $\beta$ -carotene, 150 fs,<sup>2,14</sup> is accordingly significantly shorter than observed for peridinin even in the fastest solvent, acetonitrile.

We suggest that these results have important implications in understanding the mechanism of excitation energy transfer from carotenoids to Chl acceptors in photosynthetic light-harvesting proteins. Because it strongly enhances the solvent friction suffered by torsional motions in the S<sub>2</sub> state, the carbonyl substitutent of peridinin causes the lifetime of the S<sub>x</sub> state to be extended into the >1 ps regime, well over that observed for carotenoids lacking carbonyl substitution like  $\beta$ -carotene (150 fs). Such a long lifetime allows the S<sub>x</sub> to serve as an efficient energy transfer donor to Chl acceptors in PCP. Current estimates hold that 75% of the total ~90% excitation energy transfer yield is mediated in PCP by the Förster mechanism with a time constant of 2.5–3.0 ps; the remaining 25% of the yield is carried by a very fast energy transfer channel that may involve electronic coherence or delocalized excited states.<sup>5,6,48</sup> The solvent environment of peridinin due to the surrounding protein and chromophores in PCP is roughly comparable in viscosity and polarity to ethylene glycol, as gauged by a comparison of the linebreadth, absence of resolved vibronic structure, and shift of the ground-state absorption spectra in the two environments.<sup>6</sup> The

power law models for the lifetime of S<sub>x</sub> as a function of viscosity and average solvation time (Figures 4.4a and 4.4b) predict an intrinsic lifetime for  $S_x$  in ethylene glycol in the range from 1.4 ps (from the solvation time,  $\tau_{avg} = 15.3 \text{ ps}^{24}$ ) to 5 ps (from the viscosity,  $\eta = 19.9 \text{ cP}^{23}$ ). These lifetimes are certainly long enough to allow the S<sub>x</sub> state of peridinin to make a significant contribution in PCP as an energy transfer donor state because S<sub>x</sub> would be expected to retain much of the oscillator strength of the Franck-Condon S2 state. This prediction should be compared to the presently accepted picture for the Förster energy transfer channel in PCP, which holds that the S<sub>1</sub> state of peridinin effectively borrows oscillator strength from the "bright" S<sub>2</sub> state via the ICT character it obtains from the carbonyl substitution.<sup>12,49</sup> An additional enhancement in PCP is the selection by the peridinin-binding sites of distorted ground-state conformations,<sup>49</sup> which would be expected to lengthen the effective lifetime of  $S_x$  even more by optimizing the carbonyl-induced ICT character and the resultant frictional coupling to the environment immediately upon excitation to the S<sub>2</sub> state. Note that a distorted carotenoid S<sub>2</sub> structure (X, Figure 4.5) is also implicated in energy transfer pathways to bacteriochlorophyll a detected in two-dimensional electronic spectra from the LH2 complex of purple bacteria,<sup>3</sup> but in our picture the energy transfer yield in this case is not as large as in PCP partly owing to the shorter lifetime of the S<sub>2</sub> state.

APPENDIX

#### APPENDIX

## A 4.1 Supporting Results

Heterodyne transient grating signals were obtained with 40 fs excitation pulses for peridinin dissolved in the following solvents: acetonitrile, ethyl acetate, methanol, and 2-propanol. As shown in the absorption spectra for each case, the laser was tuned to maintain a ~50% detuning to the red from the absorption maximum, which places the excitation near the wavenumber of the 0–0 vibronic transition for the S<sub>0</sub>  $(1^{1}A_{g}^{-})$  to S<sub>2</sub>  $(1^{1}B_{u}^{+})$  transition. The optical density of the samples at the center of the laser spectrum was 0.3 across the 1 mm cuvette.

For each solvent, we present in the following contour representations of the absorption (real) and dispersion (imaginary) components of the transient grating signal, as determined using Fourier transform spectral interferometry.<sup>15</sup> A global target analysis<sup>18</sup> using the Glotaran and TIMP programs<sup>50,51</sup> was employed to fit both components of the transient grating signal to a sequential decay model involving three kinetic components: the S<sub>2</sub> state prepared by the laser pulse, an S<sub>x</sub> intermediate state, and the S<sub>1</sub> state, which then yields the ground state, S<sub>0</sub>. A preliminary singular value decomposition (SVD) analysis indicated the contributions of three spectrokinetic components in all the solvents studied. The spectral time evolution in the time evolution of the EAS has been discussed in detail for β-carotene<sup>14</sup> and peridinin<sup>13</sup> in previous publications with support from a comprehensive set of numerical simulations using the response function formalism and the multimode Brownian oscillator model. Note that this work

considered a range of alternative models and considered especially the possible assignment of the  $S_x$  state in peridinin to a vibrationally excited energy level of  $S_1$ .

The lifetimes and associated confidence intervals for each of the states in the model in the four solvents were determined from the global target analysis, and the confidence intervals were found to be consistent with those obtained from the set of lifetimes from replicate runs. As noted previously,<sup>13</sup> the lifetime of the  $S_2$  state is determined with considerable indeterminacy in these experiments due to the use of 40 fs excitation pulses. We found, however, that a better estimate of the lifetime of  $S_2$  for peridinin in methanol was obtained from numerical simulations of the transient grating signal; in particular the rise time of the dispersion signal provides a bracketing of the lifetime of the  $S_2$  state between 8 fs and 16 fs. The indeterminacy for the lifetime of the  $S_2$  state in the other three solvents is similar to that in methanol.

As discussed previously,<sup>13,14</sup> the lifetimes for the  $S_1$  state returned by the models for the absorption and dispersion components are significantly different because of differential detection of a contribution from a conformationally displaced ground state species, which is produced upon nonradiative decay of the  $S_1$  state. The longer  $S_1$  lifetime observed in the absorption signal reflects the additional time required for conformational relaxation to occur on the ground-state potential surface to recover the original, photoselected ground-state conformation. The dispersion is less sensitive to the blue shift of the photoinduced absorption spectrum because the dispersion lineshapes are much broader than the absorption lineshapes. This phenomenon is comparable to that previously observed by Xu et al. in heterodyne transient grating studies of the triphenylmethane dye crystal violet.<sup>52</sup> The global models do not include this part of the dynamics because the narrow probe spectra range obtained from the 40 fs pulses does not provide enough spectral coverage of the ground-state photoinduced absorption spectrum.

# Acetonitrile



**Figure A4.1** Room-temperature (22 °C) absorption spectrum of peridinin in acetonitrile solvent. Superimposed is the spectrum of the OPA signal-beam output (505 nm center wavelength), as tuned for the heterodyne transient-grating experiments.



**Figure A4.2** Real (absorption) component of the heterodyne transient grating signal from peridinin in acetonitrile solvent at room temperature (22 °C). The yellow end of the color bar corresponds to net ground state bleaching or stimulated emission; the dark blue end indicates excited state absorption. This figure is shown in panel (a) of Figure 4.2.



**Figure A4.3** Imaginary (dispersion) component of the heterodyne transient grating signal from peridinin in acetonitrile solvent at room temperature (22 °C). The phasing of the color bar is consistent with that used in Figure A4.2 for the absorption component. At 505 nm, the yellow contours correspond to a net ESA signal.



**Figure A4.4** Time evolution of the populations for the three species in a global analysis of the heterodyne transient grating signal (Figures A4.2 and A4.3) from peridinin in acetonitrile over the -100 fs-10 ps delay range, as plotted against a linear–logarithmic time axis split at 1 ps: *black*: instantaneously formed species,  $12 \pm 4$  fs decay; *red*: first intermediate species,  $12 \pm 4$  fs rise,  $0.39 \pm 0.14$  ps decay; *blue*: second intermediate species in the absorption component,  $0.39 \pm 0.14$  ps rise,  $8.6 \pm 0.3$  ps decay back to the ground state; *dashed blue*: second intermediate species in the dispersion component,  $0.39 \pm 0.14$  ps rise,  $8.6 \pm 0.3$  ps decay back to the ground state; *dashed blue*: second intermediate species in the dispersion component,  $0.39 \pm 0.14$  ps rise,  $8.6 \pm 0.3$  ps decay back to the ground state; *dashed blue*: second intermediate species in the dispersion component,  $0.39 \pm 0.14$  ps rise,  $8.1 \pm 0.5$  ps decay.



**Figure A4.5** Evolution-associated spectra (EAS) from a global analysis of the absorption component of the heterodyne transient grating signal (Figure A4.2) from peridinin in acetonitrile over the -100 fs-10 ps delay range. The EAS assume a sequential pathway from the optically prepared S<sub>2</sub> state (black) through two intermediates (red, then blue) back to the ground state.



**Figure A4.6** Evolution associated spectra (EAS) from a global analysis of the dispersion component of the heterodyne transient-grating signal (Figure A4.3) from peridinin in acetonitrile over the -100 fs-10 ps delay range.



**Figure A4.7** Time evolution of the absorption (red) and dispersion (blue) components of the heterodyne transient grating signals from peridinin in acetonitrile solvent at 507 nm, as plotted against a linear–logarithmic time axis split at 1 ps. Both signals are superimposed with the fitted global models described in Figures A4.4–A4.6, respectively.

# **Ethyl Acetate**



**Figure A4.8** Room-temperature (22 °C) absorption spectrum of peridinin in ethyl acetate solvent. Superimposed is the spectrum of the OPA signal-beam output (505 nm center wavelength), as tuned for the heterodyne transient-grating experiments.



**Figure A4.9** Real (absorption) component of the heterodyne transient grating signal from peridinin in ethyl acetate solvent at room temperature (22 °C). The yellow end of the color bar corresponds to net ground state bleaching or stimulated emission; the dark blue end indicates excited state absorption. This figure is shown in panel (b) of Figure 4.2.



**Figure A4.10** Imaginary (dispersion) component of the heterodyne transient grating signal from peridinin in ethyl acetate solvent at room temperature (22 °C). The phasing of the color bar is consistent with that used in Figure A4.9 for the absorption component. At 505 nm, the yellow contours correspond to a net ESA signal.



**Figure A4.11** Time evolution of the populations for the three species in a global analysis of the heterodyne transient grating signal (Figures A4.9 and A4.10) from peridinin in ethyl acetate over the -100 fs-100 ps delay range, as plotted against a linear–logarithmic time axis split at 1 ps: *black*: instantaneously formed species,  $12 \pm 4$  fs decay; *red*: first intermediate species,  $12 \pm 4$  fs rise,  $0.64 \pm 0.14$  ps decay; *blue*: second intermediate species in the absorption component,  $0.64 \pm 0.14$  ps rise,  $130 \pm 3.5$  ps decay back to the ground state; *dashed blue*: second intermediate species in the dispersion component,  $0.64 \pm 0.14$  ps rise,  $130 \pm 3.5$  ps decay back to the ground state; *dashed blue*: second intermediate species in the dispersion component,  $0.64 \pm 0.14$  ps rise,  $83 \pm 10$  ps decay.



**Figure A4.12** Evolution-associated spectra (EAS) from a global analysis of the absorption component of the heterodyne transient grating signal (Figure A4.9) from peridinin in ethyl acetate over the -100 fs-100 ps delay range. The EAS assume a sequential pathway from the optically prepared S<sub>2</sub> state (black) through two intermediates (red, then blue) back to the ground state.



**Figure A4.13** Evolution associated spectra (EAS) from a global analysis of the dispersion component of the heterodyne transient-grating signal (Figure A4.10) from peridinin in ethyl acetate over the -100 fs-100 ps delay range. The EAS assume a sequential pathway from the optically prepared S<sub>2</sub> state (black) through two intermediates (red, then blue) back to the ground state.



**Figure A4.14** Time evolution of the absorption (red) and dispersion (blue) components of the heterodyne transient grating signals from peridinin in ethyl acetate solvent at 507 nm, as plotted against a linear–logarithmic time axis split at 1 ps. Both signals are superimposed with the fitted global models described in Figures A4.11–A4.13, respectively.

# 2-Propanol



**Figure A4.15** Room-temperature (22 °C) absorption spectrum of peridinin in 2-propanol solvent. Superimposed is the spectrum of the OPA signal-beam output (520 nm center wavelength), as tuned for the heterodyne transient-grating experiments.



**Figure A4.16** Real (absorption) component of the heterodyne transient grating signal from peridinin in 2-propanol solvent at room temperature (22 °C). The yellow end of the color bar corresponds to net ground state bleaching or stimulated emission; the dark blue end indicates excited state absorption. This figure is shown in panel (d) of Figure 4.2.



**Figure A4.17** Imaginary (dispersion) component of the heterodyne transient grating signal from peridinin in 2-propanol solvent at room temperature (22 °C). The phasing of the color bar is consistent with that used in Figure A4.16 for the absorption component. At 520 nm, the yellow contours correspond to a net ESA signal.



**Figure A4.18** Time evolution of the populations for the three species in a global analysis of the heterodyne transient grating signal (Figures A4.16 and A4.17) from peridinin in 2-propanol over the -100 fs–48 ps delay range, as plotted against a linear–logarithmic time axis split at 1 ps: *black*: instantaneously formed species,  $12 \pm 4$  fs decay; *red*: first intermediate species,  $12 \pm 4$  fs rise,  $1.7 \pm 0.13$  ps decay; *blue*: second intermediate species in the absorption component,  $1.7 \pm 0.13$  ps rise,  $47 \pm 1.6$  ps decay back to the ground state; *dashed blue*: second intermediate species in the dispersion component,  $1.7 \pm 0.13$  ps rise,  $32 \pm 3$  ps decay.



**Figure A4.19** EAS from a global analysis of the absorption component of the heterodyne transient grating signal (Figure A4.16) from peridinin in 2-propanol over the -100 fs–48 ps delay range. The EAS assume a sequential pathway from the optically prepared S<sub>2</sub> state (black) through two intermediates (red, then blue) back to the ground state.



**Figure A4.20** EAS from a global analysis of the dispersion component of the heterodyne transient-grating signal (Figure A4.17) from peridinin in 2-propanol over the -100 fs–48 ps delay range.



**Figure A4.21** Time evolution of the absorption (red) and dispersion (blue) components of the heterodyne transient grating signals from peridinin in 2-propanol at 520 nm, as plotted against a linear–logarithmic time axis split at 1 ps. Both signals are superimposed with the fitted global models described in Figures A4.18–A4.20, respectively.

## Solvent Dependence of the Global Model Parameters

**Table A4.1** Lifetimes of the  $S_2$ ,  $S_x$ , and  $S_1$  states, as obtained from the global models of the transient grating signal from peridinin in four solvents<sup>*a*</sup>

Solvent	S <sub>2</sub> lifetime (fs)	S <sub>x</sub> lifetime (ps)	S <sub>1</sub> lifetime (ps) (absp/disp)	Polarity <sup>c</sup>	Polarizability <sup>d</sup>
Acetonitrile	$12 \pm 4$	$0.39\pm0.14$	$8.6 \pm 0.3/8.1 \pm 0.5$	0.921	0.210
Ethylacetate	$12 \pm 4$	$0.64 \pm 0.14$	$126\pm4/83\pm10$	0.626	0.226
Methanol	$12 \pm 4$	$0.90\pm0.10$	$9.9 \pm 0.5 / 7.5 \pm 0.5$	0.913	0.202
2-propanol	$10 \pm 2$	$1.70 \pm 0.13$	$46.5 \pm 1.6/32.3 \pm 2.9$	0.852	0.230

<sup>*a*</sup>Solvent parameters are taken from Table 4.2 of reference 7;

<sup>b</sup>Lifetimes for S<sub>1</sub> as obtained from the absorption (absp) and dispersion (disp) components;

<sup>c</sup>Polarity  $P(\varepsilon)$  as calculated from the dielectric constant

<sup>d</sup>Polarizability, as calculated from the index of refraction



Figure A4.22 Lifetime of the  $S_x$  state plotted as a function of solvent polarity.



Figure A4.23 Lifetime of the  $S_x$  state plotted as a function of solvent polarizability.



Figure A4.24 Lifetime of the  $S_1$  state in the absorption component plotted as a function of solvent polarity.

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# Chapter 5: Excitation Transfer by Coherent and Incoherent Mechanisms in the Peridinin–Chlorophyll *a* Protein

Excitation transfer from peridinin to chlorophyll (Chl) *a* acceptors is unusually efficient in the peridinin–chlorophyll *a* protein (PCP) from dinoflagellates. We can show for the first time that this enhanced performance arises from the long intrinsic lifetime obtained for the  $S_2$  ( $1^1B_u^+$ ) state of peridinin that is obtained in PCP, 4.4 ps, due to the presence of its carbonyl substituent. Results from heterodyne transient grating spectroscopy show that the  $S_2$  state serves as the donor state for two channels of excitation transfer: a 30 fs process involving quantum coherence and mixed peridinin–Chl states, and an incoherent, 2.5 ps process that is initiated by dynamic localization, which accompanies the formation of a conformationally distorted  $S_2$  state structure in 45 fs. The distorted  $S_2$  state has a long lifetime in PCP due to its intramolecular charge transfer (ICT) character and enhanced system–bath coupling, which retards the torsional motions that promote nonradiative decay to the  $S_1$  ( $2^1A_g^-$ ) state. The observed lifetime of the  $S_1$  state, 18.9 ps, precludes a significant contribution to excitation transfer, but a role in nonphotochemical quenching remains a possibility.

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## **5.1 Introduction**

Of the known photosynthetic light-harvesting complexes that employ carotenoids as midvisible antenna chromophores,<sup>1</sup> the peridinin–chlorophyll *a* protein (PCP) from marine dinoflagellates is noted for its very high (~90%) quantum efficiency for excitation energy transfer from the carotenoid peridinin to Chl *a* acceptors.<sup>2–5</sup> Each subunit of PCP binds a dense cluster of eight peridinin carotenoids and two Chl *a* chromophores in a  $C_2$ -symmetric, twodomain assembly (Figure 5.1a) inside a basket of  $\alpha$  helices. The four peridinins in each domain are nearly in van der Waals contact (3.3 to 3.8 Å separation) with a central Chl *a* acceptor.<sup>6</sup> The structure of PCP indicates an elegant interplay between the electronic effects of carbonyl substitution of the conjugated polyene of peridinin<sup>7</sup> and of the strained conformations imposed on the peridinins by the binding sites in PCP.<sup>8</sup>

The states and mechanisms that mediate excitation transfer from peridinin to Chl *a* have been investigated by several laboratories using femtosecond spectroscopy.<sup>2–5,9</sup> The scheme that emerges from this work involves two excitation transfer channels. The S<sub>2</sub>  $(1^1B_u^+)$  state of peridinin is assigned a very short lifetime, ~50 fs, which limits the yield of an ultrafast excitation transfer process involving it as the donor state. The nature of this process remains in question because it has not yet been observed directly. The product of the nonradiative decay of the S<sub>2</sub> state is usually identified as a vibrationally excited (or "hot") S<sub>1</sub>  $(2^1A_g^-)$  state species. During and after vibrational cooling on the <1 ps timescale, the S<sub>1</sub> state then serves as the donor state for a 2.5–3 ps excitation transfer channel involving the Förster mechanism<sup>10,11</sup> that carries as much as two-thirds of the overall yield. This part of the scheme is intriguing because the S<sub>1</sub> state of peridinin is known to be effectively a "dark" state, lacking very much oscillator strength for transitions to and from the ground state, S<sub>0</sub>  $(1^1A_g^-)$ .<sup>12,13</sup> In addition, the low energy ICT state<sup>2,7,14</sup>

arising from peridinin's carbonyl substituent that accompanies or is formed from the  $S_1$  state is thought to have poor spectral overlap in PCP with the  $Q_x$  or  $Q_y$  transitions of the Chl *a* acceptors,<sup>15</sup> so its role as a donor state is questionable.



**Figure 5.1** Peridinin and Chl *a* chromophores in the X-ray crystal structure of PCP from *Amphidinium carterae*.<sup>6</sup> (a) Space-filling rendering of the chromophore cluster from a single subunit of PCP, with the peridinin (white) and Chl *a* (blue) chromophores numbered with residue numbers from 1PPR.pdb. (b) Ground-state absorption spectrum for the  $S_0 \rightarrow S_2$  transition from PCP at room temperature (22 °C), with the spectrum from the 520 nm (40 fs) laser excitation pulses superimposed and marked with the wavelengths for the 0–0 vibronic transition for the peridinin chromophores, as estimated in reference 16: 1, for peridinins 612 and 622; 2, for peridinins 613 and 623; 3, for peridinins 611 and 621; and 4, for peridinins 614 and 624.

In an effort to resolve this paradox, we report in this contribution some new results from femtosecond heterodyne transient grating studies that show that more than half of the optically prepared S<sub>2</sub> state population is quenched by excitation transfer to Chl *a* via an ultrafast process that very likely involves mixed peridinin–Chl *a* exciton states and a quantum coherent mechanism. Then, rather than involving the S<sub>1</sub> state as a donor, the remaining minor fraction of the excitation transfer yield is mediated by the Förster mechanism and a conformationally distorted form of the S<sub>2</sub> state, S<sub>x</sub>, which we identified in previous work on peridinin in polar solution.<sup>17,18</sup> The results indicate that dynamic localization of the optically prepared exciton states accompanies the ultrafast conformational displacement that prepares the S<sub>x</sub> state from the Franck–Condon S<sub>2</sub> state.

## **5.2 Experimental Methods**

#### 5.2.1 Sample Preparation.

PCP was isolated from *Amphidinium carterae* cells in the laboratory of Roger Hiller (Macquarie University) according to previously published methods.<sup>19</sup> For femtosecond spectroscopic experiments, PCP samples were suspended in a buffer solution containing 50 mM tricine-NaOH, 20 mM KCl, pH 7.5 to obtain an optical density of 0.3 for at the center of the laser spectrum (520 nm) in a cuvette with a 1 mm optical path length.

#### 5.2.2 Femtosecond Spectroscopy.

Transient grating signals were obtained at 22 °C as described in detail previously using a 250 kHz amplified Ti:sapphire laser and optical parametric amplifier (Coherent RegA 9040 and OPA 9450) and a diffractive-optic based, passively phase stabilized, photon-echo spectrometer.<sup>20</sup>

Following the experimental procedures and theory outlined in our previous work on  $\beta$ carotene and peridinin in polar solvents,<sup>17,18,20</sup>we characterized excitation transfer and nonradiative decay processes in PCP complexes from *Amphidinium carterae* at room temperature using a passively phase-stabilized, diffractive optic based spectrometer that employs spectral interferometric detection. Pump and probe pulses (40 fs duration) were centered at 520 nm, halfway up the red onset of the peridinin ground state absorption band of PCP. As shown in Figure 5.1b, this laser spectrum predominantly selects the  $S_0 \rightarrow S_2$  absorption transition of peridinins in PCP; absorption by Chl *a* in this region of the spectrum is negligible.<sup>5</sup> A similar detuning relative to the absorption maximum was used in the previous work on peridinin in several polar solvents in order to excite the 0–0 vibronic transition.<sup>18</sup> In PCP, the four peridinins in each domain have distinct site energies; the laser spectrum overlaps with the 0–0 regions for three of the four peridinins (2–4 in Figure 1b).

Figure 5.2 shows contour representations of the spectrally resolved absorption and dispersion components of the transient grating spectrum of PCP over the 0–500 fs probe delay range. The transient grating spectrum's appearance and time evolution is quite similar to that observed from peridinin in polar solvents under the same measurement conditions.<sup>17,18</sup> Both components of the signal are well described by a global target model (see the Appendix) defined by contributions only from three spectrokinetic species in a sequential pathway (Scheme 5.1): the S<sub>2</sub> state, which decays in 18±4 fs; an intermediate, S<sub>x</sub>, with a 1.6±0.1 ps lifetime, and the S<sub>1</sub> state, which recovers to the ground state, S<sub>0</sub> (1<sup>1</sup>A<sub>g</sub><sup>-</sup>), in 18.9±0.4 ps. Contributions to the signal from Chl *a* are negligible in the probed region, so only peridinin species were included in the model. Also, as noted previously,<sup>17,18</sup> the probed bandwidth is not broad enough to detect the excited-state absorption band near 600 nm that is observed from the S<sub>1</sub>/ICT state of peridinin in polar solvents, so a generic compartment for the S<sub>1</sub> state is also appropriate in the current model for PCP. The S<sub>x</sub> state is assigned to a distorted conformation of the S<sub>2</sub> state, which is generated from the Franck–Condon S<sub>2</sub> state via displacements along torsional and/or other out-of-plane

(pyramidal) coordinates of the conjugated polyene backbone. The rationale for not assigning the  $S_x$  species to a vibrationally excited  $S_1$  state was extensively discussed in previous work on the bases of numerical simulations<sup>17</sup> and the distinct solvent dependences of the  $S_x$  and  $S_1$  states.<sup>18</sup>



**Figure 5.2** Contour representations of the heterodyne transient grating signal from peridinin at room temperature (22 °C): (a) absorption component; (b) dispersion component. For the absorption component, the yellow end of the color bar corresponds to net ground state bleaching and/or stimulated emission; the dark blue end indicates excited state absorption. For the dispersion component, the phasing of the color bar is consistent with that used for the absorption component. At 525 nm, the yellow contours correspond to a net excited-state absorption signal.

Spectroscopic parameters for the three species that contribute to the transient grating spectrum from PCP were then determined from numerical simulations of the ground state absorption spectrum and of both components of the transient grating signal. The simulations were performed using the response function formalism and the multimode Brownian oscillator (MBO) model, as described previously,<sup>17,18,20</sup> but excitation transfer pathways from  $S_2$  and  $S_x$  to Chl *a* acceptors were added (Figure 5.3) to account for the quenching of peridinin excited states. Excitation transfer processes between the peridinins are not included in the model, so the parameters describe a population weighted average. The model parameters are tabulated in the Appendix.



**Figure 5.3** Kinetic scheme for the radiationless decay and excitation transfer pathway in PCP, as used in the numerical model (Figures 5.4 and A5.6–A5.7) for the heterodyne transient grating signal (Figure 5.2).

Although the time evolution of the transient grating signals from peridinin in solution and in PCP indicate the involvement of the same spectrokinetic intermediates, the numerical model for PCP shows that the  $S_2$  and  $S_x$  states of peridinin are quenched due to excitation transfer to Chl *a* but with time constants that are almost three orders of magnitude apart. Figure 5.4 compares with absolute intensity scaling the absorption and dispersion components of the frequency integrated transient grating signal from PCP with those of peridinin in 2-propanol. A direct comparison of the signals from the two samples can be made because the tuning of the laser spectrum relative to the absorption maximum, the pulse durations and energies, and the optical density at the center of the laser spectrum are all well matched. The signal from peridinin in 2-propanol is used here as an example because the  $S_x$  lifetime in 2-propanol is similar to that observed in PCP.<sup>18</sup>



**Figure 5.4** Comparison of the absolute intensity and time evolution of the absorption and dispersion components of the heterodyne transient grating signals from PCP (red) and from peridinin in 2-propanol (blue), as plotted with respect to a linear–logarithmic time axis split at 1 ps. The plotted absorption and dispersion transients were obtained by integrating over the laser frequency axis. The plotted data points for PCP are shown superimposed with fitted profiles from the numerical model for the transient grating signal (Figures A5.6–A5.7), as obtained with the radiationless decay and excitation transfer pathways shown in Scheme 5.1 and the MBO model parameters reported in Tables A5.1–A5.2. The time profiles for peridinin in 2-propanol were obtained from the global model reported in reference 18. The bottom panel plots the time evolution of the populations in the S<sub>2</sub> (red), S<sub>x</sub> (blue), and S<sub>1</sub> (green) states from the model for the signal from PCP.

The reduced peak intensity for the transient grating signal from PCP in Figure 5.4 can be explained only by quenching of the Franck–Condon  $S_2$  state via an ultrafast energy transfer pathway to Chl *a* acceptors on a timescale comparable to the instrument response function's width. From Scheme 5.1, the overall rate constant for the conversion of  $S_2$  to  $S_x$  obtained in the global model is the sum of the rate constants for nonradiative decay to  $S_x$  and for excitation

transfer to Chl *a*,  $k_{2x} + k_{2c} = (18 \pm 4 \text{ fs})^{-1}$ . A choice of  $1/k_{2c} = 30 \pm 4 \text{ fs}$  and  $1/k_{2x} = 45 \pm 8 \text{ fs}$  for the time constants for excitation transfer and nonradiative decay, respectively, accounts for the reduced signal intensity in PCP. These time constants determine that 60% (+6%, -8%) of the optically prepared peridinin S<sub>2</sub> state is transferred to Chl *a*; in other words, the yield of the S<sub>x</sub> state in PCP is only about 40% (+8%, -6%) of that in peridinin solution. The intrinsic lifetime of the S<sub>2</sub> state in the absence of excitation transfer, 45 fs, is more than three times as long as that estimated previously for peridinin in solution.<sup>18</sup>

Similarly, the decay of the transient grating signal in PCP over the 50 fs–5 ps range is consistent with quenching of the S<sub>x</sub> state by Chl *a*. As noted earlier, excitation transfer to Chl *a* during this part of the response has been detected in previous studies to occur with a 2.5–3 ps time constant; an exponentially rising Chl *a* GSB signal is detected at 670 nm in pump–probe experiments with >100 fs pulses.<sup>2–4</sup> From Figure 5.3, with  $k_{x1} + (k_{2c} \equiv (2.5 \text{ ps})^{-1}) = (1.6 \pm 0.1 \text{ ps})^{-1}$ , one obtains  $1/k_{x1} = 4.4 \pm 0.9 \text{ ps}$ . From the model for the transient grating signal (Figure 5.3), the integrated yield of the S<sub>x</sub> to Chl *a* excitation transfer process is 57%, which indicates an overall quantum yield of 23% (+4%, -4%) starting from the 40% (+8%, -6%) yield of the S<sub>x</sub> state.

The total quantum yield for excitation transfer from peridinin to Chl *a* obtained from the sum of the yields from  $S_2$  and  $S_x$  is 83% (+10%, -12%). Although it should be regarded as a crude estimate, this yield fully accounts for the 90% yield previously estimated from a comparison of the absorption and fluorescence excitation spectra<sup>2-4</sup> and does not involve the  $S_1$  state as an excitation donor. The yield is probably somewhat underestimated owing to the use of relatively long excitation pulses in the present experiments and the fast nonradiative decay and excitation transfer processes that deactivate  $S_2$ . Further, the long lifetime observed for the  $S_1$  state to compartment in the global model essentially rules out its being quenched by excitation transfer to

Chl *a*. No excitation transfer processes with time constants longer than 2.5 ps have been detected in PCP, and the  $18.9\pm0.4$  ps lifetime determined in this work for S<sub>1</sub> would require that the rate be very slow indeed compared to that from S<sub>x</sub>.

The slowing by almost three orders of magnitude of the excitation transfer rate that accompanies nonradiative decay of  $S_2$  to  $S_x$  in PCP can be readily explained by a decrease in the oscillator strength and a concomitant increase in the system–bath coupling or reorganization energy upon formation of the  $S_x$  state. Both effects are caused by large-amplitude conformational distortions of the conjugated polyene backbone of peridinin.

Figure 5.5 shows a schematic representation of the potential energy surfaces that account for the time evolution of the third order signals from peridinin in solution and in PCP. The potential energy curves describe a reaction coordinate for the structural displacements that follow optical preparation of the S2 state of carotenoids, with displacements first along the resonance Ramanactive C-C/C=C bond stretching coordinates that determine the bond-length alternation of the conjugated polyene backbone and then along torsional and other out-of-plane coordinates that lead towards a convergence of the energies of the S2, S1, and S0 states at a conical intersection.<sup>17,18,20,21</sup>Because optical excitations of peridinins in PCP occur from strained groundstate conformations,<sup>8</sup> vertical transitions to the S<sub>2</sub> state occur near to or perhaps even past the transition state barrier that divides planar and twisted conformations. Immediate displacements then occur along the bond-length alternation and torsional coordinates to yield the twisted structures associated with the  $S_x$  state, and further evolution along the torsional gradient promotes nonradiative decay to the S<sub>1</sub> state.<sup>17,18</sup> The excitations of peridinins in PCP are likely to be analogous to those that occur in the red tail of the ground-state absorption spectrum of peridinin in solution, which result upon formation of the S2 state in very large changes of the

permanent dipole moment,<sup>22,23</sup> an enhanced ICT character,<sup>24–26</sup> and a concomitant increase in the system–bath coupling.<sup>17,18</sup> The observation that formation of  $S_x$  and  $S_1$  in PCP is markedly slower than for peridinin in solution indicates that the friction that retards the twisting or pyramidalization of the conjugated polyene backbone of peridinin is apparently much stronger in PCP. Further, using the observed power-law dependences of the  $S_x$  lifetime for peridinin in polar solution, we estimated previously that the lifetime of  $S_x$  in PCP would be expected to fall in the range from 1.4 to 5 ps.<sup>18</sup> The intrinsic lifetime determined in the present work for  $S_x$ , 4.4 ps, is at the top of this range. This finding shows that large-amplitude conformational distortions are indeed involved in nonradiative decay for peridinins in PCP despite their being bound and conformationally strained by their protein and chromophore-derived surroundings.



**Figure 5.5** Schematic potential energy curves for peridinin in the PCP complex based on those suggested in previous work for peridinin in solution.<sup>17,18,20,21</sup> The potentials are plotted with respect to a split reaction coordinate that describes the structural displacements along bondlength alternation and torsional ( $\phi$ ) coordinates; a low barrier (‡) divides planar and twisted structures. The dashed segments show the profiles for peridinin in solution.

The 30 fs excitation transfer process that occurs from the S<sub>2</sub> state is consistent with a quantum coherent<sup>27</sup> mechanism involving peridinin-Chl *a* exciton states. In this hypothesis, the electronic interaction between peridinins and between peridinins and Chl *a* initially lies in the intermediate to strong coupling regime, in which the electronic coupling strengths determined by the oscillator strengths, *J*, are larger than the system–bath reorganization energies,  $\lambda$ .<sup>28</sup> That a manifold of exciton states actually exists in the PCP system rather than just a set of isolated (weak coupling regime) peridinin or Chl states has been repeatedly argued in analyses of the circular dichroism spectrum.<sup>29–32</sup> Downward transfer of population in this manifold of exciton states is a non-Förster type of excitation transfer; the excited state probability density moves predominantly from peridinin to Chl as nonradiative relaxation occurs to the lowest energy states. Analogous exciton relaxation processes and very similar timescales have been previously identified in systems falling in the intermediate to strong electronic coupling regime, such as in allophycocyanin trimer complexes,<sup>33–35</sup> LH1,<sup>36</sup> and in diacetylene-bridged perylenediimide dimer.<sup>37</sup>

Note that the 30 fs excitation transfer process accounts for the well documented but otherwise unexplained finding that the  $Q_y$  transition from Chl *a* in PCP is instantaneously bleached in pump-probe spectra.<sup>2,3,5</sup> As an example, the initial spectrum obtained from a global model for the time evolution of the pump–probe spectrum of PCP by van Stokkum et al.<sup>5</sup> includes a Chl *a* bleaching or stimulated emission lineshape at 670 nm riding on a much broader excited-state absorption band from the peridinin S<sub>2</sub> state. It accounts for about 70% of the final Chl *a* signal intensity; the Förster 2.5 ps excitation process accounts for an increase of 30%. Considering that the excitation pulses were not short enough to resolve the ultrafast and Förster excitation transfer processes, the 70/30 ratio for the instantaneous and final intensities for the Chl

*a* signal is fully consistent with our estimate that two-thirds of the excitation transfer yield comes from the exciton states that are initially populated optically.

Effectively competing with the 30 fs exciton relaxation process, however, is the prompt conformational distortion of the peridinins that produces the  $S_x$  state and subsequent twisted  $S_2$  structures. As the conjugated polyene backbone is distorted, the *J* couplings between peridinins and between peridinins and Chl *a* will decrease rapidly because the oscillator strength drops as the bright  $S_2$  state is increasingly mixed with the dark  $S_1$  state.<sup>8</sup> At the same time, the reorganization energies  $\lambda$  increase markedly owing to the additional ICT character and systembath coupling that develops as the conjugated polyene backbone twists.<sup>18,38–40</sup> This combination of decreasing *J* and increasing  $\lambda$  esults in dynamic exciton localization, an irreversible collapse of the initially delocalized excited state wavefunctions onto single chromophores.<sup>11,28,33,35,41,42</sup> This process initiates the slower excitation transfer process from the  $S_x$  state of peridinin to Chl *a* acceptors via the Förster mechanism.

Thus, the enhanced excitation transfer yield obtained by peridinin in PCP compared to other carotenoid-containing light-harvesting complexes is correctly attributed to the carbonyl substituent, but the mechanism for this enhancement is evidently quite different from that envisioned in prior work in which the  $S_1$  state was implicated as a donor state. The results indicate that the lifetime of the  $S_x$  state controls the minor fraction of the yield of excitation transfer to Chl *a* acceptors involving the Förster mechanism. Because the lifetime of the  $S_x$  state for carotenoids lacking carbonyl substitution is usually more than an order of magnitude shorter than observed in peridinin,<sup>18</sup> the excitation transfer yield involving  $S_x$  as a donor would be expected to be limited. This is apparently the situation in the LH2 complex, where a distorted  $S_2$  state structure serves as an excitation donor to bacteriochlorophyll *a*.<sup>43</sup> The residual fraction of
peridinin excited states that decay nonradiatively to the  $S_1$  state in PCP may actually play a role in a nonphotochemical quenching mechanism, as suggested early on by Bautista et al,<sup>7</sup> especially given that the  $S_1$  state is accompanied by a red shifted ICT state that has a poor Förster overlap with Chl *a* acceptors.<sup>15</sup> A great deal of control over the overall excitation transfer yield is accordingly possible in the PCP complex *in vivo* through protein conformational changes, especially those that alter the solvent exposure and binding sites for the peridinin chromophores when it is associated as a trimeric complex to the thylakoid membrane. APPENDIX

#### **APPENDIX**

## A5.1 Global and Target Analysis

Heterodyne transient grating signals from PCP were obtained at room temperature with 40 fs excitation pulses centered around 520 nm; the tuning of the OPA is shown in Figure 5.1b. Fourier transform spectral interferometry<sup>44</sup> was employed to separate out the absorption (real) and dispersion (imaginary) components of the transient grating signal. A global target analysis<sup>45</sup> using the Glotaran and TIMP programs<sup>46,47</sup> was then employed to fit both components of the transient grating signal to a sequential decay model involving contributions from three spectrokinetic species in a sequential pathway (Scheme 5.1): the S<sub>2</sub> state, which decays in 18±4 fs, an intermediate,  $S_x$ , with a 1.6±0.1 ps lifetime; and the  $S_1$  state, which recovers to the ground state,  $S_0$  (1<sup>1</sup>A<sub>g</sub><sup>-</sup>), in 18.9±0.4 ps. A preliminary singular value decomposition (SVD) analysis indicated the contributions of only three spectrokinetic components. Note that only a generic compartment is included for the S1 state; the probe bandwidth available in these experiments is not broad enough to probe the broad excited-state absorption spectrum that is observed from the ICT state of peridinin near 600 nm.<sup>2,7</sup> The lifetimes for each of the states in the model were as determined from the global target analysis, which gives confidence intervals that are consistent with analyses of multiple runs. As noted previously,<sup>17</sup> the lifetime of the S<sub>2</sub> state is determined with considerable indeterminacy in these experiments due to the use of 40 fs excitation pulses. We found, as in the previous work on  $\beta$ -carotene<sup>20</sup> and peridinin,<sup>17,18</sup> that a better estimate of the S<sub>2</sub> lifetime was obtained from the rise time of the dispersion signal and through numerical simulations of both components of the transient grating spectrum.

The spectral time evolution in the global model is described by the evolution-associated spectra (EAS), which are shown in Figures A5.2 and A5.3. The spectral line shapes of the three spectrokinetic species are quite similar to those for peridinin solution, which were described previously.<sup>17,18</sup>



**Figure A5.1.** Time evolution of the populations for the three species in a global analysis of the heterodyne transient grating signal (Figure 5.2) from PCP over the -100 fs-12 ps delay range, as plotted against a linear–logarithmic time axis split at 1 ps: *black*: instantaneously formed species, 18 fs decay; *red*: first intermediate species, 18 fs rise, 1.6 ps decay; *blue*: second intermediate species in the absorption component, 1.6 ps rise, 18.9 ps decay back to the ground state.



**Figure A5.2** EAS from a global analysis of the absorption component of the heterodyne transient grating signal (Figure 5.2) from PCP over the -100 fs-12 ps delay range. The EAS assume a sequential pathway from the optically prepared S<sub>2</sub> state (black) through two intermediates (red, then blue) back to the ground state.



**Figure A5.3** Evolution associated spectra (EAS) from a global analysis of the dispersion component of the heterodyne transient-grating signal (Figure 5.2) from PCP over the -100 fs-12 ps delay range. The EAS assume a sequential pathway from the optically prepared S<sub>2</sub> state (black) through two intermediates (red, then blue) back to the ground state.

## A 5.2 Numerical Simulation

We simulated the experimental absorption spectrum and heterodyne transient grating signals from PCP using nonlinear response function theory and the multimode Brownian oscillator (MBO) model.<sup>48</sup> The protocol we followed is exactly as used previously for the simulations of the signals from  $\beta$ -carotene and peridinin in methanol.<sup>17,20</sup>

Figure A5.4 shows the energy level scheme for the excited state nonradiative decay of peridinin, as used previously.<sup>17,18</sup> The vertical transition energies and the dipole strengths associated with the transitions are listed in Table A5.1.



**Figure A5.4** Schematic energy-level diagram for peridinin. Solid green arrows indicate ground state and excited-state absorption transitions. Blue wavy arrows mark internal conversion transitions. Double-headed arrows show the detuning ( $\Delta$ ) between the center of the laser spectrum and the vertical energy of an electronic transition.

Transition	$\boldsymbol{\omega}_{ij} (\mathrm{cm}^{-1})$	$\mu_{ij}$ (Debye)
$S_0 \rightarrow S_2$	21200	1
$S_2 \rightarrow S_{n2}$	14800	0.35
$S_x \rightarrow S_{nx}$	17750	1.3
$S_1 \rightarrow S_{n1}$	18500	1.23

**Table A5.1** Transition frequencies ( $\omega_{ij}$ ) and relative transition dipole strengths ( $\mu_{ij}$ ) used in the simulation of the transient-grating signal from PCP.

**Table A5.2** MBO model parameters<sup>*a*</sup> for the simulation of the peridinin  $S_0 \rightarrow S_2$  absorption spectrum of peridinin in PCP at 295 K (Figure A5.5).

Mode	$\lambda \ (cm^{-1})$	au (fs)	$\omega_v(\mathrm{cm}^{-1})$
Gaussian	800	10	
Exponential 1	700	150	
Exponential 2	1000	20000	
Vibration 1	460	2200	1150
Vibration 2	1060	2200	1520

<sup>*a*</sup>For each mode,  $\lambda$  is the reorganization energy and  $\tau$  denotes the damping time constant;  $\omega_{\nu}$  is the frequency for a vibrational mode.



Figure A5.5 Comparison of the room temperature  $S_0 \rightarrow S_2$  absorption spectrum PCP (dark blue curve) with a simulation (red dash-dotted curve) of the peridinin spectrum obtained using the MBO model and the parameters listed in Table A5.1-A5.2.

Figures A5.6 and A5.7 show the simulated absorption and dispersion components of the transient grating signal from peridinin in PCP. In the optimized model, the excited-state absorption (ESA) transition energy from the  $S_x$  state in PCP (Figure A5.4) is shifted to the blue by 450 cm<sup>-1</sup> compared to that for peridinin in methanol. Additionally, the vertical transition energy for the  $S_1$  state was adjusted (blue-shifted by 1110 cm<sup>-1</sup>) to obtain a comparable fit of the absorption and dispersion transient grating signals at long delay times. The transition dipole strengths for the ESA transitions from  $S_x$  and  $S_1$  were also slightly smaller than determined in the simulations of the signal from peridinin in methanol. This finding is consistent with photoselection of distorted ground state conformers in PCP.



**Figure A5.6**. Numerical simulation of the absorption component of the transient grating signal from PCP. The parameters used in the calculation are listed in Tables A5.1 and Table A5.2.



**Figure A5.7**. Numerical simulation of the dispersion component of the transient grating signal from PCP. The parameters used in the calculation are listed in Tables A5.1 and A5.2 and in the text.

These signals were integrated across the emission (signal) spectrum to obtain the time profiles plotted in Figure 5.4. The kinetic scheme used in the model was as used previously for  $\beta$ -carotene and peridinin in solution but with the addition of two channels of energy transfer from

 $S_2$  and  $S_x$ , as shown in Scheme 5.1. The overall lifetimes for  $S_2$ ,  $S_x$ , and  $S_1$  were as obtained from the global model. The intrinsic lifetime of  $S_2$ ,  $1/k_{2x}$ , and the energy transfer time constant  $1/k_{2c}$ were varied while maintaining the overall 18 fs decay time constant determined by the global model to match of the integrated intensity of the absorption and dispersion components with those from the global model for peridinin in 2-propanol,<sup>18</sup> as plotted in Figure 5.4. A 2.5 ps time constant was assumed for  $1/k_{xc}$ , so a 4.4 ps intrinsic lifetime was chosen for  $1/k_{x1}$  to maintain the overall lifetime for  $S_x$ , 1.6 ps, as determined by the global model.

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# **Chapter 6: Conclusions and Future Directions**

In this dissertation, the mechanisms of nonradiative decay and excitation energy transfer by carotenoids were investigated using femtosecond heterodyne transient grating spectroscopy. The specific goal was to determine the role of intermediate electronic states and conformational motions in the mechanisms that mediate carotenoid to chlorophyll energy transfer. The experimental findings were corroborated by numerical simulations using nonlinear response function formalism and the multimode Brownian oscillator model.

The controversy regarding the possible presence of an intermediate electronic state ( $S_x$ ) mediating the  $S_2$ - $S_1$  nonradiative decay pathway of carotenoids was addressed. In  $\beta$ -carotene, the ultrafast rise of the dispersion signal and detection of different ground state recovery timescales observed in the absorption and dispersion channels have provided direct evidence for the formation of conformationally distorted intermediates. The dark intermediate labelled  $S_x$  in previous work is assigned to a torsionally distorted structure evolving on the  $S_2$  ( $1^1B_u^+$ ) potential surface. These findings were extended to studies of peridinin. Nonradiative decay of the  $S_x$  state in peridinin is hindered by solvent friction owing to the development of an ICT character. This work identifies the role of torsional dynamics of the conjugated backbone in promoting nonradiative decay and ICT character in carotenoids. In the peridinin–chlorophyll *a* protein, the torsional evolution on the  $S_2$  state is accompanied by the formation of ICT character and dynamic exciton localization, which controls the mechanism of excitation energy transfer to chlorophyll *a* acceptors.

The results presented in this thesis have raised several important questions that need further investigation. In our work on the peridinin–chlorophyll a protein, we found that the S<sub>2</sub> to Chl a

pathway accounts for a significant fraction of the quantum yield for energy transfer. This pathway, however, has never been observed directly due to the very short lifetime of the  $S_2$  state. The present work suggests that the lifetime of the  $S_2$  state of peridinin in methanol is only 12 fs, which is much shorter than the previously estimated value of 50 fs.<sup>1,2</sup> The near instantaneous rise of the bleaching signal from the  $Q_x$  state of Chl *a* observed upon excitation of peridinin chromophores led Zigmantas et al.<sup>2</sup> led us to propose that a coherent energy transfer process is responsible for the majority of the energy transfer yield. The ultrafast timescale of energy transfer from the  $S_2$  state (<30 fs) in our work also points to this mechanism.

In future work, broadband two-dimensional electronic spectroscopy could be employed to determine if coherent energy transfer is involved in PCP. An incoherent Förster type downhill energy transfer process manifests itself as a single cross peak in the two-dimensional electronic spectrum, whereas coherent energy transfer would be manifested as cross peaks above and below the diagonal.<sup>3,4</sup> These cross peaks would be expected to oscillate at frequencies corresponding to the strength of the electronic interaction between the donor and acceptor.<sup>5–7</sup>

Our finding that out-of-plane torsional motions promote nonradiative decay and formation of an intramolecular charge transfer character in carotenoids may have important implications in understanding the photoactivation and nonphotochemical quenching mechanism in the orange carotenoid protein (OCP) from cyanobacteria.<sup>8–13</sup> The OCP employs a carbonyl-substituted carotenoid, 3'-hydroxyechinenone<sup>14,15</sup> or canthaxanthin as a sensor for incident light levels and as a quencher of bilin excited states in the phycobilisome. The NPQ mechanism involving the OCP works under high light conditions by switching from a dark, orange state (OCP<sup>O</sup>) to an active, red state (OCP<sup>R</sup>); binding of OCP<sup>R</sup> to the phycobilisome results in a reduction of the rate of excitation energy transfer to the PSII reaction center.<sup>16</sup> How the carotenoid in OCP<sup>O</sup> senses ambient light levels with its strong absorption feature to the  $S_2$  state and then decays nonradiatively to trigger the photoactivation response is not yet understood. It has been proposed that an ICT state serves as a low lying quenching electronic state that is crucial to the photoprotection function.<sup>17,18</sup> It is reasonable to suggest, however, that OCP<sup>O</sup> undergoes a nonradiative decay pathway after optical excitation starting from a twisted S2 structure having some of the properties suggested for the  $S_x$  or X states in the potential-energy scheme as discussed in the previous chapters. The key event would be the relaxation pathway from the  $S_1$ state to the ground state, S<sub>0</sub>, and the preparation of a conformationally displaced protein structure. Whether or not a photoactivation reaction trajectory is launched or not would be determined by which side of the twisted ( $\phi = \pi/2$ ) S<sub>0</sub> maximum that the system lands upon nonradiative decay. Most of the time, the system would be expected to relax by returning to the originally photoselected ground state. A minor fraction of events, however, would involve relaxation to an alternate ground-state conformation, which would begin the process of photoactivation. These ideas could also be explored using transient grating spectroscopy or twodimensional electronic spectroscopy.

To conclude, the work presented in this dissertation has definitively established the role of conformational dynamics and intermediate electronic states in the photosynthetic light harvesting function of carotenoids. The new picture of carotenoid photophysics that has emerged from this work is likely to be useful in understanding the photoprotection mechanism of carotenoids. The new insights about light harvesting obtained from this work will provide the necessary guiding principles for designing an efficient chromophore for mid-visible solar harvesting.

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