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Heat-induced Gelation of Myosin with Native or Heat-Denatured B-Lactoglobulin

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Manee Vittayanont

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Denise M. Smith
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HEAT-INDUCED GELATION OF MYOSIN WITH NATIVE OR HEAT-DENATURED β-LACTOGLOBULIN

By

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ABSTRACT

HEAT-INDUCED GELATION OF MYOSIN WITH NATIVE OR HEAT-DENATURED β-LACTOGLOBULIN

By

Manee Vittayanont

Whey protein is widely used to enhance the textural qualities of processed meat products, but its effectiveness is often variable as the major protein, β-lactoglobulin (β-LG), does not gel at typical processing temperatures (68-71°C). Preheated whey protein aggregates form gels at lower temperatures than the native protein. The functions of native or preheated whey protein in meat systems have not been clearly explained due to the multi-component nature of the proteins. The objective of this research was to study gelation mechanisms of myosin with native or preheated β-lactoglobulin (β-LG) in a model system.

There was no interaction between myosin and β-LG during heating as indicated by the unique denaturation peaks of each protein in the thermogram of the mixed protein solution. Use of β-LG in the mixed system increased initial aggregation temperatures and decreased maximum aggregation rates when compared to myosin. Storage modulus or stiffness of myosin/β-LG decreased during heating between 55-75°C, then increased above 80°C and after cooling. β-LG interrupted gel network formation of myosin at 55-75°C, but enhanced stiffness of the co-gels when heated above its gelling temperatures of 80°C.

Preheating (80°C for 30 min) caused irreversible denaturation and formation of soluble β-LG aggregates (HDLG). The minimum concentration of HDLG required for gel formation in 0.6M NaCl, 0.05M sodium phosphate buffer at pH 7.0, was 2%, whereas β-LG required 4% protein for gel formation. The HDLG gelled at a lower temperature and had higher stiffness than native β-LG. Decreasing the pH from 7.0 to 6.0 decreased the gel point of HDLG. The HDLG gel networks were comprised of strands and clumps of small globular aggregates in contrast to β-LG gels which contained coarse, particulate networks of compacted globules with large voids.

When heated to 71°C, mixed solutions of 1% myosin/1% HDLG had the same gel point (48°C) and a higher storage modulus after cooling when compared to 2% myosin. Myosin/β-LG gelled at 49°C and the G' was much lower than myosin and myosin/HDLG gels. Gels of 2% myosin and myosin/HDLG had a similar fine stranded networks, whereas myosin/β-LG networks were thicker with larger void spaces. HDLG was incorporated within the myosin networks when heated to 71°C, whereas β-LG remained soluble.

This work revealed that β-LG interrupted network formation of myosin during heat-induced gelation. Preheating at 80°C for 30 min modified the gelling properties of β-LG, allowing strong co-gel formation with myosin at lower temperatures than that needed for unheated β-LG. This work provides direction for the design or modification of whey protein with optimum properties needed by meat processors and may contribute to enhanced applications of whey products where protein gelation at low temperature is required.

Dedication

To papa, mama, and other sevens

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CHAPTER 1: INTRODUCTION

Heat induced gelation of salt soluble proteins (SSP) in meat is an important functional property responsible for texture, water holding, binding and appearance of meat products (Smith, 1994). Many studies have been performed to understand muscle protein gelation mechanisms, so that textural qualities and yields of meat products can be modified and controlled. Non-meat proteins can be used to substitute for muscle proteins by maintaining desired texture of meat products and controlling costs.

Whey proteins possess high nutrient and functional properties and can form emulsions, foams and heat-induced gels. Whey proteins are widely used by the poultry and meat industries as non-meat binders to enhance yield and textural quality of their products. However, results from the use of whey protein in meat products are highly variable due, in part, to differences in source and processing history of whey proteins and to differences in processing conditions of the meat products in which the whey proteins are added. Functions of whey proteins in meat systems need to be defined and characterized to provide direction for designing or modifying whey protein with optimum properties needed by the meat industry.

Several researchers have attempted to elucidate the heat induced gelling mechanisms of whey proteins. The studies looked at factors affecting gel properties such as heating temperature, heating rate, pH, ionic strength, ion type, protein concentration, other food components i.e. salt and sugar, and solubility of whey protein (Kuhn and

Foegeding, 1991; Beuschel et al., 1992; Xiong et al., 1993; Smith and Rose, 1995; Boye et al., 1997). Some studies have investigated the interaction and influence of individual whey proteins i.e. β-lactoglobulin (β-LG), α- lactalbumin (α-LA) and bovine serum albumin, on gel properties (Hines and Foegeding, 1993; Matsudomi et al., 1993, 1994).

Other studies have looked at mixed gel systems of salt soluble proteins and whey protein concentrates (WPC). The solubility of WPC affects meat protein gelation. The hardness and deformability of a co-gel of 4% SSP and 12% WPC heated at 65°C were enhanced using WPC of low solubility, whereas mixtures of highly soluble WPC increased the hardness and deformability of gels heated to higher temperatures (Beuschel et al., 1992). Hung and Smith (1993) reported that WPC shifted the transition temperatures of storage and loss modulus (G′ and G") of SSP to a higher temperature and increased G′ and G" magnitudes of gels heated to 90°C. In isothermal experiments at 65°C, the elasticity of a combination gel of SSP and low solubility WPC was higher than those contain highly soluble WPC. The microstructure of gels prepared from highly soluble WPC and SSP was fibrous at 65°C and globular at 90°C. The proportion of each protein and the concentration of NaCl were found to influence the gelling properties of SSP and WPC co-gels (Smith and Rose, 1995).

In the past few years, cold-set gelation of heat-denatured soluble whey protein aggregates have been intensively investigated (Barbut and Foegeding, 1993; McClements and Keogh, 1995; Hongsprabhus and Barbut, 1997a; 1997b; Ju and Kilara, 1998b; 1998e). Heat-denatured whey protein aggregates form gels at low temperature upon addition of salts, acid or proteases (Ju and Kilara, 1998b). Gelling properties of

heat-denatured whey protein aggregates are greatly influenced by heat treatment and environmental conditions both during preheat treatment and gelation. Cold-set gels were reported to be stronger and less opaque than heat-set gels (Bryant and McClements, 1998).

Preheated whey protein could be a very useful ingredient in many food products, especially in low heat processed foods. Recently, the use of heat-denatured whey proteins in poultry meat batters has shown promising results (Hongsprabhus and Barbut, 1999). Soluble aggregates of heated-denatured whey protein were produced by heating a solution of 10% WPC in distilled water, pH 7.0, at 80 °C for 30 min. Two percent substitution of heat-denatured whey protein in combination with cold-set gelation (16h at 1.0°C) was found to improved the texture parameters of raw and cooked poultry meat batters, especially at ≤ 1.5% salt.

Interactions involved in formation of mixed gels from heat-denatured whey proteins and SSP remain unclear because of the multi-component nature of the protein preparations. Studies using pure proteins are needed to simplify the models and allow for a greater understanding of the mechanisms of co-gel formation. Myosin and β-LG are the major functional components of meat and whey, respectively. Use of these proteins in model systems is expected to ease the interpretation of the results. The objective of this project was to investigate the effects of native β-LG and heat-denatured β-LG on the heat-induced gelation of myosin in mixed systems.

CHAPTER 2: OBJECTIVES

The overall goal of this project was to investigate the mechanisms involved in the formation of myosin and β-lactoglobulin (β-LG) co-gels. The effects of β-LG and heat-denatured β-LG aggregates (HDLG) on the gelation of myosin were also examined. Our specific objectives were to:

1. Study interactions between β-LG and myosin during heating by comparing the denaturation profiles (endotherms), aggregation patterns, rheological properties, and microstructures of mixed protein systems to those of each pure protein;
2. Compare the gelling properties of HDLG to gels formed from native β-LG; and
3. Compare the rheological and microstructural properties of co-gels formed from myosin/HDLG to those of myosin/β-LG gels.

In Chapter 5, studies of thermally induced gelation of myosin, β-LG and the protein mixtures were studied. The interactions between myosin and β-LG in the mixed systems were interpreted by comparing the denaturation, aggregation and rheological properties of the mixed proteins to those of each pure protein. Results from this experiment led to the study of heat-denatured β-LG presented in Chapter 6. Thermal denaturation, rheological properties and microstructures of native and preheated β-LG were compared. The gelation mechanisms of mixed systems of myosin/native β-LG and myosin/HDLG were investigated in Chapter 7. Comparison of their thermal denaturation, rheological and

microstructural properties led to an understanding of the influences of HDLG on myosin gelation. An understanding of the functional properties of HDLG, mechanisms of heat-induced gelation of mixed protein systems of myosin with β-LG and HDLG were integrated to develop suggestions for modifying whey protein to achieve the optimum functional properties needed by the meat industries.

CHAPTER 3: SIGNIFICANCE AND JUSTIFICATION

Heat induced gelation is the most significant functional property of muscle proteins and is responsible for the textural quality of many meat products. Protein gels are characterized as a three-dimensional network of partially unfolded protein, which retains liquid within the network to form a viscoelastic solid. Whey proteins may interact with muscle proteins through a variety of mechanisms to enhance water holding capacity, reduce cook loss and improve textural qualities of processed meat products. Several mechanisms may be responsible for these functions:

1) Whey proteins dissolved in the entrapped liquid phase of the muscle protein gel may result in increased water retention and viscosity. This more viscous phase enhances strength of the protein network.
2) Whey protein has the ability to form heat induced gels, thus it might form a second gel network within the muscle protein gel structure (phase separated network) or form an interpenetrating network. These networks will strengthen the final gel matrix.
3) Whey proteins influence the formation of the three dimensional muscle protein networks leading to an alteration in the myosin matrix.

The effects of whey proteins on textural quality and yield of meat products have been extensively studied. The results were highly variable due to differences in source and processing history of the whey, which affects protein composition and gelling

properties of the proteins. The processing conditions and formulations (salt and pH) of meat products in which the whey proteins were added also greatly affected the gelation of the proteins. Muscle proteins gel at about 55-70°C which are typical processing temperatures for many meat products. Meanwhile whey proteins need to be heated over 80°C to form a gel. Therefore, gel enhancing effects of whey protein are limited when used in meat products. The mechanism of interaction between whey proteins and muscle proteins has never been completely understood.

Heating whey protein in a dilute salt solution at neutral pH denatures whey proteins and produces soluble aggregates. These soluble whey protein aggregates can form cold-set gels at ambient or refrigeration temperatures. Preheated or heat-denatured whey proteins were proposed for use in food products processed at temperatures lower than the gelling point of native whey. Some examples of these products are comminuted meat and fish products, deserts, sauces and dips (Bryant and McClements, 1998).

In this project, we focused on the ability of β-lactoglobulin (β-LG) and heat-denatured β-LG to form a gel network and/or influence the gelation of myosin. The stability of a gel depends on the strength of the protein matrix, which is determined by the size, shape, concentration and cross-linking of the protein polymers. Hence, it was very interesting to investigate network formation in a mixed gel system to understand how whey protein influences the gelation of meat proteins. This information on gelling behavior of whey and meat protein could provide significant new basic knowledge for further improving the functional properties of whey proteins for expanded use in meat products.

Both muscle proteins and whey proteins are multi-component systems. The large number of proteins in each system may cause confusion when interpreting results. To minimize these problems, we used myosin and β-LG in a model system. Myosin and β-LG form strong heat-induced gels and are the major protein components of meat and whey, respectively.

CHAPTER 4: LITERATURE REVIEW

4.1. Heat induced gel formation of protein

4.1.1. Definition and types of protein gels. Protein gels have been described as three dimensional matrices or networks in which polymer-polymer and polymer-solvent interactions occur in an ordered manner resulting in the immobilization of large amount of water by a small proportion of protein (Flory, 1974; Hermaanson, 1979; Mulvihill and Kinsella, 1987). Heat induced gel formation is one of the most important functional properties of many proteins such as egg white proteins, plant proteins, milk proteins and meat proteins. Functional properties contribute to desired textural characteristics and quality of various kinds of food products containing these proteins.

Protein gel structure may be categorized into 2 main types: fine stranded networks and particulate aggregates (Figure 4.1). A fine stranded gel is usually formed by long chain structure or fibrillar protein, such as muscle protein and gelatin, whereas most globular proteins, such as whey and egg white proteins, can form both gel types depending on gelling condition. The protein network of the fine strand gels is formed by cross-linking at points where the interaction occurs between specific locations of molecules (Figure 4.1a), or connection at junction zones which occurs via association

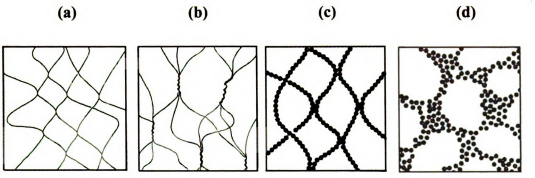


Figure 4.1. Network structure of protein gels (a) fine stranded network formed by connection at points; (b) fine stranded network formed by junction zone connection; (c) fine stranded string of beads; (d) particulate randomly aggregated gel network.

between segments of molecules (Figure 4.1b) (Matsumura and Mori, 1996). Tombs (1974) described fine strand gel formation of globular proteins as a highly oriented string of beads (Figure 4.1c), whereas random aggregation is responsible for the particulate structure (Figure 4.1d). The pore size of a protein network was suggested as a way to distinguish the type of gel. Fine strand gels have pore sizes in the order of nanometers, whereas pore sizes of particulate gels are in the micrometer range (Langton and Hermansson, 1992).

4.1.2. Mechanism of heat-induced gel formation. Protein gel formation has been suggested to be comprised of two main steps. The first step involves thermal unfolding or denaturation of protein molecules. The second step is the aggregation process of the unfolded proteins resulting in a development of a cross-linked gel network with viscoelastic properties (Ferry, 1948). Foegeding and Hamann (1992) proposed a theoretical model, listing a series of steps within the aggregation process leading to final gel structure (Figure 4.2). In this model, proteins unfold and aggregate to form a progressively more viscous solution. Once the gel point is reached, a continuous viscoelastic gel network is formed. The viscoelastic properties of the gels may change with time to form an equilibrium matrix (Smith, 1994).

The gelling process and final gel characteristics or properties depend on both intrinsic and extrinsic factors that significantly influence each step throughout the heat induced gelation of the protein. The gelling ability of a protein is influenced by its concentration, amino acid composition, size, and shape. Meanwhile, the development of the protein network, governed by the denaturation and aggregation properties, is dictated

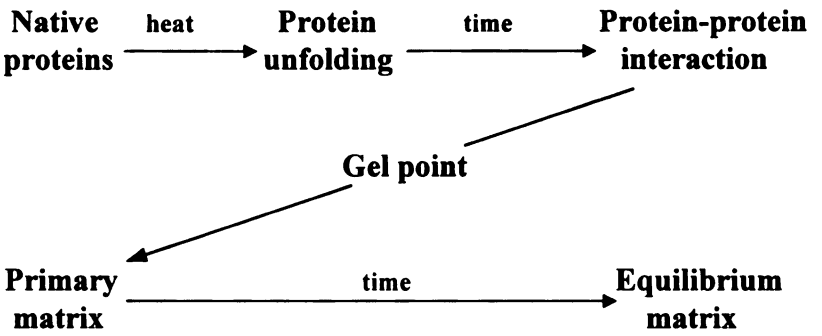


Figure 4.2. Schematic model of heat-induced gelation of protein (from Foegeding and Hamann, 1992)

by the processing conditions, including length and rate of heating and cooling, and environmental factors, such as ionic strength, pH and buffer composition (Mulvihill and Kinsella, 1987). The textural properties of gels are largely influenced by the interactions between proteins which ultimately cross-link to develop gel network structures.

4.1.3. Protein-protein interaction in gels. A gel matrix is formed via the assembly of individual molecules into extensive three-dimensional networks. This assembly requires a balance of attraction and repulsion between protein molecules. Normally, denaturation or partial unfolding and, in some cases, dissociation of the native protein subunits, is a prerequisite. The molecular interactions between proteins and their general characteristics are summarized in Table 4.1.

Heat treatment causes proteins to unfold and expose some hydrophobic residues which become available for intermolecular bonding and network formation (Oakenfull et al., 1997). Shimada and Matsushita (1980) showed a correlation between hydrophobic amino acid content of proteins and a subsequent gel formation. They found that proteins containing more than 31.5 mole percent of non-polar amino acid residues formed coagulated type gels, whereas those containing less than this level of non-polar residues formed transparent gels. However, the hydrophobic amino acid content of a protein is not the only factor affecting its gelation. The amino acid sequence and local conformation also influence protein interactions. Physical/chemical treatments and buffer conditions can alter the surface hydrophobic areas of proteins and therefore influence intermolecular hydrophobic interactions.

Table 4.1. General characteristics of molecular interactions between proteins in aqueous solution (adapted from Bryant and McClements, 1998)

Type	Force direction	Relative strength	Effect of pH	Effect of ionic strength	Effect of temperature
Hydrophobic	attractive	Strong	no	no	Increase
Electrostatic	repulsive	weak→strong[a]	yes	yes	Increase
Hydrogen bonding	attractive	Weak	no	no	Decrease
Van der Waals	attractive	Weak	no	no	-
Steric repulsion	repulsive	Strong	no	no	-
Disulfide bond	attractive	Weak	no	no	-

[a] Depends on pH and ionic strength

It has been suggested that heat treatment results in cleavage of existing disulfide bonds or activation of buried sulfhydryl groups through the unfolding of the protein. These sulfhydryl groups then can form new intermolecular disulfide linkages which are essential for heat induced gel formation in some globular proteins (Boye et al., 1997). The role of disulfide/sulfhydryl interchange reactions in the cross-linking of protein gels is illustrated in Figure 4.3.

Electrostatic interactions depend on the exposure of charged groups on the protein. The surface charge of protein molecules is affected by both the presences of dissolved salts and solvent pH. Electrostatic repulsion contributes to the dispersion of protein molecules in solution. The charge repulsion of proteins is enhanced as the solution pH is adjusted away from the isoelectric point. However, the addition of salts can alter the net charge of the protein by shielding the excess charges and leading to inhibition of the exposure of hydrophobic residues buried within the proteins. Divalent cations may form cross-bridges between adjacent protein molecules contributing to coagulation and/or gelation.

4.1.4. Methods to study heat-induced gel formation. The gelling mechanisms of proteins have been investigated by various biochemical and physicochemical techniques. The choice of appropriate methods is important for obtaining desired results. The sensitivity of instruments is a determining factor when selecting experimental conditions.

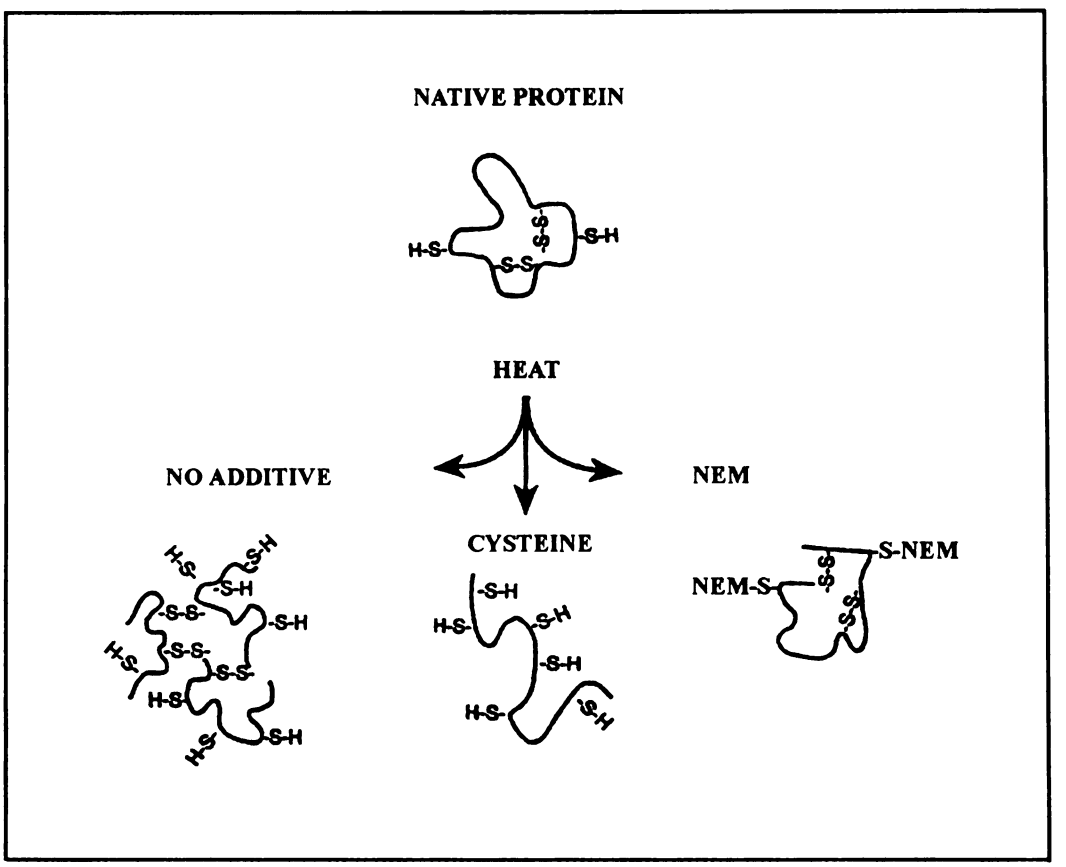


Figure 4.3. Role of sulfhydryl/disulfide interchange reactions during protein gelation in the presence and absence of N-ethylmaleimide (NEM) (from Boye et al., 1997).

4.1.4.1. Thermal denaturation. Differential scanning calorimetry (DSC) is most commonly used to follow protein unfolding during heating. With this technique, thermodynamic parameters related to thermally induced conformational changes of proteins are measured during various rates of heating (Krishnan and Brandts, 1978). Changes in heat capacity of a protein solution with temperature are recorded as a thermogram. The temperature at which 50% of the molecule has been unfolded is called the melting temperature for a single domain protein.

Spectroscopic techniques, such as Fourier transform infrared spectroscopy, Raman spectroscopy, nuclear magnetic resonance and circular dichroism also have been reported as tools for studying changes in protein secondary structure (Nonaka et al., 1993; Li et al., 1994; Matsuura and Manning, 1994). Monoclonal antibodies have been used as precise probes for specific conformational changes during unfolding of protein (Wang and Smith, 1994c).

4.1.4.2. Thermal aggregation. Protein aggregation has been followed using turbidity measurements, light scattering or other forms of spectroscopy. Electrophoresis and chromatography are useful tools for investigating bond formation of proteins during thermal aggregation. The most convenient way to follow thermal aggregation is by measuring turbidity. With this technique, the differential change in optical density as a function of temperature is used to determine the initial temperature of aggregation (thermal transition: T_o) and transition peak temperature (T_m) of protein-protein association (temperature at which protein-protein interaction was maximum) (Xiong et al., 1993).

In dynamic light scattering, a laser beam is used to detect molecular size of particles. The Brownian motion of particles results in a time-dependent fluctuation in the scattering intensity. The auto-correlation function of fluctuating intensity is derived into the diffusion coefficient of the scattering particles, providing information of the hydrodynamic radius of particles. Hoffmann et al. (1996) studied the effects of temperatures, salt type and concentration, and initial protein concentration on heat-induced aggregation of β-lactoglobulin (β-LG) using *in situ* dynamic light scattering (a measurement of scattering light from a sample while it is heated in the instrument). The results showed that aggregate size and aggregation rate of β-LG increased when the protein concentration was increased. Increasing the heating temperature from 61.5 to 70.0°C resulted in an increase in initial aggregation rate of β-LG. The aggregate size increased when the concentrations of salts were increased from 0 to 10 mM. The aggregate size (30 nm) of β-LG was similar when heated at 68.5°C for ≥1h in either 0.5 mM $CaCl_2$ or 10 mM NaCl.

4.1.4.3. Viscoelastic network formation. The formation of a gel network, as defined by the phase transition of a protein sol to gel upon heating, can be observed by small deformation dynamic testing. This technique involves continuously recording the changes in viscoelastic properties of protein solutions at small deformation, thus avoiding interruption of network formation during measurement. The complex shear modulus (G*) is resolved into two components. The storage modulus (G′) is a measurement of the elastic properties, and loss modulus (G") is a measurement of viscous properties of the

material. The transition from sol to gel can be monitored by the change in these two parameters. The phase transition temperature or gel point is usually defined as the temperature at which the G' and G" crosses over (Stading and Hermansson, 1990). The ratio of G"/G' or tan delta (tan δ)) is another parameter used to describe viscoelastic behavior. Polymer systems show high tan δ for dilute solutions, 0.2-0.3 for amorphous polymers and low (near 0.01) for glassy crystalline polymers and gels (steffe, 1996).

4.1.4.4. Final gel structure. The microstructure of the final gel matrix is often studied by light microscopy, transmission electron microscopy, and scanning electron microscopy. The transmission electron microscopy is relevant for description of a dense fine-stranded structure, whereas scanning electron microscopy or light microscopy is better for observing a coarse particulate structure (Hermansson et al., 1993). However, SEM observation is preferable to TEM because of the easier specimen preparation and more realistic imaging (three-dimensional imaging) due to the higher depth of field.

Concomitantly with electron microscopic observation, final gel strength and character are usually evaluated by large deformation or destructive studies. In large deformation testing, apparent strain and stress at failure by compression and stress and strain at failure by torsion analysis are commonly used in model gel systems. Stress at failure can indicate the firmness or hardness of the gel and strain at failure is an indication of gel deformability (Hamann et al., 1987).

Type of gel can be characterized by frequency dependence of the complex shear modulus (G*) (Clark and Ross-Murphy, 1987). The slope of log-log plots between G' or

G* and frequency usually indicate strength of gels. The slope value of a strong gel is less than that of a weak gel. The G* of a strong cross-linked gel is frequency independent, whereas G* is slightly dependent on frequency for a physically entanglement gel network. Stading et al. (1992) discriminated homogeneous and heterogeneous β-LG gel structures, formed at different pHs, based on the frequency dependence of G'. An in-homogeneous network structure containing regions of different density showed a two-slope frequency dependence (broken) curve, one at low frequency and another at high frequency. The homogeneous structure was frequency independent and exhibited a straight line over the frequency sweep range.

4.2. Myosin

There are three fractions of proteins in skeletal muscle: myofibrillar, sarcoplasmic and stromal. Myosin and actin, defined as contractile proteins, account for about 50% and 20%, respectively, of the myofibrillar fraction (Obinata et al., 1991; Yates and Greaser, 1983). Myosin is the most abundant myofibrillar protein in skeletal muscle. It is also mainly responsible for functional properties of processed meats (Smith, 1988).

4.2.1. Molecular characteristics. Myosin is a highly asymmetric protein molecule with dimensions of about 150 nm in length and 1.5-2.0 nm diameter in the rod portion and 8 nm diameter in the globular head (Peason and Young, 1989). Myosin is composed of two heavy chains (MHC) and two pairs of light chains. Each MHC of chicken pectoralis myosin is constructed from 1,938 amino acids with a molecular weight

of 222.971 KDa (Hayashida et al., 1991). The amino-terminal end of MHC forms the globular or head region, whereas the remainder of the MHC forms the rod or tail region. In nature, the rod regions of the two heavy chains form a dimeric, extended coiled-coiled helical structure.

Each pair of light chains (LC) of myosin is non-covalently associated with the head of the molecule (Smith et al., 1983). Myosin LC can be divided into 2 types based on releasing reagent: alkaline LC and 5,5'-dithiobis-(2-nitrobenzoate) or DTNB LC. The alkaline LC is released from myosin by treatment in an alkaline solution, pH 11. These LC are non-phosphorylated and are essential for ATPase activity (Pearson and Young, 1989). They are designated as alkaline LC1 (A1) or LC-1 and alkaline LC2 (A2) or LC-3. Chicken skeletal muscle myosin LC-1 and LC-3 contain 192 and 150 residues with molecular mass of 20.913 and 16.710 KDa, respectively (Maita et al., 1981; Umegane,et al., 1982). DTNB LC or LC-2 are dissociated from the myosin molecule by the sulfhydryl reagent 5,5'-dithiobis-(2-nitrobenzoate) and are not essential for ATPase activity, but affect the calcium binding ability of myosin. The LC-2 of chicken skeletal muscle myosin contains 166 amino acid residues with a molecular mass of 18.673 KDa (Suzuyama et al., 1980).

Myosin can also be divided into sub-fragments by enzymatic digestion. With limited digestion by trypsin, myosin is cleaved into two subfragments: heavy meromyosin (HMM) and light meromyosin (LMM) (Lowey et al., 1969). In the same way, papain hydrolyzes myosin into subfragment-1, which comprises the two globular heads of myosin, and a rod region, which contains the α-helix (tail) of the molecule.

When digested by papain, HMM is cleaved into two subfragments: subfragment-1 and subfragment-2, which is the rod region of HMM (Figure 4.4) (Smith et al., 1983). The isoelectric point of myosin is about 5.3 reflecting its high content of aspartic and glutamic acid residues (Harrington, 1979).

4.2.2. Heat induced gel formation of myosin. Myosin has a multi-domain structure resulting in a very complex denaturation and aggregation process. The mechanisms of myosin gelation during heating have been extensively studied. During the heating process, myosin gel network is first formed between 30-44°C by the rod region. The second step of gel formation is attributed to the head portion of myosin between 51-80°C (Zayas, 1997). Sano et al. (1990) reported that the aggregation and sol-gel transition of LMM (extracted from the dorsal lateral muscle of live carp) in 0.6M KCl, pH 6.0 occurred at 32-50°C, whereas HMM aggregated above 45°C. The role of six myosin sub-fragments (LC, S-1, HMM, LMM, rod, and MHC) in gel network formation of chicken breast muscle myosin in 0.6M NaCl, 0.05M phosphate buffer pH 6.5 were studied by Smyth et al. (1996). Thermal stability and thermal aggregation profiles showed that the rod was the main fragment involved in myosin gelation. LMM and S-1 were responsible for denaturation and aggregation below 55°C, while S-2 unfolded and aggregated above 55°C. The alkaline LC was less heat stable than the DTNB LC and neither aggregated during heating, suggesting that LC themselves do not gel. However, the thermal stability and aggregation of myosin were found to be influenced by LC when bound to MHC.

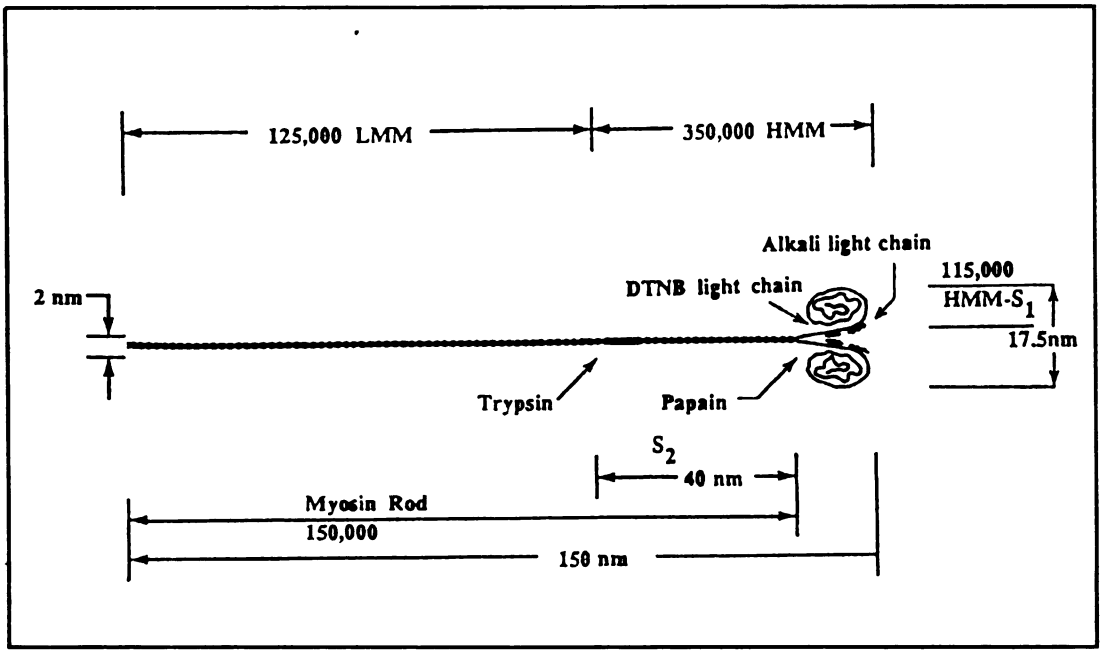


Figure 4.4. Schematic representation of the myosin molecule: HMM, heavy meromyosin; LMM, light meromyosin; HMM-S1, subfragment-1; S2, subfragment- 2; DTNB, 5,5-dithiobis-(2-nitrobenzoate) light chain (Adapted from Smith et al., 1983).

4.2.3. Factors influencing myosin gelation. The gelling mechanism of myosin is also influenced by other factors, including protein concentration, environmental conditions, and heat treatment. These factors are known to have significant effects on both gel formation and the final gel characteristics of myosin.

4.2.3.1. Protein concentration. As a gel network is formed by the interaction of denatured proteins, the amount of protein needs to be large enough to form intermolecular bonds. At low concentrations, proteins tend to form intramolecular bonds resulting in protein precipitation instead of a gel network. The critical or minimum concentration to form a gel depends on the molecular size and structures. Wang and Damodaran (1990) demonstrated the importance of polypeptide chain length on network formation of protein gels. They suggested that increasing the chain length of the protein increases molecular entanglement and reduces the diffusion coefficient of the gel structures, therefore increasing the total amount of stable bonds formed between the peptides per unit cell of the gel. Myosin is a fibrillar protein having a long tail (~130 nm) that facilitates contact and intermolecular binding, thus promoting interactions. Therefore, the critical concentration of myosin to form a self supporting gel structure is quite low when compared to globular proteins, such as β-LG, which has a diameter of 2-3 nm. Acton et al. (1983) reported that actomyosin required at least 0.6% protein to form a self supporting gel network in 0.6M KCl, pH 6.0, whereas Paulsson et al. (1986) suggested that the minimum concentration needed for β-LG gel formation is about 2% at pH 6.6. Above the critical concentration, hardness of myosin gels increased with increasing

protein concentration as a result of an increase in the number of cross-links within the gel matrix (Zayas, 1997).

4.2.3.2. Environmental conditions. The pH and ionic strength of the protein environment have been reported to be the most important factors that influence myosin gelation. Charge distribution among amino acid side chains of the myosin molecule is altered by pH and ionic strength, and hence the attractive and repulsive forces between molecules are changed. This event subsequently affects the type and stability of the final gel. Hermansson et al. (1986) reported that bovine *semimembranosus* myosin formed two types of gels depending on the ionic strength of the solution. At low ionic strength (0.1-0.2 M KCl and pH 6.0), myosin formed a thicker filament and finer gel matrix than those formed at higher ionic strength (0.6M KCl and pH 6.0). Boye et al. (1997) studied thermal gelation of myosin from fast and slow switch rabbit muscles and found that the direct suspension of protein at a low ionic strength (0.2 M KCl, pH 6.0) led to very weak gels, whereas a gradual lowering of ionic strength, from 0.6 M KCl to 0.2 M KCl, by a dialysis technique, produced strong heat induced gels. Maximum gel rigidity of myosin from chicken breast was found at pH 5.4 to 5.9 in 0.6 M KCl (Asghar et al., 1984; Morita et al., 1987).

4.2.3.3. Heat treatment. Time, temperature and the rate of heating during preparation influence the gelation of myosin. The maximum rigidity of myosin gels occurs when heated to 65°C in 0.2 and 0.6 M KCl at pH 5.5 and 6.0, respectively (Zayas, 1997). In this temperature range, incubation time is an important factor determining

completion of network formation. However, protein concentration will affect the time period for gel formation. Zayas (1997) suggested that above a critical concentration, the time necessary to form a gel decreases as protein concentration is increased. Wang and Smith (1994a) studied the dynamic rheological properties of chicken breast myosin during isothermal heating at 55, 65 and 75°C in 0.6M NaCl at pH 6.5. They found that myosin heated at 75°C had higher viscous-like properties as indicated by a higher G", while myosin heated at 55°C and 65°C formed elastic-like gels. During heating, a three-dimensional protein network is formed by aggregation of partially unfolded proteins. Since myosin is a multi-domain molecule, denaturation and aggregation during gel formation is very complex.

Network formation of strong gels occurs when the proteins are heated at appropriate heating rates that allow the orientation of unfolded molecules and facilitate the aggregation process. Foegeding et al. (1986) studied the effect of heating rates on thermal gelation of myosin and found that myosin gels prepared at a heating rate of 12 °C/h were harder than those prepared at a heating rate of 50 °C/h. Camou et al. (1989) reported that after cooling to 1°C, a higher force was needed to compress salt soluble protein gels heated at a rate of 17 °C/h when compared to those prepared by heating at 38 or 85°C/h.

4.3. β-Lactoglobulin

The proteins found in whey include β-LG, α-lactalbumin (α-LA), bovine serum albumin, immunoglobulins and a proteose-peptone fraction. Among these components,

β-LG is the predominant protein, constituting up to 50% of total whey proteins, and plays the major role in forming heat induced gels (Swaisgood, 1982; Mulvihill and Kinsella, 1987). There are seven known genetic variants of bovine (*Bos taurus*) β-LG. The most common in milk are variants A and B (Eigel et al., 1984).

4.3.1. Molecular characteristics. β- LG is a globular protein with a radius of about 2-3 nm. It has a single polypeptide chain of 162 residues. The A variant has a monomeric molecular mass of 18.363 KDa (Oakenfull et al., 1997). The isoelectric point of β-LG is around 5.2. Under physiological conditions, β-LG exists as a dimer consisting of 10-15% α-helix, ~50% β-sheets, 20% turns and the remaining 15% in a random arrangement (Timasheff et al., 1966; Casal et al., 1988). Below pH 3, β-LG has been reported to exist as a monomer. It reversibly aggregates to form octamers between pH 3.7-6.5. Above pH 9.0, the protein undergoes time dependent, irreversible denaturation and aggregation (Boye et al., 1996). Native monomers contain two disulfide bridges between C66/C160 and C106/C119, and one free sulfhydryl group (SH-group) at C121 (Creamer et al., 1983; Papiz et al., 1986). The disulfide bonds and free SH-group play an important role in heat-induced gel formation of β-LG.

4.3.2. Heat induced gelation. The heat-induced gelation of β-LG has been extensively investigated in many laboratories. The mechanism of gelation during heating involves two main steps; 1) thermal dissociation of dimers into monomers and unfolding of monomers, and 2) the aggregation of unfolded proteins resulting in network formation.

Denaturation of β-LG during heating has been studied under various conditions and by using different methods, such as DSC, Fourier transform infrared spectroscopy, nuclear magnetic resonance, circular dichroism and other spectroscopic techniques (Li et al., 1994; Matsuura and manning, 1994; Qi et al., 1995; Iametti et al., 1996). When heated above 25°C, native β-LG dissociates into monomers which are further unfolded as temperature is increased. This step is irreversible at temperatures higher than 65°C (Iametti et al., 1996).

Sawyer (1968) reported a two step aggregation process for β-LG at neutral pH. It was suggested that small aggregates were first formed by the unfolded proteins which further associated into bigger aggregates by physical interaction. More details on the aggregation of β-LG were found using light scattering techniques, size exclusion chromatography and electrophoresis (Griffin et al., 1993; Roefs and de Kruif, 1994; Aymard et al., 1996; Hoffmann et al., 1996 Prabakaran and Damodaran, 1997). During heating, the two step aggregation process began with the formation of small aggregates of disulfide linked β-LG polymers from molten globules. Prabakaran and Damodaran (1997) suggested that sulfhydryl-disulfide interchange reactions between two reactive monomers initiated further propagation of β-LG polymerization at 60-65°C. In the second step, the small aggregates formed in the initial stage associated into larger aggregates by physical interactions. The rate of aggregation depended on heating temperature, pH and concentration of the monomeric form of β-LG. The size of aggregates depended on pH, salt type and concentration, and initial concentration of β-LG (Elofsson et al., 1996; Hoffmann et al., 1996). Roefs and De Kruif (1994) predicted

the size of β-LG aggregates to be a proportional to the square root of the initial protein concentration by using a kinetic model obtained in low salt (<1.0%) conditions. Aggregates formed in the early stage were suggested to be small rod-like particles (Griffin and Griffin, 1993). However Aymard et al. (1996) claimed that the shape of aggregates was not rod-like, but globular. They found that monomer concentration decreased by a 2 order kinetic model indicative of the association of monomers into dimers before formation of the globular aggregates. The globules finally aggregated to form fractal structures. The two-step aggregation mechanism of β-LG during heating is shown in Figure 4.5.

An important chemical reaction during the early stage of β-LG aggregation is the formation of intermolecular disulfide bonds. During thermal denaturation, the cleavage of disulfide bonds and exposure of reactive SH-groups on the surface of the unfolded proteins facilitate intermolecular disulfide bonds and sulfhydryl/disulfide interchange reactions (Shimada and Cheftel, 1988). A sulfhydryl-blocking agent such as N-methylmaleimide (NEM) has been used to demonstrate the effect of disulfide bonding on protein gelation. When NEM was added to a solution of β-LG, heat-induced gel hardness increased slightly after heating at concentrations up to 5 mM NEM, and then rapidly decreased when the concentration of NEM exceeded 10 mM (Phillips et al., 1994). Non-covalent interactions, such as electrostatic, hydrophobic and hydrogen bonding, also play a major role in the aggregation of β-LG. Aggregation of β-LG leads to gel network formation. The initial gelling temperature of β-LG is found over a broad range, depending on protein concentration, pH and salt conditions. Paulsson et al. (1986)

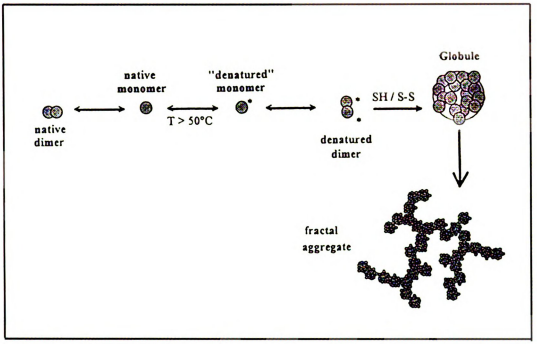


Figure 4.5. Schematic representation of the two step aggregation of β-lactoglobulin at pH 7.0 (from Aymard et al., 1996).

reported that 3-9% β-LG in 1% NaCl, pH 6.6 started to gel at 75-80°C.

4.3.3. Factors influencing β-lactoglobulin gelation. The appearance and properties of β-LG gels strongly depend on protein concentration, medium conditions, and heat treatments used in the preparation. The combination of these conditions influences the denaturation and aggregation mechanisms during gel formation, which further alter the final gel characteristics. Gels formed under different combinations of pH and salt concentration may vary from weak to strong, brittle to elastic in texture or opaque to transparent in appearance.

4.3.3.1. Protein concentration. Increasing the protein concentration increases the probability of intermolecular interactions, therefore promoting gel formation. The minimum concentration of β-LG needed to form a gel is highly pH dependent, with the minimum at the isoelectric point (Stading and Hermansson, 1990). The critical concentration of β-LG needed to form a gel at temperatures over 80°C was 2.0% in 1.0% NaCl, pH 6.6 (Paulsson et al., 1986), or 5.0% in 0.1M NaCl, 0.02M imidazole buffer, pH 7.0 (McSwiney et al., 1994) and pH 8.0 (Matsudomi et al., 1991). The initial gelling temperatures decrease and the gelling rates increase with an increase of rotein concentration. Above the critical concentration, the gels become more rigid and opaque as protein concentration was increased.

4.3.3.2. Protein modification. β-LG can be modified to alter the gel point and rheological properties of the protein. Chen et al. (1994) found that partially hydrolyzed β-LG gelled more rapidly at the same protein concentration at 80°C. The G' after cooling of the gels formed from partially hydrolyzed β-LG (7% protein in 20 mM $CaCl_2$, pH 7.0) was twice less than that of the native protein gel heated at 80°C for 3h. However, when heated at 60°C, the G' of hydrolyzed β-LG gel was 10 time higher than that of the intact β-LG. Rector et al. (1991) investigated the gelling properties of β-LG preheated with hot air at 80°C. Preheating decreased the concentration of monomeric β-LG and the hardness (force needed for 80% deformation) of the preheated protein gels (10% protein, 0.1M Tris-HCl buffer, pH 8.0).

4.3.3.3. Environmental conditions. β-LG forms a gel upon heating, and develops different network structures depending on pH, ionic strength, and type of salt. The change in pH alters hydrogen bonding and electrostatic interactions, while salts might neutralize charge and increase non-polar interactions of proteins to further induce local unfolding or aggregation of β-LG. Xiong et al. (1993) demonstrated the sensitivity of β-LG aggregation to pH and ionic environment by following turbidity changes during heating. The aggregation profiles of β-LG during heating were displayed by plotting turbidity changes over time (dA_{600}/dT) against temperature. The maximum aggregation rate was determined at the transition peak and temperature at the peak was defined as transition peak temperature. The results showed that an increase in pH from 5.5 to 6.5 decreased the aggregation rate of 0.12% β-LG in distilled water as indicated by a

decrease of the transition peaks. Addition of $CaCl_2$ (5-20 mM) decreased transition peak temperature, indicating a promotion of β-LG aggregation. The addition of 0.02-1.0M NaCl increased aggregation rate, but increased transition peak temperature of β-LG. Mulvihill and Kinsella (1988) reported that at neutral pH, salt was required for gel formation. Maximum gel hardness was obtained in 10 mM $CaCl_2$, pH 7.0 when β-LG was heated at 80°C. A lower gel point and more rapid gel formation was observed in 20 mM $CaCl_2$ as compared to 100 mM NaCl (Foegeding et al., 1992).

Ca^{2+} was more effective at a lower concentration than Na^+ at inducing β-LG aggregation. However, the exact role of these cations in the gelation of β-LG is not clear. Generally, cations modulate thermal gelation by decreasing electrostatic repulsion, forming salt bridges and facilitating intermolecular disulfide bond formation and hydrophobic interactions. Li et al. (1994) used nuclear magnetic resonance to look at the involvement of Na^+ and Ca^{2+} in the aggregation of β-LG between 10-85°C at pH 7.0. They found that Ca^{2+}caused conformational changes in β-LG above 40°C. The results suggested that divalent cations may cross-link anionic carboxyl groups during the unfolding of polypeptides and stabilize the unfolded form.

Jeyarajah et al. (1994) studied calcium binding to native and preheated β-LG using an ion selective electrode. Changes in hydrophobic regions and reactive sulfhydryl content of the proteins were also monitored by fluorescence spectroscopy using 8-anilinonaphthalene sulfonic acid and fluorescein mercuric acetate, respectively. Reactions of mercury with sulfhydryl groups of amino acids quench the fluorescence of murcurial derivatives of fluoresceins, therefore an increase in the content of reactive

sulfhydryl groups can be determined by measuring fluorescence quenching of the fluorescein mercuric acetate. Heat treatment (80°C for 15 min) slightly increased calcium binding to β-LG. Addition of $CaCl_2$ (1-15 mM) increased 8-anilinonaphthalene sulfonic acid fluorescence of β-LG. The fluorescence intensity of β-LG preheated at 80°C was higher than that of β-LG preheated at 60°C and the unheated form. This suggested that heat treatment unfolded the molecule promoting the exposure of hydrophobic regions while calcium binding magnified the structural changes of β-LG. A smaller increase in 8-anilinonaphthalene sulfonic acid fluorescence was observed upon the addition of NaCl compared to addition of $CaCl_2$, indicative of different effects of calcium and sodium on the structure of β-LG. The reactive sulfhydryl group of preheated β-LG was less than that of unheated β-LG, indicating that sulfhydryl/disulfide interchanges occurred during heating. The reactive sulfhydryl content of β-LG increased as $CaCl_2$ was increased from 0 to 10 mM. The authors suggested that addition of $CaCl_2$ might cause local unfolding of β-LG which exposed the reactive sulfhydryl groups buried at the sheet-helix interface. Addition of NaCl (25-50 mM) decreased the reactive sulfhydryl group content of preheated β-LG, suggesting that disulfide linkages may have been formed.

Foegeding et al. (1998) summarized the network characteristics of whey protein gels formed at different pHs (Table 4.2). Stranded protein networks, which have a diameter of one to several molecules, are formed at pHs above and below the pI. The gels formed at pH > pI are strong and elastic, whereas those formed at pH < pI are weak and brittle. Particulate gel networks of protein clusters with diameters of 100-1000 times molecular size are formed at pHs close to the pI (5.1). The network structures of β-LG

Table 4.2. Network types and physical properties of whey protein gels (from Foegeding et al., 1998)

Gel type	Fracture rheology			Appearance	Water holding
	Stress	Strain	Rigidity		
Stranded					
i) pH > pI	+++	+++	++	Translucent	+++
ii) pH < pI	+	+	++	Translucent	NA
Mixed	++++	++	++++	Cloudy/opaque	++
Particulate	+++	+++	++	Opaque	+

The number of "+" symbols represents the relative magnitude of the property among gel types, with the greater number indicating a greater magnitude.
" NA " indicates no relevant data.

gels formed at different pHs follow a similar trend as the whey protein gels. A fine stranded structure of β-LG was found at pH 4.0 and lower, whereas both fine stranded and particulate gel structures were formed at pH 6.0 or higher. At pH 4.0-6.0, white particulate gels were formed (Langton and Hermansson, 1992). Large deformation measurements indicated that β-LG gels formed at low pH were brittle with low stress and strain at fracture, whereas those formed at high pH were elastic with high stress and strain at fracture (Stading and Hermansson, 1991).

4.4. Mixed protein gel systems

Gelling mechanisms of polymers, such as proteins and polysaccharides, are usually studied in a single component system because it provides a simple ideal model for the investigation of the gelling process. However, most food gels contain a complex mixture of different gelling polymers, i.e. different proteins or proteins mixed with polysaccharides. The gel formation and final gel properties of these mixtures are very complex and are greatly influenced by the individual gelling characteristics of each polymer under the processing conditions employed. Researchers are confronted with difficult interpretations of results obtained from complex gel systems. To begin to understand the gelation of mixed polymers, experiments have been designed to study model composite gels of two biopolymers.

4.4.1. Models of two component mixed gel structures. Brownsey and Morris (1988) proposed two types of two component mixed gels. Type I is called a filled gel.

Only one of the two components forms the gel network. The second polymer remains soluble or exists as dispersed particles, and is entrapped inside the network as a gel filler (Figure 4.6). The filler component may influence gelation of the main network forming protein.

A type II gel is formed when both components independently form a gel network. The structure of the final gel may be divided into three forms (Figure 4.7):

1) Coupled network: two polymers interact to form a gel network, therefore at least some junction zones involve both polymers.
2) Interpenetrating network: a combination of two separate polymeric networks is formed. These networks are entangled but there is no common junction zone.
3) A phase-separate network: one polymer separately forms a network that is interspersed within another polymeric network.

The properties of two component heat-set gels are the net result of the complex molecular interactions within each polymer, between each polymer and the solvent, and between both polymers. Each polymer has a structure that determines its gelling behavior, thus the gelling mechanism of a mixed system mainly depends on the intrinsic character of each polymer and the heat treatment employed.

4.4.2. Interactions between meat protein and other proteins. A wide variety of protein additives from plant and animal origin are used to improve water retention and textural properties of comminuted meat products. Many studies have been conducted

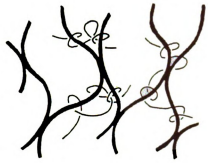


Figure 4.6. Type I filled gel network showing network formation by one protein with soluble polymer entrapped inside (from Brownsey and Morris, 1988).

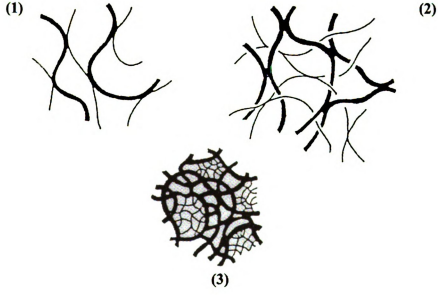


Figure 4.7. Schematic diagrams of possible structure of type II mixed gel (1) coupled network; (2) phase separated network; (3) interpenetrating network (from Brownsey and Morris, 1988).

with these additives in meat systems. Burgarella et al. (1985) looked at the gel strength (measured from penetration force) of surimi (minced fish) in combination with egg white proteins and whey protein concentrate (WPC). The results showed that the addition of egg white proteins at a 1:4 ratio with surimi (12% total protein and 2% NaCl w/w) lowered the rigidity modulus of the gels below 40°C, but strengthened gel rigidity at temperatures above the gelling temperature of both proteins (70°C). Addition of WPC increased the rigidity modulus of gels throughout the heat treatment. The authors suggested egg white protein interfered with network formation of the myofibrillar protein of surimi, resulting in a decrease in rigidity of the co-gels below 40°C. Egg white protein gelled above 70°C, leading to enhanced rigidity of the co-gel. Soluble WPC increased the interstitial fluid viscosity, imparting additional rigidity to the myofibril protein throughout the heat treatment, and did not interrupt the gelation of surimi protein. Combinations of surimi and egg white or whey proteins were suggested to form filled gels.

The formation of myosin and albumin or fibrinogen co-gels in 0.5M NaCl pH 6.0 was investigated. Foegeding et al. (1986) found that strength (penetration force) of myosin-albumin and myosin-fibrinogen gels was affected by heating rate. The myosin-fibrinogen combination did not form a gel when heated at 70°C for 20 min. When slowly heated at a rate of 12°C/min to 55°C or 70°C, myosin interacted with fibrinogen resulting in gels with higher hardness than the sum of the gel hardness of each individual protein. In myosin-albumin blends, there was no interaction between the proteins until 85°C where sufficient thermal alteration of the albumin had occurred. The strength of the

combination gel formed by isothermal heating at 90 or 95°C was half that of the gel formed when heated to 90 or 95°C at 12°C/min.

Peng et al. (1982) studied the interactions between soybean 11S protein and myosin heated at 34.5, 70, 85, 90, 95 and 100°C for 30 min in 58.3 mM $NaPO_4$, 0.4M NaCl, pH 6.55, 10 mM 2-mercaptoethanol, 1 mM $MgCl_2$, 0.1 mM ATP, and 0.02% NaN_3. This buffer condition was supposed to mimic the pH and ionic environment of a processed meat system. Gel filtration chromatography and electrophoresis of the supernatants collected after centrifugation (100,000 x g, 30 min) of the proteins after heating and cooling indicated that 11S soy interacted with myosin only in the temperature range of 85-100°C. Interactions occurred between the myosin heavy chain and partially dissociated or fully dissociated 11S soy protein.

Shiga et al. (1985) studied interactions of acid precipitated soybean protein (pre-heated in100°C water bath for 0-15 min) and ground chicken meat. YounG's modulus and the water holding capacity of the ground chicken meat and preheated soy protein mixtures heated at 70 or 100°C increased with an increase of preheating time (0-4 min) of soy protein. These changes in physical properties of the mixed gels were related, in part, to an increase in the SH-group concentration of soy protein during the first 4 min of preheating. These results indicated that unfolding of soy protein was needed for interaction with meat protein to enhance the gel strength.

The properties of co-gels of chicken SSP and WPC of various solubilities have been studied. WPC solubility had different effects on meat protein gelation depending on heating temperature. Beuschel et al. (1992) reported that hardness and deformability of co-gels formed with 4% SSP and 12% WPC in 0.6M NaCl, pH 7.0, heated at 65°C were

enhanced by low solubility WPC. Mixtures with highly soluble WPC required a higher temperature (90°C) to enhance the hardness of the co-gels. Hung and Smith (1993) studied the rheological properties and microstructure of the same proteins, and found that WPC shifted the first storage and loss moduli (G' and G") transitions of SSP to higher temperatures and increased the magnitude of G' and G" of gels heated to 90°C. In isothermal studies at 65°C, the elasticity of a combination gel of SSP and low solubility WPC was higher than that of the combination containing highly soluble WPC. Microstructure of the co-gels formed with highly soluble WPC showed a typical SSP fibrous structure at 65°C. When heated at 90°C, the globular structure of WPC was observed in the co-gel networks. The authors suggested that at 65°C, SSP and WPC co-gels formed a filled gel network where the WPC was trapped within the SSP network and competed for available water and increased the viscosity of the liquid phase. When heated to 90°C, WPC aggregated and probably formed either a filled gel with low solubility WPC or a phase separated gel network with highly soluble WPC, leading to an increase of G' in both co-gels. Addition of WPC, however interrupted the network formation of SSP as indicated by an increase of G' and G" transition temperatures, and distortion of the co-gel matrix when compared to SSP gel structures. WPC was added to SSP at a high concentration (12% WPC + 4% SSP), so the texture enhancement could be a result of a higher total protein concentration when compared to a control of 4% SSP. Moreover, the multi-component nature of SSP and WPC caused difficulties in the interpretation of results.

Smyth et al. (1998a) investigated the effects of β-LG and α-LA on the heat-induced gelation of chicken breast SSP in 0.6M NaCl, pH 6.5, using dynamic rheology and a water holding capacity test. SSP, β-LG, α-LA and the mixtures of SSP with β-LG or α-LA at ratios of 80:20, 60:40, 40:60 and 20:80 at a total protein content of 2% were used. SSP had greater G' at 70°C, but lower G' at 90°C than those of 80:20 and 60:40 SSP/β-LG mixtures. However, the G' of SSP gels were greater than all combinations of SSP and β-LG after cooling. The water holding capacity of SSP/β-LG co-gels were not different from SSP gels, whereas water holding capacity of SSP/α-LA co-gels were lower than those of SSP gels. SSP was primarily responsible for network formation in the mixed protein systems. Denaturation and aggregation of β-LG above 70°C facilitated a more rigid gel structure, whereas α-lactalbumin did not participate in the network formation of the co-gels. The decrease of G' of all mixed protein solutions observed below 70°C could be due to the dilution of SSP and interruption of SSP network formation by β-LG or α-LA.

Hongsprabhus and Barbut (1999) reported that addition of 2% preheated (30 min at 80°C, pH 7.0) WPI followed by cold set gelation (16h at 1.0°C) increased hardness (penetration force) and water holding capacity of chicken meat batters after cooking, especially at low salt concentration (≤1.5%) when compared to the use of unheated whey proteins. The function of preheated WPI in gel formation of chicken meat is unclear. The authors suggested that the texture enhancing effects were probably caused by the electrostatic interactions of meat and whey proteins during the incubation period.

4.5. Pre-heated whey protein and a two-step gelation process

Barbut and Foegeding (1993) reported that 4% preheated WPI solution (at 80°C for 10-30 min) formed a fine stranded gel by adding 10 mM $CaCl_2$ at ambient temperature. The gel was more transparent and higher in shear stress at fracture than a WPI gel formed at 80°C. McClements and Keogh (1995) reported similar results when 200 mM NaCl was added to 9% WPI preheated at 90°C for 30 min. The author also found that the magnitude of the complex modulus increased with the increasing of temperature during gelation. It was suggested that preheating whey proteins in absence of salt caused irreversible denaturation as indicated by a disappearance of the denaturation peak in a heat capacity profile of the preheated protein. The viscosity of preheated whey protein solutions suggested the formation of fibrous aggregates or polymers.

Re-heating the preheated protein solutions (two-step heating method) resulted in the formation of stronger and more transparent gels over a wider range of pHs and ionic strengths when compared to gels formed by single step heating of the same protein in presence of salt. The two-step heating method has been reported to alter the gelling properties of other globular proteins, such as ovalbumin, bovine serum albumin and egg lysozyme (Doi, 1993). Mechanisms of aggregate formation and gel network formation of the aggregates are unclear. Doi (1993) proposed a possible mechanism for ovalbumin aggregate formation during preheating at 80°C for 6h in 0.02M phosphate buffer, pH 7.0. Based on the aggregate size observed by TEM and molecular size of the native protein, the ovalbumin aggregates were formed by partially unfolded globules which first bind together as dimers then arrange into a worm-like cylinder (Figure 4.8). This model

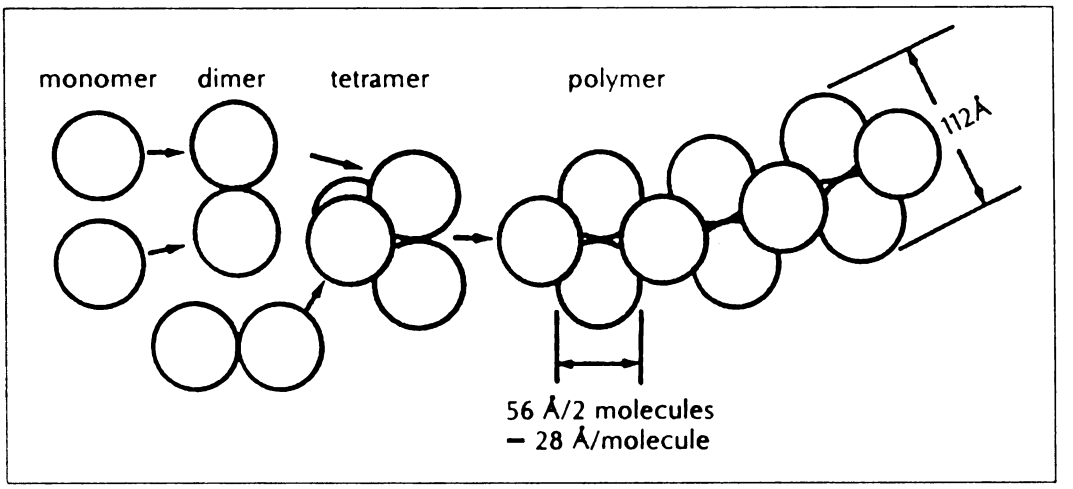


Figure 4.8. Model for linear polymer formation of ovalbumin (from Doi, 1993).

explains the formation of ordered linear polymers which form strong transparent gels. More evidence is needed to confirm this proposed mechanism.

The pre-aggregation or pre-polymerization alters the gelling properties of globular proteins, especially whey protein isolate or concentrate. Much research has been conducted to study various methods to form soluble whey protein aggregates and to induce gelation of the aggregates.

4.6. Cold-set whey protein gels

Several researchers have indicated that soluble whey protein aggregates can be formed using acidulants (Ju and Kilara, 1998c), calcium salts (Ju and Kilara, 1998a; 1998d) enzymes (Otte et al., 1996), or heat treatment (Ju and Kilara, 1998b). These soluble aggregates can gel upon the addition of salts or by changing pH. Since gelation occurs at temperatures below the gel point of native whey proteins, the gels formed are called cold-set gels (McClements and Keogh, 1995). As native whey proteins do not form gels until about 80°C, their influences on the texture of products that have been processed at lower temperatures are limited. Therefore, soluble whey protein gels have potential for applications where protein gelation at low temperature is desirable, such as in comminuted meat products.

Preparation of cold-set whey protein gels involves two main steps. In the first step, soluble aggregates of whey proteins are formed. The second step involves the formation of a gel network by the soluble aggregates, which can be initiated by addition of salt or by changing the pH of the solution.

4.6.1. Formation of soluble whey protein aggregates.

Acid-induced aggregates of whey proteins were largest when prepared at pH 5.2, the isoelectric point of β-LG. The aggregate size decreased when pH was decreased below the pI, indicating a dissociation of the aggregates (Ju and Kilara, 1998c). Incubation of WPI in $CaCl_2$ produced stable whey protein aggregates (Ju and Kilara, 1998d). The calcium-induced aggregation rate of WPI increased as the incubation temperature was increased from 21 to 45°C, while the aggregate size increased with increased protein and $CaCl_2$ concentrations (Ju and Kilara, 1998a). Acid-induced aggregation of WPI was more rapid than calcium-induced aggregation.

Otte et al. (1996) studied the protease-induced aggregation of whey proteins. Particle sizes of 12% WPI were increased when the protein was hydrolyzed by a protease as indicated by an increase in turbidity. Size exclusion chromatography of whey proteins before and after 3h hydrolysis at 40°C showed 35% degradation of β-LG and α-LA. Average aggregate size, detected by dynamic light scattering, increased from 130-200 nm to infinity after a lag period of about 50 min, suggesting that the aggregates were formed from the hydrolyzed proteins.

Soluble whey protein aggregates can also be prepared by heat treatment and does not require the use of chemicals. This approach is relatively more convenient and less harsh when compared to other treatments that have been investigated. The treatment parameters (temperature and time) and the conditions of the initial protein solutions (pH, ionic strength and protein concentration) need to be controlled to prevent gelation during heating. Soluble whey protein aggregates were formed when heated above the

denaturation temperature of β-LG (70-90°C) and held for 5-60 min. Higher preheating temperatures and longer times produced larger aggregates than those prepared at the lower preheating temperatures and shorter times (Ju and Kilara, 1998b). An adequate time/temperature combination allows denaturation of the whey proteins leading to the exposure of non-polar amino acids and reactive sulfhydryl groups buried in the native molecules. As a result, hydrophobic attractions and sulfhydryl-disulfide interchange reactions between unfolded protein increase and the protein aggregates are formed. Prabakaran and Damodaran (1997) reported that sulfhydryl-disulfide interchange among β-LG monomers was initiated at 60-65°C. The pH of the β-LG solution needs to be far from the isoelectric point (pH 5.2) of the protein and the salt concentration should be very low so that the aggregation process is limited, resulting in filamentous aggregates which remain soluble due to electrostatic repulsion between their surfaces. Most of the experiments were performed between pH 7.0-8.0. In addition, the concentration of $CaCl_2$ was less than 10 mM or kept within the inherent mineral content of the whey protein preparations (Hongsprabhus and Barbut, 1998; Ju and Kilara, 1998b).

Ju and Kilara (1998b) studied the influence of protein concentration on the formation of soluble aggregates of whey protein isolate during heating at 80°C at pH 7.0. Dynamic light scattering revealed formation of soluble aggregates in solutions of protein concentration higher than 3%. Size of whey protein aggregates increased from 21 nm to 61 nm when the protein concentration was increased from 3 to 9%. However, above a critical concentration, gel formation occurred. Solutions of 12% protein were reported to form self supporting gels after heating at 80°C for 30 min, pH 7.0, without addition of salt

(Ju et al., 1995; Ju and Kilara, 1998e). The viscosity of the heated solution significantly increased when compared to that of the initial protein solution indicative of the formation of filamentous aggregates (Barbut and Foegeding, 1993; McClements and Keogh, 1995; Mleko and Foegeding, 1999b; Vardhanabhuti and Foegeding, 1999b). More research is needed to understand the mechanisms and factors influencing the formation of soluble aggregates, and to understand how the properties of the soluble aggregates influence the characteristics of the cold-set gel. This knowledge is important for manipulating the functional properties of whey protein products.

4.6.2. Network formation of cold-set gels of heat-induced whey protein aggregates.

A solution of heat-induced soluble whey protein aggregates can form a gel network upon addition of proteolytic enzyme, salts or changing the pH at ambient temperature. The cold-set gel network formation and properties are dependent on aggregate formation and gelling conditions. Heat treatment is a common process used in whey manufacture and the gel properties of heat-induced aggregates, such as clarity, hardness and water holding capacity are similar to those needed by food processors. More research is needed to study the network formation and properties of gels formed from heat-induced whey protein aggregates.

4.6.2.1. Enzyme-induced gelation. Heat-induced whey protein aggregates have been reported to form gels upon proteolytic digestion. The proteolytic digestion of pre-heated soluble aggregates induced network formation via a different mechanism from

acid- and salt-induced gelation. Sato et al. (1995) studied gelation of heat-denatured whey protein by proteolytic digestion at 37°C using several enzymes (pepsin, papain, pronase, protease and trypsin). Weak gels were obtained when protein solutions were incubated with all enzymes, except pepsin. Pepsin did not hydrolyze the whey protein after 8h incubation. Hardness of the gels increased as the degree of hydrolysis was increased over the incubation time, suggesting gel networks were formed from the protein hydrolysates. The interactions between the hydrolysates of preheated whey proteins, which led to a gel network formation, were not known.

4.6.2.2. Salt-induced cold-set gelation. Bryant and McClements (1998) compared the heat-induced gelation of native whey proteins with cold-set gel formation of heated whey protein aggregates (Figure 4.9). During heat-induced gelation of native whey protein in the presence of salts (at a protein concentration higher than the critical concentration for inducing gelation), the proteins undergo unfolding and aggregation almost simultaneously when the temperature reaches the gel point. Salts accelerate the rate of aggregation resulting in large aggregates which cross-link to form particulate gel structures. In contrast, soluble whey protein polymers with highly negatively charged surfaces form a fine stranded gel network upon the introduction of salts, at or below ambient temperature. Salts decrease electrostatic repulsion and form salt bridges between the soluble aggregates leading to three-dimentional network formation.

Salt type and concentration affected gel properties, suggesting different mechanisms were involved in network formation. Divalent cations may form intra- and/or intermolecular salt bridges which affect the texture of the final gels (Ju and Kilara,

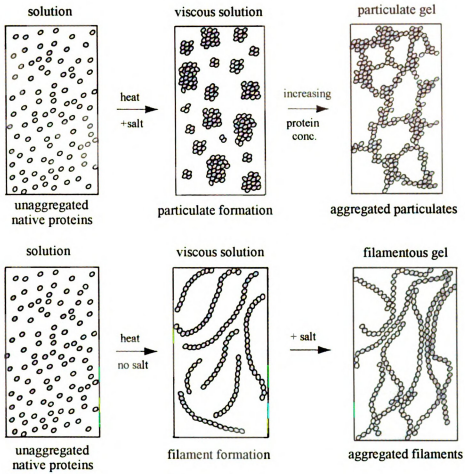


Figure 4.9. Development of a particulate gel structure from heat-induced gelation and a filamentous gel structure from cold-set gelation of whey protein (from Bryant and McClements, 1998).

1998e). According to Vardhanabhuti and Foegeding (1999a), the mechanism of cold-set gel formation is "salt specific." A lower concentration of Ca^{2+} is needed to induce cold set gelation, when compared to Na^{+}. The most rigid gels were obtained by addition of 20-30 mM $CaCl_2$ or 200 mM NaCl to 8-10% preheated whey protein. Gel networks became coarser with decreased water holding capacity as the concentration of salts was increased above 30 mM $CaCl_2$ or 200 mM NaCl (Hongsprabhus and Barbut, 1997b; 1998; Ju and Kilara, 1998a; Vardhanabhuti and Foegeding, 1999a).

4.6.2.3. Acid-induced gelation. Lowering the pH of pre-heated whey protein isolate (soluble aggregates) induced gel formation at or below ambient temperature by decreasing electrostatic repulsion. Mleko and Foegeding (1999a) reported that weak gels were formed at 7°C by decreasing the pH of soluble whey protein polymers (preheated at 80°C for 15-53 min) from pH 8.0 to 6.0. Ju and Kilara (1998c) found that addition of glucono-delta-lactone induced gelation of 8% pre-heated whey protein (preheated at 80 °C for 30 min) when the pH was decreased from 7.0 to 5.3 at 45 °C. Acid-induced gels had a maximum hardness at pH 4.7 and were harder than salt-induced gels prepared from the same protein solutions. No studies have been reported on cold-set gelation induced by salts at a pH lower than 7.0.

Factors affecting textural properties of cold-set gels induced by salts and acid are protein concentration and incubation time and temperature. The minimum protein concentration needed to form a salt or acid induced cold-set gel has been reported to be 3% (diluted from 9% whey protein solution heated at 80°C for 30 min, pH 7.0) (Ju and Kilara, 1998b). An increase in the concentration of soluble protein aggregates led to the

formation of a harder gel (Hongsprabhus and Barbut, 1998; Ju and Kilara, 1998b; 1998e). However, the hardness of the final gels was also affected by the concentration of protein during the preheating step. Increasing the protein concentration from 3% to 9% during preheating increased the size and content of the soluble aggregates resulting in the formation of a stronger gel network (Ju and Kilara, 1998b).

Time and temperature have a great effect on the rate of gelation and the final properties of both salt- and acid-induced gels of soluble whey protein aggregates. The rate of network formation of soluble whey protein aggregates is increased by increasing temperature (Kitabatake et.al., 1996; Mleko and Foegeding, 1999a). Opacity and hardness of salt-induced gels increased with an increase in gelling temperature from 20 to 50°C (McClements and Keogh, 1995), suggesting that hydrophobic interactions and disulfide bond formation, may be involved in cold-set gelation at higher temperatures. A slow addition of calcium ions by dialysis resulted in more translucent gels than direct addition of calcium to the protein aggregate solution (Roff and Foegeding, 1996). More research is needed to clarify the factors influencing gelation of pre-heated whey proteins.

Most research has been focused on cold-set gelation of whey protein aggregates formed at ambient temperature or lower. However, heat is used during food processing to destroy pathogenic microorganisms and to produce desired sensory attributes in many products. Re-heating by the consumer is also a common food preparation practice. This heating process can affect the properties of gels formed from whey protein aggregates. Hongsprabhus and Barbut (1998) reported that re-heating at 80°C for 30 min increased YounG's modulus and shear stress, and reduced shear strain, extensibility and water holding capacity of a cold-set whey protein gel initially formed by incubation with $CaCl_2$

at 24°C for 16h. In some food products, such as sausages, salts, low pH and heat are used. Therefore, the effects of these factors on the gelation of pre-heated whey protein polymers need to be studied.

CHAPTER 5: HEAT-INDUCED GELATION OF MYOSIN/β-LACTOGLOBULIN MIXED PROTEINS

5.1. ABSTRACT

The thermal denaturation, aggregation and rheological properties of chicken breast muscle myosin, β-lactoglobulin (β-LG) and mixed myosin/β-LG systems at various concentrations in 0.6M NaCl, 0.05 mM sodium phosphate buffer, pH 7.0, were studied. There was no interaction between myosin and β-LG during heating as indicated by the unique denaturation peaks of each protein in the thermogram of the mixed protein solution. The maximum aggregation rate (AR_{max}) of both proteins increased, and the temperature at the AR_{max} (T_m) and initial aggregation temperature (T_o) decreased as the protein concentration was increased. Above 0.5% protein, concentration had no effect on T_o, T_m and AR_{max} of myosin. Aggregation of myosin at <0.5% protein was interrupted by the presence of 0.25% β-LG in the mixtures. Addition of 0.5-3.0% β-LG decreased storage moduli of 1% myosin between 55-75°C, but increased storage moduli when heated to 90°C and after cooling. β-LG had no effect on the gel point of ≥1.0% myosin. Overall, β-LG interrupted the aggregation and gel formation of myosin at 55-75°C, but enhanced gel strength above 80°C and after cooling. The magnitude of the effects was concentration dependent.

5.2. INTRODUCTION

Salt soluble proteins form heat-induced gels which are responsible for texture, water holding, binding and appearance of meat products (Smith, 1994). To improve yields, reduce costs, or improve quality of meat products, meat processors often use non-meat proteins to substitute for muscle proteins. Whey proteins possess high nutritional and functional properties and are used in meat products as binders to enhance yield and textural quality. However, results from the use of whey proteins in meat products are highly variable due to differences in the source and processing history of whey protein concentrates or isolates, and variation in processing conditions used for meat products. The source and processing history of whey products affects protein composition and functional properties. While differences in processing condition used for meat products (time/temperature, salt and pH) greatly affect gelling properties of both proteins.

Many studies have looked at mixed gel systems of salt soluble protein and whey protein concentrate (Beuschel et. al, 1992; Hung and Smith, 1993; Smith and Rose, 1995; McCord et al., 1998). The functions of whey proteins in meat systems have not been clearly explained due to the multi-component nature of these proteins. Since myosin and β-lactoglobulin (β-LG) are the major functional proteins of meat and whey products, respectively, the use of these proteins in a model system might help elucidate the interactions that occur when these proteins are used.

The mechanism of protein gelation is a multi-step process involving denaturation, aggregation and network formation of the protein molecules. Bringing together the information from each step of the process and comparing the data between mixed and

pure protein systems will lead to a better understanding of interactions between the proteins throughout the gelation process. The objective of this experiment was to elucidate the mechanisms involved in the formation of myosin and β-LG co-gels by: 1) observing the thermal stability of myosin and β-LG during heating of mixed systems, 2) investigating the influence of β-LG on aggregation of myosin during heat-induced gelation, and 3) monitoring rheological properties of myosin and β-LG in mixed protein gel systems.

5.3. MATERIALS AND METHODS

5.3.1. Protein solution preparation

Myosin from breast muscle (*M. pectoralis*) was extracted immediately after sacrifice from 8-12 week old commercial type broilers (Wang and Smith, 1994b). Myosin was stored in 48% saturation ammonium sulfate with 30% glycerol (v/w) at -20°C. Prior to use, myosin was suspended in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 7.0 (PBS), dialyzed against three changes of the same buffer for 48h and centrifuged at 78,000x g to precipitate denatured protein. Bovine milk β-LG (L0130, lot #114H0755) containing variants A and B, was purchased from Sigma chemical Co. (St. Louis, MO). β-LG solutions were prepared by dissolving the protein in PBS overnight at 4°C. The purity of both myosin and β-LG were assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis using 4% and 12% acrylamide for stacking and resolving gels, respectively (Smyth et al., 1996). Protein concentrations were determined by absorption using extinction coefficient ($E^{1\%}$) of 5.5 at 280 nm for myosin (Smyth et al,

1996) and 9.55 at 278 nm for β-LG (Foegeding et al., 1992). Mixed protein solutions were prepared by mixing an equal volume of each protein prepared at twice the desired concentration in PBS. The concentrations of myosin, β-LG, and mixed solutions used in denaturation, aggregation, and dynamic oscillatory experiments are presented in Table 5.1.

5.3.2. Differential scanning calorimetry

The thermal denaturation of myosin, β-LG, and myosin/β-LG in PBS, pH 7.0, were investigated using an MC-2 differential scanning calorimeter (DSC) (Microcal Inc., Amherst, MA). The proteins and blank solutions (PBS) were degassed in a vacuum chamber before loading 1.24 mL into the DSC. Experiments were conducted at a scan rate of 1°C/min from 25°C to 90°C. Calorimetric enthalpy (ΔH_{cal}) and endothermic peak or melting temperature (T_m) were determined from heat capacity profiles (C_p vs temperature) using the software (DA-2 Data Acquisition and Analysis system) provided by the manufacturer. The experiments were conducted using at least three replications for each sample.

5.3.3. Thermal aggregation of protein

Thermal aggregation of proteins was followed by turbidity measurement at 340 nm using a Lamda 20 UV-visible spectrophotometer connected to a PTP-6 peltier temperature programmer (Perkin Elmer Inc., Norwalk, CT). Protein solutions of 1.4 mL

Table 5.1. Concentrations of myosin and β-lactoglobulin (β-LG) in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 7.0, used in thermal denaturation (DSC), thermal aggregation and dynamic oscillatory experiments

Protein type	Protein concentration (%)			
	DSC Experiment	Thermal aggregation Experiment	Dynamic oscillatory experiment	
Myosin	1.0	0.05, 0.1, 0.25, 0.5, 1.0, 1.5	0.5, 1.0, 1.5, 2.0	
β-LG	1.0	0.1, 0.18, 0.25, 0.5, 1.0, 1.5	1.0, 2.0, 3.0	
Myosin : β-LG	1.0 : 1.0	0.1 : 0.1	0.1 : 0.25	1.0 : 0.5	0.5 : 1.0
		0.1 : 0.25	0.25 : 0.25	1.0 : 1.0	1.0 : 1.0
		0.1 : 0.5	0.5 : 0.25	1.0 : 2.0	1.5 : 1.0
		0.1 : 1.0	1.0 : 0.25	1.0 : 3.0	2.0 : 1.0

were placed in quartz cuvettes (1-cm path length) and sealed with Teflon™ tape to prevent evaporation. A blank containing the same buffer was used as the control. Solutions were equilibrated at 25°C for 5 min, then heated to 90°C at 1°C/min, held at 90°C for 30 min, cooled to 25°C within 15 min, followed by a 5 min holding at 25°C (Figure 5.1a). The change in turbidity of protein solutions was recorded every 0.5°C. Each study was performed in triplicate. A plot of aggregation rate (Δabsorbance/Δtime) against time or temperature was constructed for determination of the initial aggregation temperature (T_o), maximum aggregation rate (AR_{max}), and temperature at AR_{max} (T_m) (Figure 5.1b). T_o was defined as the temperature at which the aggregation rate was ≥ 0.01 min^{-1}. Peak of the plot determined AR_{max} and temperature at AR_{max} was determined as T_m.

5.3.4. Small amplitude dynamic oscillatory testing

Dynamic oscillatory tests were performed using a controlled stress rheometer (RS 100, Haake, Karlsruhe, Germany) equipped with a 35 mm diameter stainless steel parallel plat. Storage (G') and loss (G") moduli were recorded continuously at a fixed frequency of 0.464 Hz using constant stresses (producing strains from 0.1-0.3%) within the range of linear viscoelastic behavior determined from stress sweeps performed for each protein at 90°C and after cooling to 25°C. Protein solutions were loaded between the plate and base with a gap between 1.0 and 1.2 mm. A few drops of corn oil (Mazola, Best Food, CPC International, Inc., Englewood Cliffs, NJ) were used to cover the edge of the gap to prevent evaporation. Solutions were heated using the same temperature profile used in aggregation experiments (Figure 5.1a). The temperature was controlled by a circulating

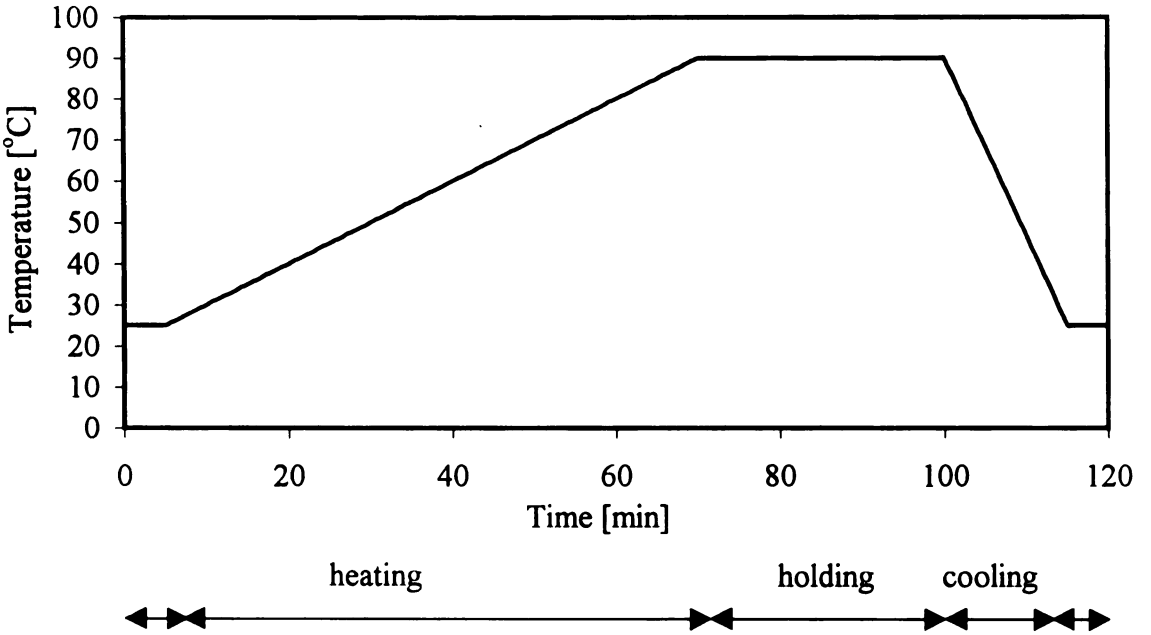


Figure 5.1a. Heating profile for thermal aggregation and rheological studies.

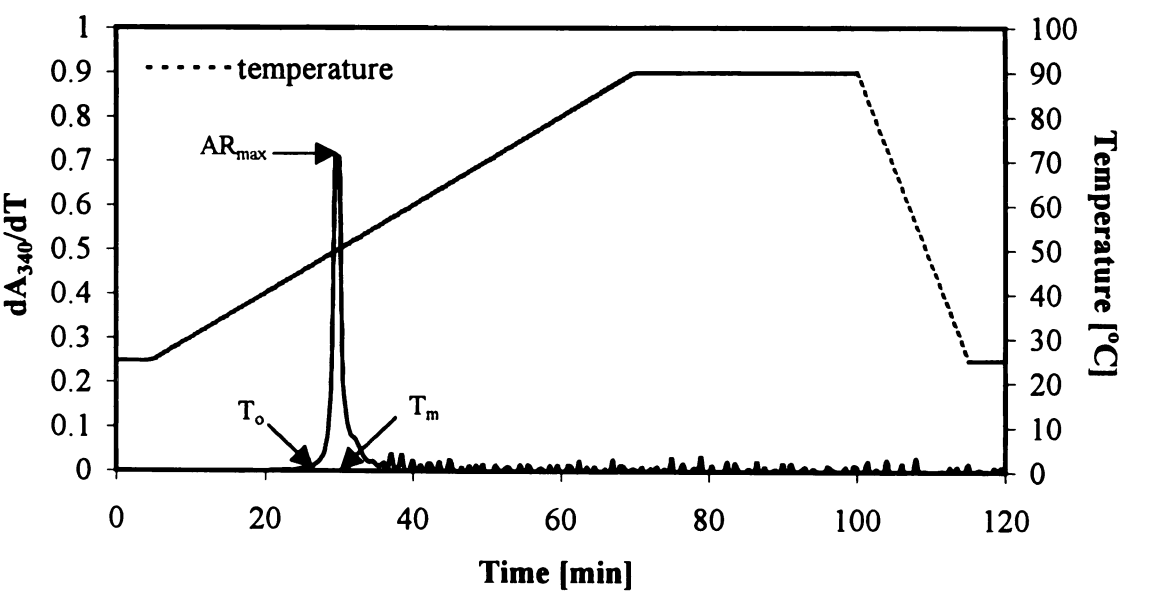


Figure 5.1b. Derivative curve of protein aggregation for determination of initial aggregation temperature (T_o), maximum aggregation rate (AR_{max}), and temperature at AR_{max} (T_m).

water bath attached to the rheometer. Frequency sweeps (0.01-100 radian/second) were performed after cooling using constant stress resulting in strain of 0.1-0.3%. Protein solutions used in sweep tests were 2% myosin, 1% myosin/1% β-LG, and 4% β-LG. The gel point of protein solutions was defined as the temperature at which G' and G" crossed over in the fixed frequency test (Stading and Hermansson, 1990). Each solution was tested in triplicate.

5.3.5. Experimental design and statistical analysis

Each experiment was performed in triplicate. Differences between aggregation and rheological properties (T_o, T_m, AR_{max}, G' and gel point) affected by protein type or concentration were statistical analyzed using one way analysis of variance (ANOVA). Means were compared using the Tukey-Kramer HSD test with the mean square error at 5% probability (JMP, Version 3.2.2, SAS Institute Inc., Cary, NC).

5.4. RESULTS AND DISCUSSION

5.4.1. Thermal denaturation of myosin and β-lactoglobulin

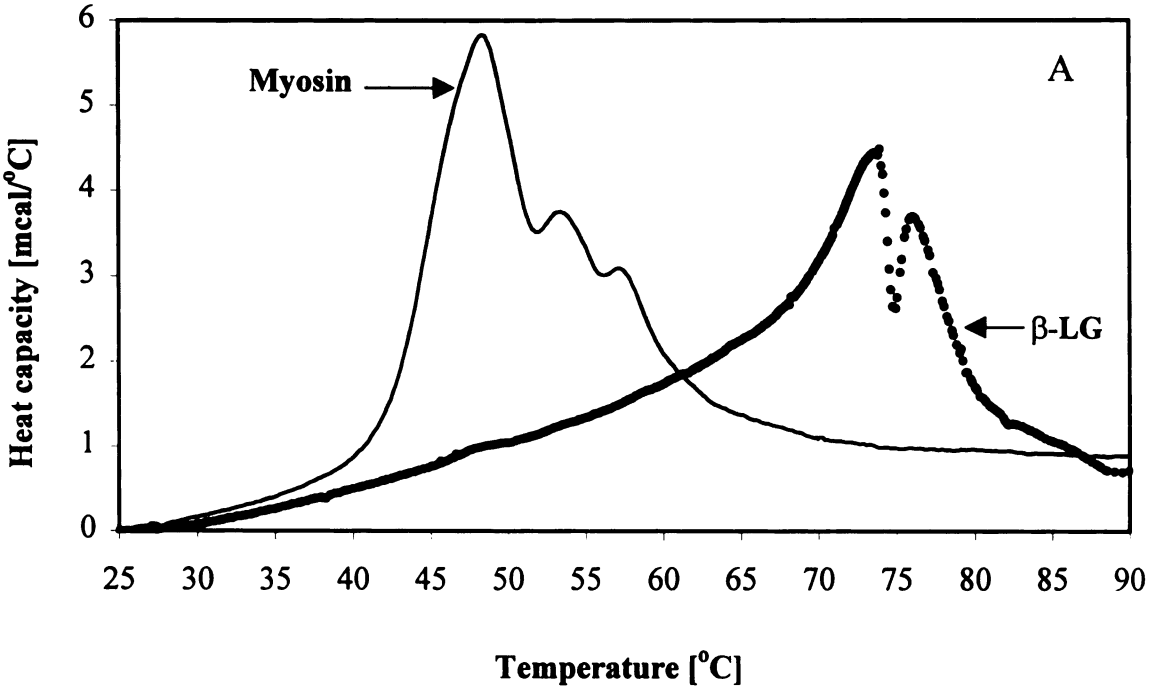
The enthalpy profile of 1% chicken breast myosin in PBS, pH 7.0, contained three endothermic peaks (T_m) at 48.5, 53.2 and 57.0°C (Figure 5.2a). The myosin endotherm was similar to those reported by other researchers. Chicken breast myosin heated at 1°C/min in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 6.5, exhibited three endothermic peaks at 47.5, 54.0 and 57.4°C (Smyth et al., 1996). Lui et al (1996) reported transition temperatures of 48.1, 49.9 and 67°C for chicken breast myosin in 0.6M

NaCl at pH 6.0. The denaturation ΔH_{cal} of myosin (2130 ± 80 kcal/mole) was close to the ΔH_{cal} of chicken *pectoralis* myosin reported by Wang and Smith (1994b) (2,140 kcal/mole) and Lui et al. (1996) (2,216 kcal/mole).

The endotherm of 1% β-LG showed a broad T_m peak at 73.6°C (Figure 5.2A). The result was close to a T_m of 73.4°C reported by Foegeding et al. (1992) using 10% β-LG in 0.1M NaCl, 0.05M TES buffer, pH 7.0 and heating at 10°C/min.

A rapid decrease in heat capacity was observed at 76.0°C. The intensity of this peak increased as the concentration of β-LG was decreased from 2% to 0.5% (data not shown). The sudden drop of heat capacity was previously reported in DSC studies of myosin at concentrations ≤ 0.523% (Lui et al., 1996) and was attributed to the aggregation and precipitation of the unfolded proteins at low concentration. However, a DSC study using 0.4% β-LG in 0.07M phosphate buffer, pH 6.75, showed no precipitation when heated at 1.5°C/min (Qi et al., 1995). Moreover, no such phenomenon occurred when 1% β-LG in distilled water was heated at 1°C/min (data not shown). Therefore, the rapid decrease of the heat capacity could be a result of the aggregation and precipitation of β-LG at high ionic strength (0.6M NaCl).

The endotherm of myosin and β-LG mixtures was identical to that expected if the endotherm of each protein was overlaid on the same axis (Figure 5.2B). Wang and Smith (1995) studied the influence of weight ratio of actin to myosin on the gelation of the reconstituted chicken breast actomyosin. The results revealed interactions between myosin and actin, which led to changes in the denaturation profile of actomyosin when



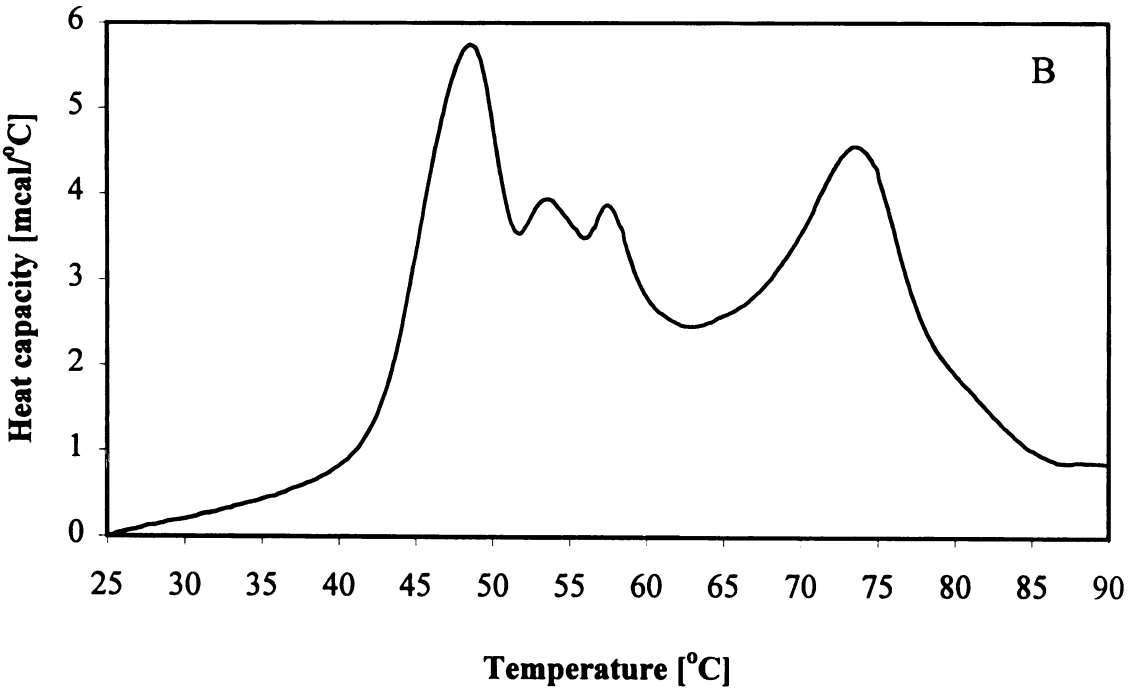


Figure 5.2. Heat capacity profiles of (A) 1% myosin and 1% β-lactoglobulin (β-LG) analyzed separately, and (B) a mixture of 1% myosin/1% β-LG mixture in 0.6M NaCl, 0.05M sodium phosphate buffer pH 7.0.

compared to pure myosin and pure actin. In this experiment, DSC results indicated that no interaction occurred between myosin and β-LG during heating as each protein maintained its unique transition profile. However, β-LG did not precipitate when heated in the mixed solution with myosin. Since myosin is denatured at a lower temperature than β-LG, it may form networks which help prevent aggregation and precipitation of β-LG during heating in mixed solutions.

5.4.2. Thermal aggregation

UV-visible spectroscopy was employed to investigate the aggregation of pure and mixed protein solutions at different concentrations. The aggregation patterns of myosin (Figure 5.3) had a sigmoidal shape similar to those reported by previous researchers (Lui et al., 1996; Smyth et al., 1996). Myosin rapidly aggregated after the onset temperature. The turbidity remained constant after reaching a maximum at around 67°C regardless of concentration, suggesting that myosin aggregation was complete.

The initial aggregation temperature of myosin (T_o) decreased ($p<0.05$) from 50.6 to 46.3°C as the protein concentration was increased from 0.1 to 0.5% (Table 5.2). The maximum aggregation rate (AR_{max}) and the temperature at maximum aggregation rate (T_m) also increased with increasing concentrations of myosin from 0.1 to 0.5%. Above 0.5%, concentration had no significant effect on either AR_{max}, T_m or T_o of myosin, suggesting that myosin aggregation was concentration dependent below 1.0% protein. The AR_{max} of 0.1% myosin was 0.44 /min, much lower than 1.96 /min reported by Lui et

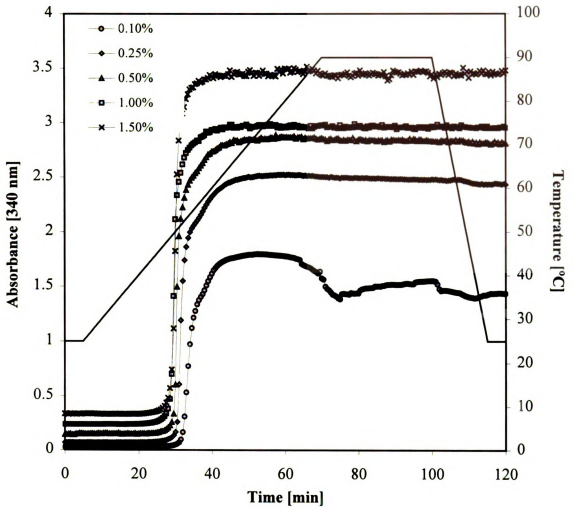


Figure 5.3. Aggregation of 0.1-1.5% myosin in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 7.0, during heating at 1°C/min from 25 to 90°C, holding at 90°C for 30 min and cooling to 25°C.

Table 5.2. Initial aggregation temperature (T_o), maximum aggregation rate (AR_{max}), and temperature at maximum aggregation rate (T_m) of myosin, and mixed solutions of 0.25% β-lactoglobulin (β-LG) and 0.1-1.0% myosin in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 7.0, during heating from 25 to 90°C, holding at 90°C for 30 min and cooling to 25°C

Protein type	Myosin concentration (%)	T_o (°C)	T_m (°C)	AR_{max} (°C)
Myosin	0.1	50.6 ±0.2^{b}	53.2 ±0.3^{b}	0.44 ±0.051^{b}
	0.25	49.2 ±0.4^{c}	51.7 ±0.7^{c}	1.07 ±0.077^{d}
	0.5	46.3 ±0.2^{d}	50.1 ±0.2^{cd}	1.36 ±0.150^{e}
	1.0	45.8 ±0.7^{d}	49.9 ±0.6^{d}	1.61 ±0.136^{f}
	1.5	45.3 ±0.2^{d}	49.3± 0.2^{d}	1.60 ±0.167^{f}
0.25% β-LG/ myosin	0.1	77.5±0.5^{a}	90[1]^{a}	0.03±0.002^{a}
	0.25	49.2 ±0.7^{c}	54.2 ±0.3^{b}	0.71 ±0.180^{c}
	0.5	46.3 ±0.3^{d}	52.3 ±0.5^{bc}	1.35±0.180^{e}
	1.0	46.1 ±0.2^{d}	50.9 ±0.5^{cd}	1.79 ±0.250^{f}

[1] T_m occurred during holding at 90°C.

[a-f] Values are means ± standard deviations of three observations. Means with different superscripts in each column are significantly different ($p<0.05$).

al. (1996) using 0.07% chicken pectoralis myosin and heating at 55°C in 0.6M NaCl, pH 6.0. The difference is probably due to differences in pH of the protein solutions.

The aggregation pattern of 0.1-1.5% β-LG (Figure 5.4) was similar to that of myosin except that β-LG began to aggregate later and had lower AR_{max} than myosin. Thermal denaturation of proteins precede thermal aggregation (Sano et al., 1990; Smyth et al., 1996). DSC results showed that myosin denatured at 48-57°C, whereas β-LG melted at 73.6°C. Myosin is a fibrillar protein of 150 nm in length, whereas β-LG is a globular protein with a radius of about 2-3 nm. Increasing the chain length of protein increases molecular entanglement and facilitates contact or intermolecular binding, promoting protein aggregation (Wang and Damodaran, 1990). Therefore, myosin had a lower T_o and higher AR_{max} than β-LG.

T_o of β-LG decreased from 79.3°C at 0.25% protein to 63.5°C at 1.0% protein (Table 5.3). Initial aggregation of 1% β-LG was detected at a temperature (63.5°C) lower than its melting point (73.6°C), indicating that aggregation occurred between partially unfolded molecules. Prabakaran and Damodaran (1997) reported that small β-LG particles were first formed via the sulfhydryl-disulfide interchange reaction between two reactive monomers (molten globules) at 60-65°C. The small particles further associated into larger aggregates in the propagation stage where the aggregation rate reach its maximum. The AR_{max} and T_m of β-LG increased ($p<0.05$) as the protein concentration was increased from 0.1 to 1.0%. Many studies have shown that the rate of β-LG aggregation depended on reactive β-LG monomer concentration (Elofsson et al., 1996; Hoffmann et al., 1996; Verheul et al., 1998). The aggregation of β-LG at concentrations

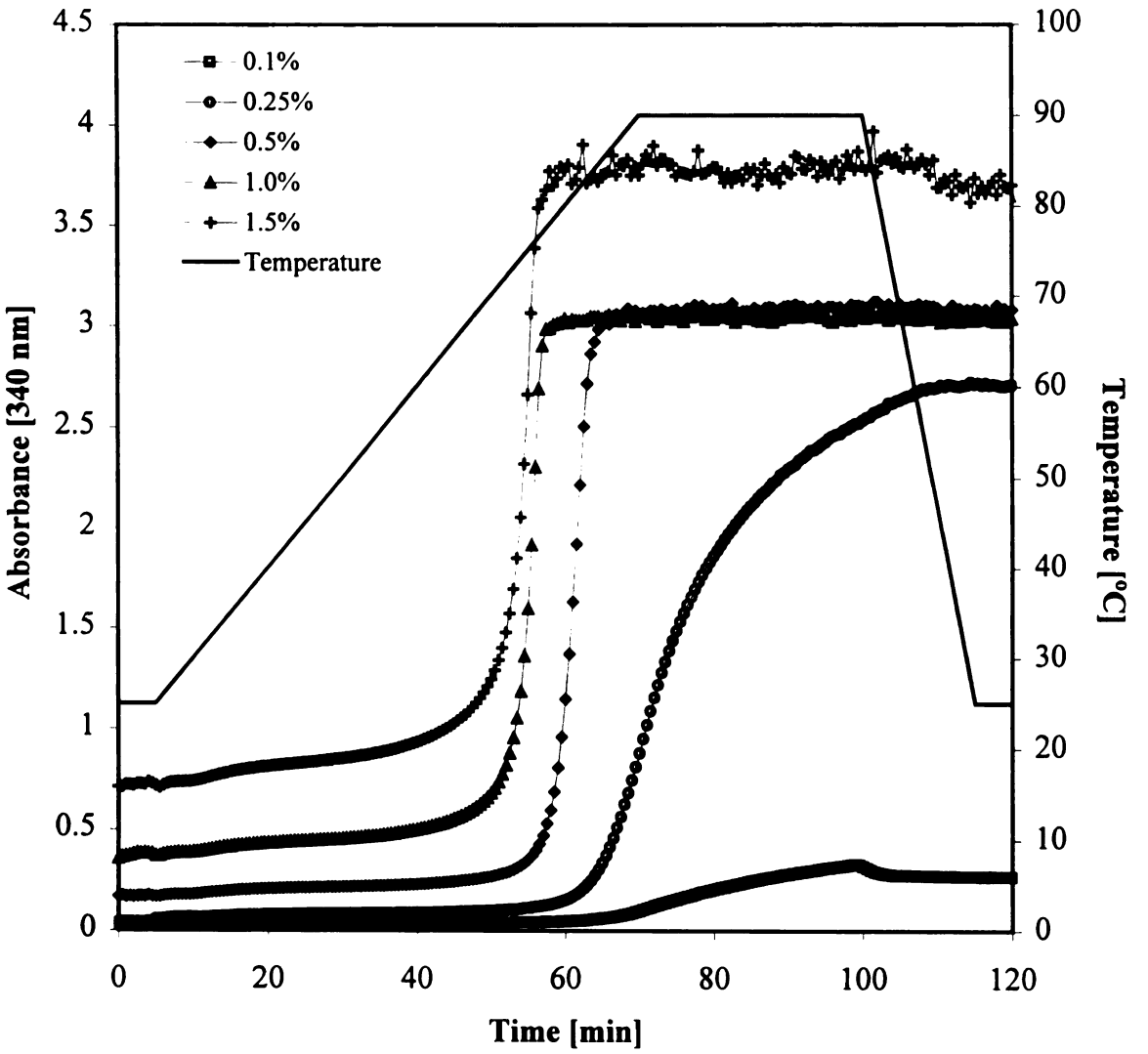


Figure 5.4. Aggregation of 0.1-1.5% β-lactoglobulin in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 7.0, during heating at 1°C/min from 25 to 90°C, holding at 90°C for 30 min and cooling to 25°C.

Table 5.3. Initial aggregation temperature (T_o), maximum aggregation rate (AR_{max}), and temperature at maximum aggregation rate (T_m) of β-lactoglobulin (β-LG), and mixed solutions of myosin and 0.1-1.0% β-LG in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 7.0, during heating from 25 to 90°C, holding at 90°C for 30 min and cooling to 25°C

Protein type	β-LG concentration (%)	T_o (°C)	T_m (°C)	AR_{max} (min^{-1})
β-LG	0.1	84.5±0.5[a]	90[1a]	0.02 ±0.002[a]
	0.25	79.3 ±1.0[b]	89.3 ±0.7[ab]	0.21 ±0.099[b]
	0.5	73.5 ±0.5[d]	84.0 ±1.6[b]	0.57 ±0.046[c]
	1.0	63.5 ±0.5[f]	78.3 ±0.2[c]	0.76 ±0.025[d]
	1.5	63.0 ±0.5[f]	77.3±0.2[c]	0.77 ±0.058[d]
0.1% myosin /β-LG	0.1	85 ±0.6[a]	89.8 ±1.0[a]	0.02 ±0.003[a]
	0.25	77.5 ±0.5[c]	90[1a]	0.03 ±0.002[a]
	0.5	70.0 ±0.4[e]	88 ±0.2[ab]	0.11 ±0.006[ab]
	1.0	63.2±0.8[f]	79.5±0.1[bc]	0.38 ±0.002[bc]

[1] T_m occurred during holding at 90°C.
[a-f] Result values are means ± standard deviations of three observations. Means with different superscripts in each column are significantly different ($p<0.05$).

higher than 1.5% was not studied due to limitations of the spectophotometric technique. Both myosin and β-LG formed opaque gels with absorbance values >5.

The effects of β-LG on aggregation of myosin at different concentrations are illustrated in Figure 5.5. Addition of 0.25% β-LG increased T_o of 0.1% myosin from 50.6 to 77.5°C and decreased AR_{max} from 0.44 to 0.03 /min (Table 5.2). When myosin concentration was increased above 0.25%, the T_o, AR_{max}, and T_m of the mixed systems were not ($p>0.05$) different from those of myosin at the same concentration. The results indicated that 0.25% β-LG interfered with myosin aggregation in the mixed system containing ≤0.25% myosin.

The aggregation patterns of mixed protein solutions containing 0.1% myosin and 0.1-1.0% β-LG compared to those of 0.1-1.0% β-LG are presented in Figure 5.6. Mixed solutions of 0.1% myosin and 0.1% β-LG exhibited two aggregation steps with the first T_o, T_m and AR_{max} at 51.4°C, 55°C and 0.08 /min, respectively. The first aggregation step was attributed to myosin aggregation. However, T_o and T_m were higher and AR_{max} was lower than those of 0.1% myosin, indicating interruption of myosin aggregation. In the second step, T_o, T_m and AR_{max} occurred at 85°C, 89.8°C and 0.02 /min, respectively, and were similar to those of 0.1% β-LG (Table 5.3). The results suggested that both proteins aggregated independently in mixed systems of low protein concentration. When β-LG concentration was increased above 0.25%, aggregation of the mixed protein solutions exhibited a pattern more typical of β-LG aggregation. However, AR_{max} of all the mixed proteins were lower than that of β-LG at the same concentration. Results suggested that both proteins interfered with the aggregation of the other and the proportion of the

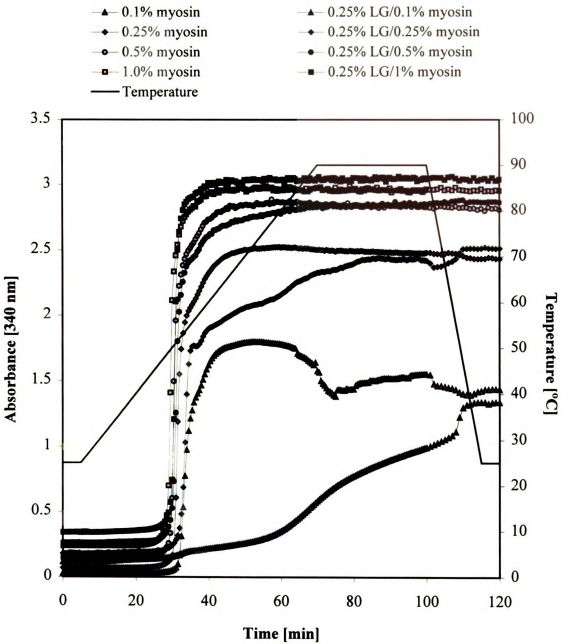


Figure 5.5. Aggregation of myosin and mixed solutions of myosin with 0.25% β-lactoglobulin (β-LG) in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 7.0, during heating at 1°C/min from 25 to 90°C, holding at 90°C for 30 min and cooling to 25°C.

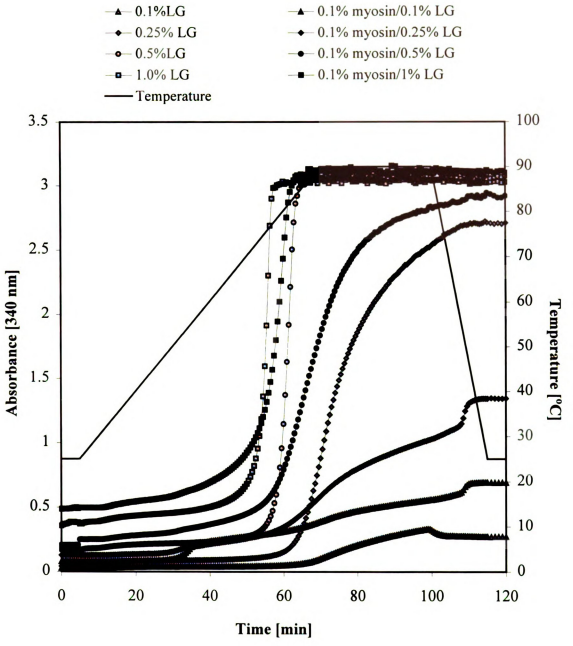


Figure 5.6. Aggregation of β-lactoglobulin (β-LG) and mixed solutions of β-LG with 0.1% myosin in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 7.0, during heating at 1°C/min from 25 to 90°C, holding at 90°C for 30 min and cooling to 25°C.

proteins determined the magnitude of the effects. Since myosin gelled at a lower temperature, its aggregation was dominant in the mixed system.

5.4.3. Rheological properties

Gel points of β-LG solutions in PBS, pH 7.0, decreased from 79°C at 0.5% protein to 73°C at 3% protein (Table 5.4). Data could not be obtained during holding at 90°C and cooling at any β-LG concentration due to shrinkage of the gel. The result suggested that the minimum concentration of β-LG to form a set gel in 0.6M NaCl, pH 7.0, is higher than 3%. McSwiney et al. (1994) reported that a minimum concentration of 5.0% was required for β-LG gel formation at pH 7.0 in 0.02M imidazole buffer, 0.1M NaCl.

The gel point of myosin in PBS, pH 7.0, decreased ($p<0.05$) from 50.3 to 47.8°C as the concentration was increased from 0.5-2.0% (Table 5.4). Storage modulus (G') of myosin increased rapidly above its gel point (Figure 5.7). The rheograms of G' contained two peaks at all myosin concentrations. The G' at each transition peak increased as the concentrations were increased. The first transition peak was observed at about 53-54°C and a second peak occurred at 73°C. A two-transition temperature pattern was previously reported in heat-induced gelation of myosin from live carp dorsal lateral muscle (Sano et al., 1990), chicken breast muscle (Lui, et al., 1996), rabbit *Psaos* major (Boyer, et al., 1996), and chicken breast muscle salt soluble proteins (Smyth et al., 1996). Smyth et al. (1996) suggested that light meromyosin (LMM) and S-1 of chicken breast myosin were responsible for denaturation and aggregation below 55°C, while S-2 unfolded and

Table 5.4. Gel points, storage moduli (G') and tan δ of myosin, and mixed protein solutions of myosin and β-lactoglobulin (β-LG) during heating in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 7.0, during heating from 25 to 90°C, holding at 90°C for 30 min and after cooling to 25°C

Protein Type	Protein concentration (%)	Gel point (°C)	G' at 53°C (Pa)	G' at 73°C (Pa)	G' at 25°C (Pa)	Tan δ at 25°C
Myosin	0.5	50.3 ± 0.01^{b}	4.8 ± 0.7^{b}	29 ± 5.6^{b}	40.4 ± 1.0^{a}	0.16 ± 0.03^{a}
	1.0	49.3 ± 0.40^{c}	17.0 ± 2.0^{c}	83 ± 6.3^{c}	119 ± 6.0^{ab}	0.12 ± 0.01^{ab}
	1.5	48.5 ± 0.15^{d}	36.5 ± 5.0^{d}	147 ± 20.0^{d}	205 ± 49.0^{bc}	0.11 ± 0.02^{ab}
	2.0	47.8 ± 0.10^{e}	76.0 ± 6.2^{e}	306 ± 4.0^{f}	501 ± 37.0^{e}	0.08 ± 0.02^{b}
1% β-LG/ myosin	0.5	51.0 ± 0.06^{a}	2.5 ± 0.2^{a}	4.7 ± 1.0^{a}	341 ± 21.0^{d}	0.09 ± 0.01^{ab}
	1.0	49.3 ± 0.15^{c}	16.0 ± 0.6^{c}	32 ± 1.0^{b}	507 ± 29.0^{e}	0.10 ± 0.01^{ab}
	1.5	48.4 ± 0.20^{d}	42.0 ± 2.5^{d}	92 ± 15.0^{c}	730 ± 33.0^{f}	0.11 ± 0.01^{ab}
	2.0	47.7 ± 0.20^{e}	88.3 ± 5.1^{f}	208 ± 31.0^{e}	835 ± 46.0^{g}	0.10 ± 0.01^{ab}
1% myosin/ β-LG	0.5	49.2 ± 0.40^{c}	16.0 ± 0.8^{c}	49 ± 2.4^{bc}	235 ± 12.0^{c}	0.11 ± 0.01^{ab}
	1.0	49.3 ± 0.15^{c}	15.5 ± 0.6^{c}	32 ± 1.0^{b}	$507 \pm 29.0e$	0.10 ± 0.01^{ab}
	2.0	49.6 ± 0.05^{c}	15.0 ± 3.0^{c}	23 ± 2.0^{ab}	1447 ± 76.0^{h}	0.09 ± 0.02^{ab}
	3.0	49.7 ± 0.30^{c}	14.0 ± 0.9^{c}	17 ± 1.3^{ab}	$3{,}367 \pm 51.0^{i}$	0.09 ± 0.01^{ab}

[a-i] Values are means of three determinations ± standard deviation. Means with different superscripts in each column are significantly different ($p<0.05$).

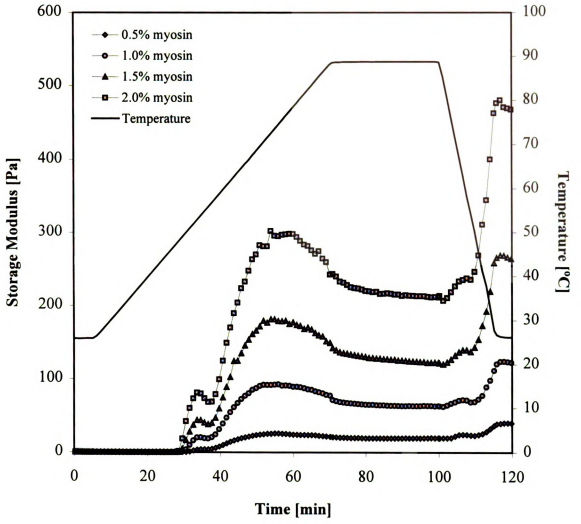


Figure 5.7. Storage moduli of 0.5-2.0% myosin in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 7.0, during heating at 1°C/min from 25 to 90°C, holding at 90°C for 30 min and cooling to 25°C.

aggregated above 55°C. Therefore, the first peak of G' possibly reflected rigidity of the network formed by LMM and S-1, while the aggregation of S-2 completed the protein network and led to the increase of G' at the second peak.

The G' of myosin decreased above 75°C and during holding at 90°C. In contrast, Lui et al. (1996) reported that G' of chicken breast myosin in 0.6M NaCl, pH 6.0 increased during holding at 75°C. Heating at 90°C may extensively unfold protein molecules, facilitating intramolecular hydrophobic interactions and leading to the lowering of gel elasticity. Syneresis was also detected during holding at 90°C, suggesting extensive intramolecular interactions within the protein networks.

The G' of myosin increased sharply during cooling, indicating the formation of hydrogen bonds which enhanced the final rigidity or stiffness of the gels. The G' after cooling of myosin at each concentration were 1.4-1.6 times greater than G' at 73 °C (Table 5.4), indicating the important role of network cross-linking by hydrogen bonding.

In mixed protein solutions containing 0.5% myosin, addition of 1% β-LG increased the gel point and decreased G' of the first transition peak when compared to that of 0.5% myosin (Table 5.4), indicating interruption of myosin network formation. When myosin in mixed systems was increased ≥1.0%, 1% β-LG had no effect on the gel point and the G' at the first transition of myosin. The G' at 73°C and after cooling of mixed solutions increased as myosin concentration was increased, but the G' was still lower than that of myosin at the same concentration. At 90°C, the G' of all mixed solutions were greater than those of myosin at the same concentrations (Figure 5.8). The results suggested that in the mixed system containing ≥ 1% myosin, β-LG had no effect on gel

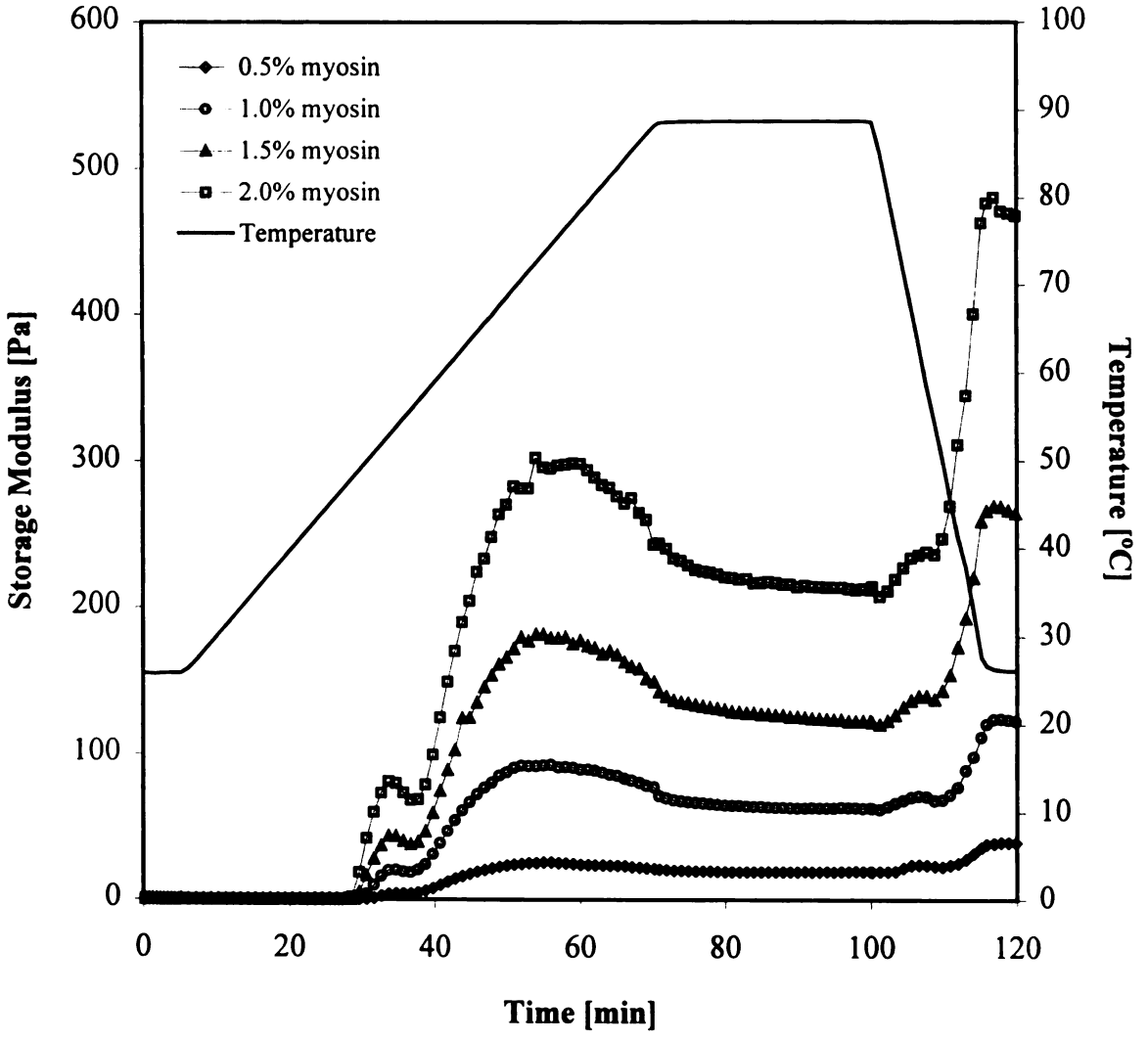


Figure 5.8. Storage moduli of myosin and mixed solutions of myosin and 1.0% β-lactoglobulin (β-LG) in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 7.0, during heating at 1°C/min from 25 to 90°C, holding at 90°C for 30 min and cooling to 25°C.

point but interrupted network formation of myosin during heating at 55-75°C. The gel enhancing effects were obtained when the mixed protein solutions were heated to the temperatures over the gel point of β-LG (80°C). Burgarella et al. (1985) showed that the addition of egg white proteins at a 1:4 ratio with surimi (12% total protein) lowered the rigidity modulus of the gels below 40°C, but strengthened gel rigidity at temperatures above the gelling temperature of both proteins (70°C). Hung and Smith (1993) reported that addition of 4% WPC increased the first G' transition temperature, and increased the magnitude of G' of 12% chicken salt soluble protein in 0.6M NaCl, pH 7.0 when heated to 90°C.

A log scale was used in Figure 5.9 to depict the effects of β-LG concentration on the G' during heating of mixed protein containing 1.0% myosin compared to that of 1.0% myosin. The G' of mixed protein decreased at 55-75°C and increased above 80°C when β-LG concentration was increased from 1% to 3%. The magnitude of the effects increased with increasing β-LG concentration. The final G' of co-gels from 1% myosin mixed with 0.5%, 1%, 2% and 3% β-LG are 2, 4, 12 and 28 times greater than G' of 1% myosin. It was noticed that G' after cooling of 1% myosin/2% β-LG gels was 1447 Pa, almost 2 times greater than 835 Pa of 2% myosin/1% β-LG co-gel. This suggested that G' or stiffness of co-gels could be controlled by an appropriate proportion of myosin and β-LG. The G' enhancing effects could be a result of β-LG aggregation at temperature higher than 80°C. These aggregates filled up the voids within the myosin gel matrix to form a filled gel structure, enhancing rigidity of the co-gels.

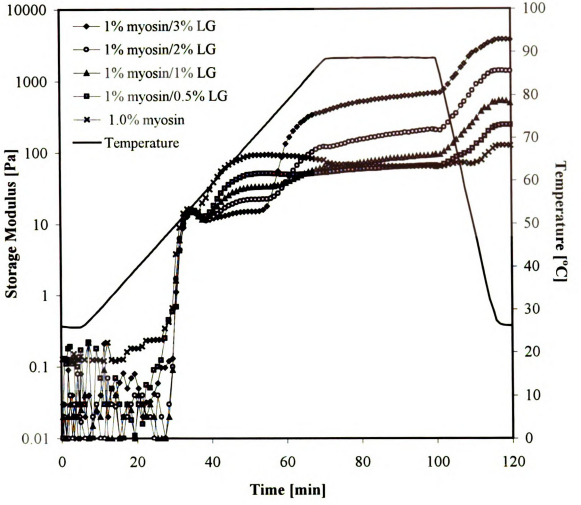


Figure 5.9. Storage moduli of myosin and mixed solutions of 1.0% myosin and 0.5-3% β-lactoglobulin (β-LG) in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 7.0, during heating at 1°C/min from 25 to 90°C, holding at 90°C for 30 min and cooling to 25°C.

Myosin at 1.0% protein or above and myosin/β-LG solution at all proportions exhibited viscoelastic gel characteristic as indicated by the low values of tan δ after cooling (Table 5.4). Observation of polymer shows very high tan δ for dilute solutions, 0.2-0.3 for amorphous polymers, and low (near 0.01) for glassy crystalline polymers and gels (Steffe, 1996).

Gels formed from 2.0% myosin, 4% β-LG and the mixture of 1% myosin/1% β-LG were strong as G' and G" were independent of frequency with G' 8-10 times greater than G" (Figure 5.10). A strong cross-linked gel network is characterized by G' and G" independent of frequency, whereas an entangled weak gel is strongly dependent on frequency (Stading et al., 1992). Myosin had a stronger gel structure than those of β-LG and myosin/β-LG gels as indicated by the larger difference between G' and G". The difference between moduli indicates recovery of the stored energy of the gel. A bigger difference indicates a stronger gel network (Rao, 1999).

5.5. CONCLUSIONS

Based on our results, we propose the following gelation process for mixed solutions of myosin and β-LG. During heating, myosin begins to denature and aggregate below 50°C. The myosin aggregates to form a gel network beginning about 53°C. β-LG remains soluble until its gel point of 73-79°C is reached. In systems containing less than 0.5% myosin, β-LG interrupted myosin gelation at 50-53°C during the aggregation and network formation of the rod region. At myosin concentrations above 1.0%, β-LG

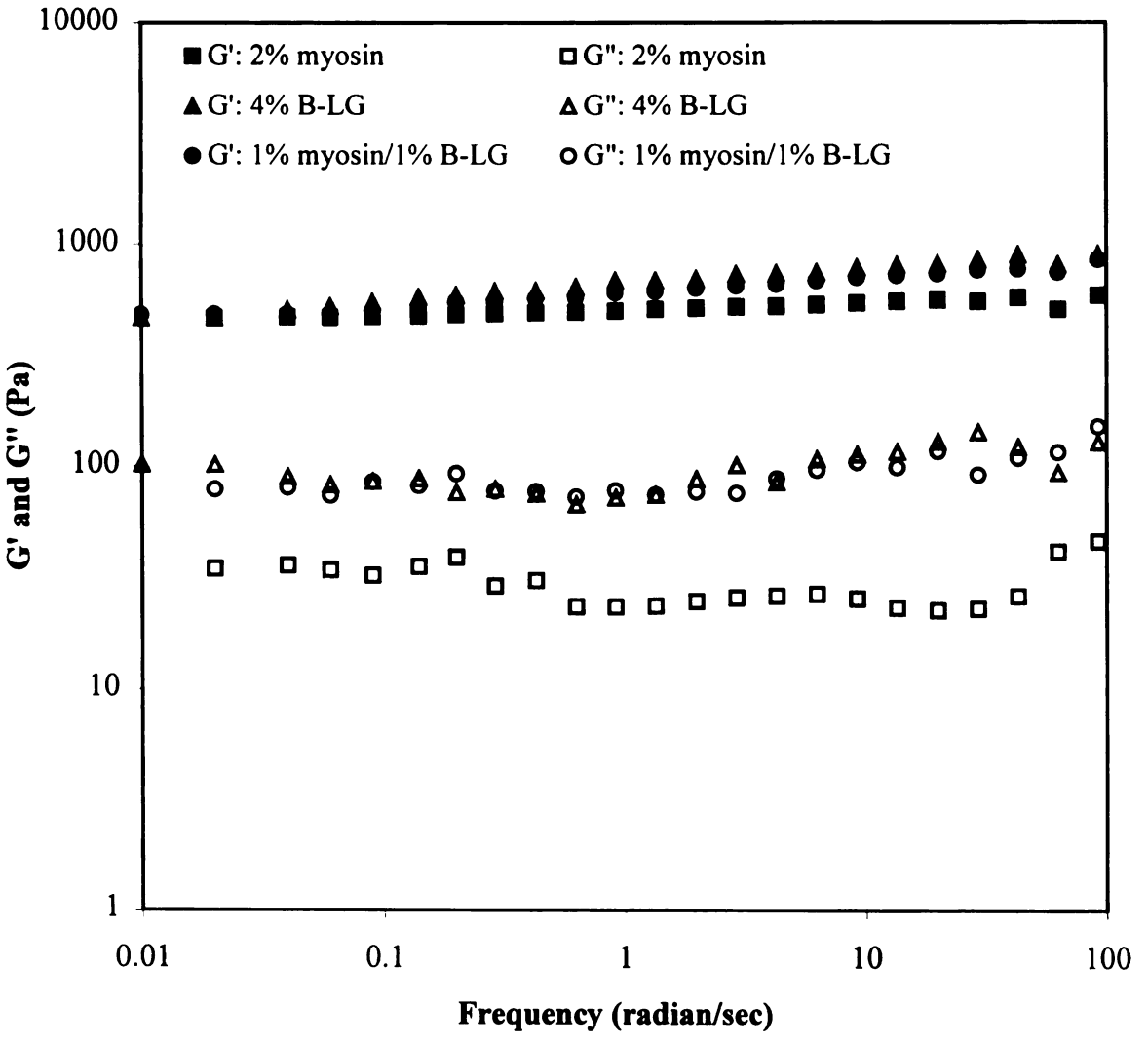


Figure 5.10. Frequency dependence of storage (G') and loss (G") moduli of 2% myosin, 4% β-lactoglobulin (β-LG) and 1% myosin/1% β-LG gels in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 7.0, measured at 25°C. Gels were prepared by heating at 1°C/min from 25 to 90°C, holding at 90°C for 30 min and cooling to 25°C.

interfered with network formation of myosin between 55-75°C. At higher temperatures (>80°C), β-LG began to aggregate which enhanced the G' of the mixed gels. More research is needed to elucidate the type of interactions between β-LG and myosin during gel network formation. As β-LG gels at a higher temperature than myosin, the full synergistic effect expected from addition of β-LG to myosin solutions is not likely to be achieved until the protein mixture is heated to the gelling point of β-LG (>75°C). This experiment showed that β-LG did not co-aggregate with myosin, but interrupted the gel formation of myosin during heat treatment at 55-75°C. This finding indicates a reduced potential for use of whey protein in meat products or in other applications where protein gelation at low temperature is desirable. We hypothesize that rigidity of myosin/β-LG co-gels could be enhanced if the protein networks can be formed simultaneously. A modified β-LG, which has lower gelling temperature than that of the native protein, is needed.

CHAPTER 6: GELLING PROPERTIES OF HEAT-DENATURED β-LACTOGLOBULIN

6.1. ABSTRACT

The thermal denaturation, rheological and microstructural properties of gels prepared from β-lactoglobulin (β-LG) and heat-denatured β-LG (HDLG) were studied. The HDLG was prepared by heating solutions of 4% β-LG in deionized water, pH 7.0, at 80°C for 30 min. This heat treatment caused irreversible denaturation and formation of soluble β-LG aggregates. In 0.6M NaCl, 0.05M sodium phosphate buffer at pH 7.0, HDLG formed a set gel at a minimum concentration of 2% when heated to 71°C, whereas ≥4% protein was needed for β-LG to form set gels at 90°C. The HDLG (3%) gelled at 52°C and had a storage modulus (G') of 2200 Pa after cooling, whereas 3% β-LG had a G' of 160 Pa upon cooling. When the pH was decreased from 7.0 to 6.0, 3% HDLG gelled at 42°C and G' was 3100 Pa after cooling. The HDLG gel microstructure was comprised of strands and clumps of small globular aggregates in contrast to β-LG gels which contained a coarse, particulate network of compacted globules with large voids. The HDLG formed a gel at lower concentration and lower temperature than β-LG, suggesting that thermal denaturation and aggregation during preheating might improve the textural enhancing effect of whey proteins when used in food products processed to 71°C or less.

6.2. INTRODUCTION

Whey protein is highly nutritious and is widely used as a functional ingredient in comminuted and restructured meat products. Heat-induced gelation is an important functional property of whey protein. However, commercial whey protein normally gels above 75°C, which is higher than typical processing temperatures of many food products, such as comminuted meat products. Therefore, the functional effects expected from whey protein gelation in these food systems are limited.

The ability to form stong gels at low temperature broadens the potential use of whey products. Researchers have looked at several ways to lower the gelling temperature of whey protein. Application of hydrostatic pressure to whey protein concentrate (WPC) and whey protein isolate (WPI) promoted sulfhydryl/disulfide interchange reactions, formation of hydrogen bonds and rupture of hydrophobic interactions to induce gel formation of the protein at ambient temperature (Funtenberger et al., 1997). Partially hydrolyzed β-LG prepared by limited proteolysis with trypsin had a lower gel point than native β-LG (Chen et al., 1994). Preheating whey protein produced a product which formed cold-set gels upon addition of salt or acid at ambient temperature or lower (Barbut and Foegeding, 1993; McClements and Keogh, 1995; Roff and Foegeding, 1996; Hongsprabhas and Barbut, 1998; Ju and Kilara, 1998b). The preheated whey proteins formed stranded transparent gels with greater strength and water holding ability than a heat-set whey protein gel prepared with the same concentration of salts.

Cold-set gelation of pre-heated whey protein involves a two-step process. In the first step, heating whey protein at neutral pH under low salt conditions results in the

formation of soluble aggregates of denatured protein. In the second step, gel network formation is induced by increasing ionic strength or changing pH to allow interactions of the soluble aggregates. Ionic strength, pH, protein concentration, time and temperature were reported to be factors affecting soluble aggregate formation of whey protein during preheat treatment (Ju and Kilara, 1998b; Hongsprabhus and Barbut, 1996; Mleko and Foegeding, 1999b; Vardhanabhuti and Foegeding, 1999b). The mechanisms of soluble aggregate formation and gelling properties of heat-denatured whey protein are not yet fully understood.

Doi (1993) reported that a two-step heating method (re-heating the preheated protein) formed stronger and more transparent gels of some globular proteins, such as ovalbumin, bovine serum albumin and egg lysozyme when compared to those formed by single-step heating of the protein in the same NaCl concentration.

Salt-induced cold-set gelation of whey protein aggregates could be beneficial when used in products containing salt and processed at low temperature, such as comminuted meat. Since myosin is the major functional component of meat protein, buffer conditions and heat treatment for myosin gelation (heating to 71°C at 1 °C/min for 60 min in 0.6M NaCl, 0.05M sodium phosphate buffer) was used to study gelling properties of β-LG and HDLG. The objective of this experiment was to compare the thermal denaturation, rheological and microstructural properties of β-LG and HDLG gels prepared by heating to 71°C at 1 °C/min for 60 min in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 6.0, 6.5 and 7.0.

6.3. MATERIALS AND METHODS

6.3.1. Preparation of protein solutions

Bovine milk β-LG (L0310 lot # 114H7055) containing variants A and B was purchased from Sigma Chemical Co. (St. Louis, MO). β-LG was dissolved in 0.6M NaCl, 0.05M sodium phosphate buffer (PBS) at pH 6.0, 6.5 or 7.0 and kept at 4°C before use. Protein concentration was determined by spectrometric absorption using an extinction coefficient ($E^{1\%}$) of 9.55 at 278 nm (Foegeding et al., 1992). Heat-denatured β-LG was prepared based on Ju and Kilara (1998b) by dissolving 4% β-LG in deionized water and adjusting to pH 7.0 with 0.1N NaOH or HCl. The solutions were placed in 16 x 125 mm glass tubes, heated at 80°C for 30 min in a water bath, cooled in an ice bath, and kept at 4°C. The HDLG was diluted with concentrated buffer to prepare solutions of 1.0, 2.0, and 3.0% protein in PBS. Protein solutions were adjusted to the desired concentration immediately before use to avoid gel formation during holding at ambient temperature.

6.3.2. Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to assess the purity of β-LG and to characterize the molecular weight of β-LG aggregates. Electrophoresis was run in a mini-Protean II Dual Slab Cell (Bio Rad Laboratories, Hercules, CA) using a Tris (hydroxymethyl) aminomethane glycine electrode buffer, pH 8.3, and 0.1% SDS as described by Laemmli (1970). The acrylamide concentration of stacking and resolving gels were 4% and 14%, respectively.

β-LG and HDLG solutions were diluted to 4 mg/mL with sample buffer (0.0625M Tris pH 6.8, 2% SDS, 10% glycerol, and 0.2% bromophenol blue), mixed well using a vortex mixer, heated in boiling water for 5 min, and stored at -20°C until use. Sample buffer for reducing conditions also contained 5% of 2-mercaptoethanol. About 5 μL of each protein sample was loaded into a sample well and the gels were run at 85 mA current and 200 constant voltage for 45 min. Gels were stained for at least 20 min with Coomassie Brilliant Blue R250 solution (0.25% in 9:45:45 v/v/v of acetic acid: methanol: water) and were de-stained overnight in acetic acid-methanol-water (6:4:7 v/v/v) solution. Molecular weight of the proteins was determined from their relative mobilities compared to those of standard molecular weight markers (Bio-Rad Laboratories, Hercules, CA) under the same electrophoretic conditions (Weber and Osborn, 1969).

6.3.3. Scanning electron microscopy (SEM) of aggregates

Soluble HDLG aggregates were mixed with an equal volume of 4% glutaldehyde in 0.1M sodium phosphate buffer, pH 7.0, for 1h at 4°C. After fixation, one drop of the suspension was placed on a cover slip coated with poly-L-lysine (Sigma, P1399) and allowed to stand for 5 min. The cover slip was then carefully washed with several drops of distilled water, follow by dehydration, critical drying and coating as previously described except that a 15 nm layer of gold was applied. The HDLG aggregates were examined at an 8 mm working distance using an accelerating voltage of 10 kV and 20,000 x magnification. The HDLG samples were prepared in duplicate and three samples from each preparation were examined.

6.3.4. Differential scanning calorimetry

The thermal denaturation patterns of 1% β-LG and 1% HDLG in deionized water, pH 7.0, and in PBS, pH 6.0, 6.5, and 7.0, were investigated using a MC-2 differential scanning calorimeter (Microcal Inc., Amherst, MA). The protein and blank solutions (deionized water or PBS) were degassed in a vacuum chamber (Nalgene, Fisher Scientific, Pittsburgh, PA) and 1.24 mL were immediately loaded into the DSC using a syringe. Each experiment was conducted in triplicate at a heating rate of 1 °C/min from 25°C to 90°C. Heat capacity profiles (C_p vs temperature) was used to define calorimetric enthalpy (ΔH_{cal}) and endothermic peak or melting temperature (T_m) using the software (DA-2 Data Acquisition and Analysis System) provided by the manufacturer.

6.3.5. Rheological measurement

Dynamic oscillatory tests of 1-3% HDLG and β-LG in PBS at pH 6.0, 6.5 and 7.0 were performed using a controlled stress rheometer (RS 100, Haake, Karlsruhe, Germany) equipped with a 35 mm diameter stainless steel parallel plate. A circulating water bath was used to control temperature. Storage (G') and loss (G") moduli were recorded continuously at a fixed frequency of 0.464 Hz. using constant stresses (producing strains from 0.1-0.3%) within the range of linear viscoelastic behavior determined from stress sweeps performed for each protein at 90°C and after cooling to 25°C. Protein solutions were loaded between the parallel plate and base of 1.0-1.1 mm gap. A few drops of maize oil (Mazola, Best Food, CPC International, Inc., Englewood Cliffs, NJ) were used to cover the edge of the plate to prevent evaporation. Solutions

were equilibrated at 25°C for 5 min, heated from 25 to 71°C at 1°C/min, held at 71°C for 60 min, cooled to 25°C within 10 min and held at 25°C for 5 min. In addition, the β-LG solutions were also heated to 90°C, held at 90°C for 30 min and cooled to 25°C within 15 min. Each experiment was performed in triplicate. The gel point was determined as the temperature at which G' and G" crossed over in the fixed frequency test.

6.3.6. Scanning electron microscopy (SEM) of gels

The β-LG and HDLG gels were prepared at 4% and 2% protein, respectively, in PBS, pH 6.0, 6.5, and 7.0. One milliliter of each protein solution was transferred into a 12 mm x 75 mm glass tube and sealed with Teflon™ tape. The HDLG were heated in a programmable water bath (Model 9510, PolyScience, Niles, IL) from 25°C to 71°C at 1°C/min, held for 60 min, and cooled in an ice bath. β-LG solutions were heated from 25 to 90°C at 1°C/min and held for 30 min before cooling. Protein gels were cut into 1 x 2 x 2 mm pieces, fixed in 2.0% glutaldehyde in 0.1M sodium phosphate buffer, pH 7.0, for 3h at 4°C and post-fixed in 0.1% osmium tetraoxide overnight at 4°C. Fixed gels were rinsed with 0.1M sodium phosphate buffer and dehydrated by immersion in a graded ethanol series of 25, 50, 75, and 95% for 20 min per step, followed by three steps in 100% ethanol. Gels were dried using a Balzers carbon dioxide-critical point dryer (Balzers CPD, FL-9496, Balzers, Liechtenstein) and coated with a 25-30 nm gold layer in an Emscope ion-sputter coater (Emscope Laboratories Ltd., Ashford, Kent, UK). Gel structures were observed with a JEOL scanning microscope (Model JSM-6400V, version 96-2, Tokyo, Japan) at 15 mm working distance using an accelerating voltage of 10-12

kV and 5,000x magnification. Gels were prepared in duplicate and three samples from each preparation were examined in the SEM.

6.3.7. Experimental design and statistical analysis

All experiments were conducted in triplicate. Differences in gel point and storage moduli of gels due to treatments (protein concentration and pH) were statistically analyzed using one way analysis of variance (ANOVA). Means were compared to determine significant differences between treatments using the Tukey-Kramer HSD test with the mean square error at 5% level of probability (JMP Version 3.2.2, SAS Institute Inc., Cary, NC).

6.4. RESULTS AND DISCUSSION

6.4.1. Electrophoresis

The electrophoretic patterns of β-LG and HDLG resolved by SDS-PAGE under non-reducing and reducing condition are shown in Figure 6.1. A band corresponding to β-LG monomer (18.4 KDa) was observed under both conditions. SDS-PAGE of HDLG contained a similar protein band under reducing condition, while only a minor band of β-LG at 18.4 KDa and some proteins too large to pass through the stacking gel were observed using non-reduced SDS-PAGE. The results suggested that a small amount of β-LG was present in HDLG. A weak band of about 37 KDa was observed in both native β-LG and HDLG. It may be a dimeric form of β-LG.

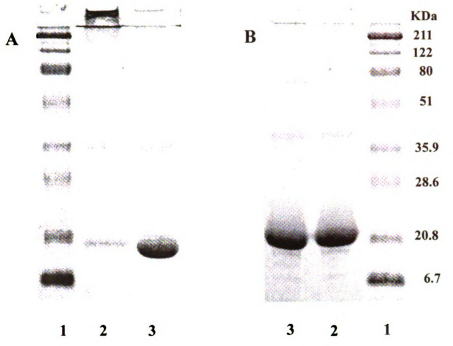


Figure 6.1. Electrophoregram of (A) nonreduced and (B) reduced native and heat-denature β-lactoglobulin: Lane 1, molecular weight markers; Lane 2, heat-denature β-lactoglobulin; and Lane 3, β-lactoglobulin.

Prabakaran and Damodaran (1997) reported that β-LG dimers were formed via disulfide linkages between monomers during heating at 60-65°C, pH 7.0. A similar protein band was found in β-LG A, B, and C heated to 72°C for 12.5 min (Manderson et al., 1998). When heated to higher temperatures, these dimers further formed larger aggregates or polymers which did not pass through a 4% stacking gel of SDS-PAGE under non-reducing conditions, suggesting that disulfide bonding played a dominant role in the formation of HDLG aggregates.

6.4.2. Microstructure of heat-denatured β-lactoglobulin aggregates

Heat-denatured β-LG was comprised primarily of small globular aggregates of 30-50 nm, although some of the globules appeared to associate into larger clumps and long strands (Figure 6.2). Native β-LG has a diameter of 3-5 nm (Elofsson et al., 1996), suggesting HDLG aggregates were formed by several β-LG molecules. The HDLG aggregates were similar in size to those of whey protein aggregates (21-61 nm) prepared by heating 3-9% WPI at 80°C for 30 min, although WPI aggregates were comprised of β-LG, α-LG, bovine serum albumin and immunoglobulin (Ju and Kilara, 1998b; 1998e). Since disulfide bonds and hydrophobic interactions were found to be important in polymerization of whey protein and β-LG (Prabakaran and Damodaran, 1997; Mleko and Foegeding, 1999b; Vardhanabhuti and Foegeding, 1999a), the mechanisms and factors affecting the formation of soluble aggregates of whey protein and β-LG could be similar. The size and shape of protein polymers greatly affect network formation of gels (Wang and Damodaran, 1990). Studies of optimum conditions for the formation of β-LG

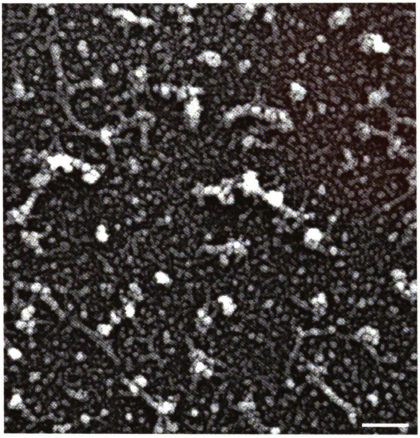


Figure 6.2. Scanning electron microscopic image of heat-denatured β-lactoglobulin aggregates at 20,000x magnification. Bar = 1.0 μm.

polymers could provide information for manipulation of gelling properties of whey protein polymers.

6.4.3. Thermal denaturation of β-lactoglobulin and heat-denatured β-lactoglobulin

The thermograms of 1% β-LG and 1% HDLG in deionized water, pH 7.0, were different (Figure 6.3A). β-LG melted at 79.0°C. Foegeding et al. (1992) reported a denaturation temperature (T_m) of 77.3 °C when 10% β-LG was heated in deionized water at 10°C/min. The difference of T_m is probably due to different protein concentrations used in the experiments. A more cooperative denaturation peak (narrower peak) and lower T_m are often observed as the protein concentration is increased (Relkin and Launay, 1990; Qi et al., 1995). No thermal transitions were found for HDLG, suggesting that pre-heating of β-LG at 80°C for 30 min resulted in irreversible denaturation of the protein molecules. The irreversible denaturation of β-LG and whey protein above 80°C has been previously reported (McClements and Keogh, 1995). However, De Wit and Klarenbeek (1981) and Paulsson et al. (1985) suggested that the complete denaturation of β-LG probably occurred between 130-140°C as indicated by a second distinct denaturation peak of β-LG. It is possible that HDLG was a stabilized form of partially denatured or molten globular form of β-LG occurred during preheating. The molten globule was defined as a unique molecular state with partially folded conformation, retaining some native structures, but being distinctive from fully forms (Li-Chan, 1995).

The denaturation profiles of HDLG in PBS at pH 6.0, 6.5 and 7.0 showed small exothermic peaks at about 56, 67 and 71°C, respectively (Figure 6.3B). Since DSC

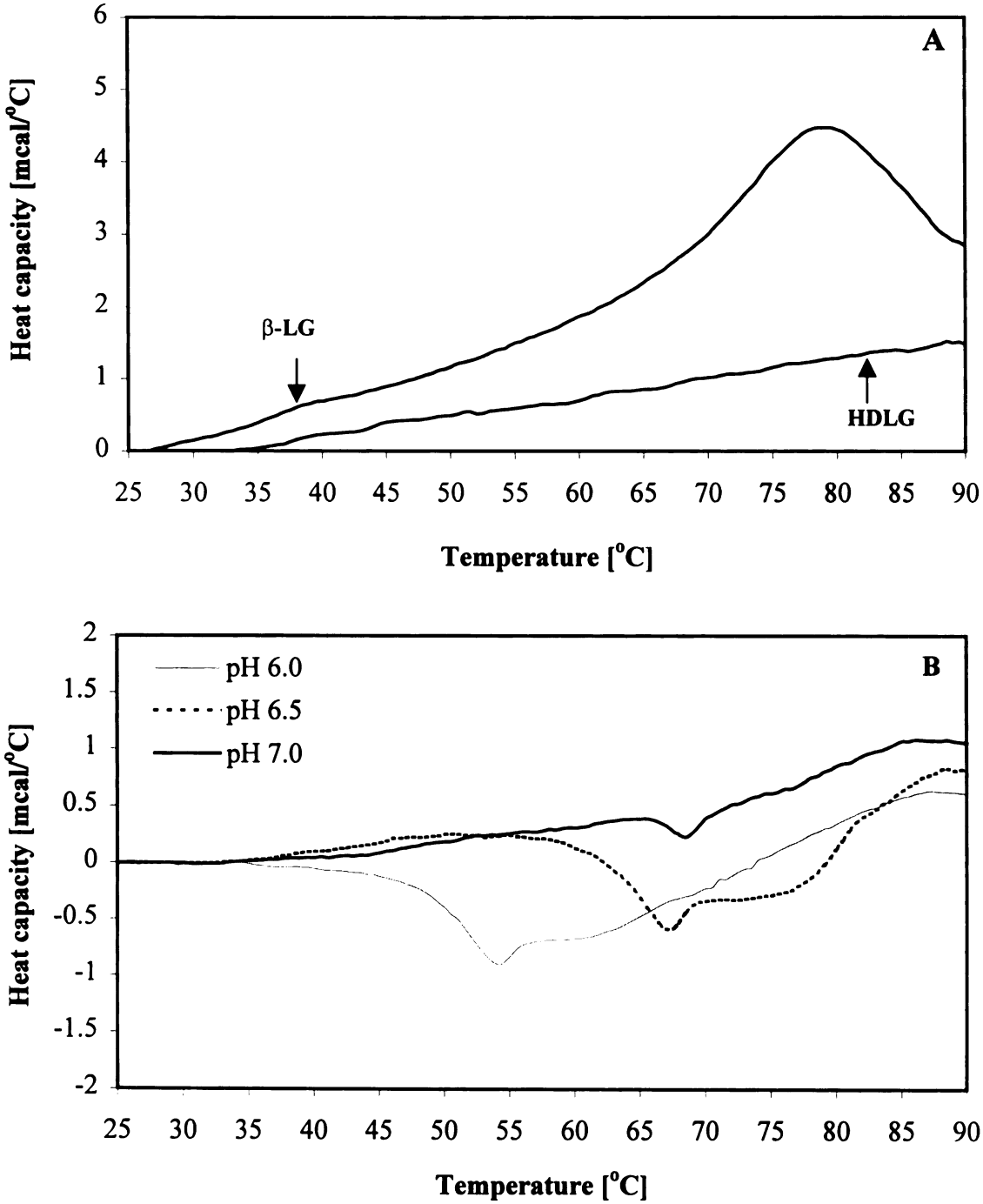


Figure 6.3. Heat capacity profiles of (A) 1.0% β-lactoglobulin (β-LG) and 1.0% heat-denatured β-LG (HDLG) in deionized water, pH 7.0, and (B) 1.0% HDLG in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 6.0, 6.5 and 7.0.

simultaneously detects denaturation and aggregation enthalpy (Gotham et al., 1992), the exothermic peaks were possibly a reflection of HDLG aggregation in the presence of 0.6M NaCl. Additional experiments using a higher concentration of HDLG and/or different concentrations and types of salts are needed to reveal whether or not these exothermic peaks indicate HDLG aggregation.

6.4.4. Rheological properties of heat-denatured β-lactoglobulin gels

During heating to 90°C and holding for 30 min, the gel points of β-LG in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 7.0, decreased from 79°C at 1.0% protein to 73°C at 3.0% protein. The G' of β-LG could not be measured accurately during holding and cooling due to precipitation and shrinkage of the gels which led to rapid fluctuations of G' (Figure 6.4). This may be a result of using a lower concentration of β-LG than optimal to set a gel. McSwiney et al. (1994) reported that 5% is the critical concentration of β-LG to form gels at 80°C in 0.1M NaCl at pH 7.0. When heated to 71°C and held at 71°C for 60 min in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 7.0, G' of 1-3% β-LG were very low (4-9 Pa), indicating lack of network formation when heated at a temperature lower than its gel point.

Heat-denatured β-LG formed gel networks without precipitation as indicated by a continuous increase of G' during heating at 71°C and cooling (Figure 6.5). At pH 7.0, the gel point of HDLG decreased ($p<0.05$) by 19°C and G' after cooling increased 137 fold as protein concentration was increased from 1% to 3% (Table 6.1). Gels formed by

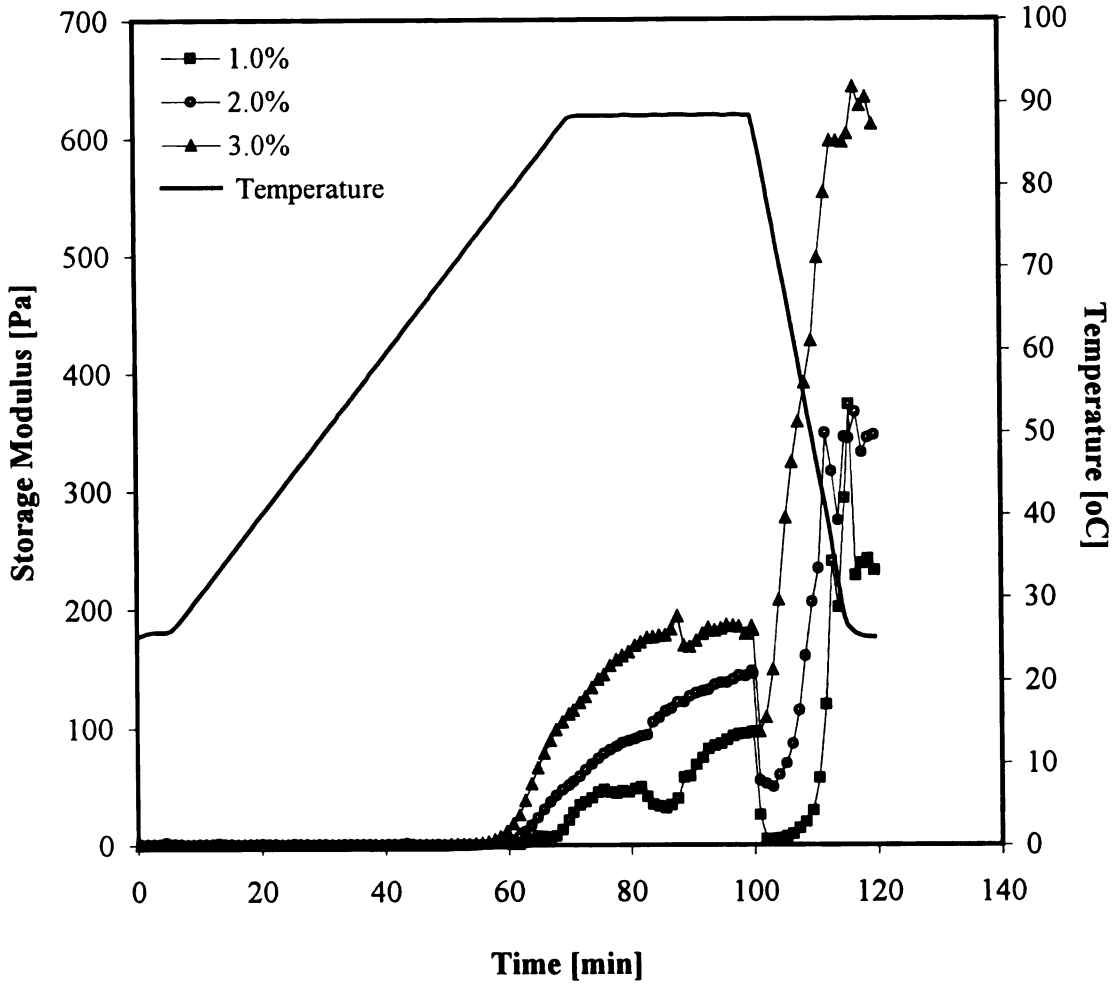


Figure 6.4. Storage moduli of β-lactoglobulin in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 7.0, during heating at 1°C/min from 25 to 90°C, holding at 90°C for 30 min and cooling to 25°C.

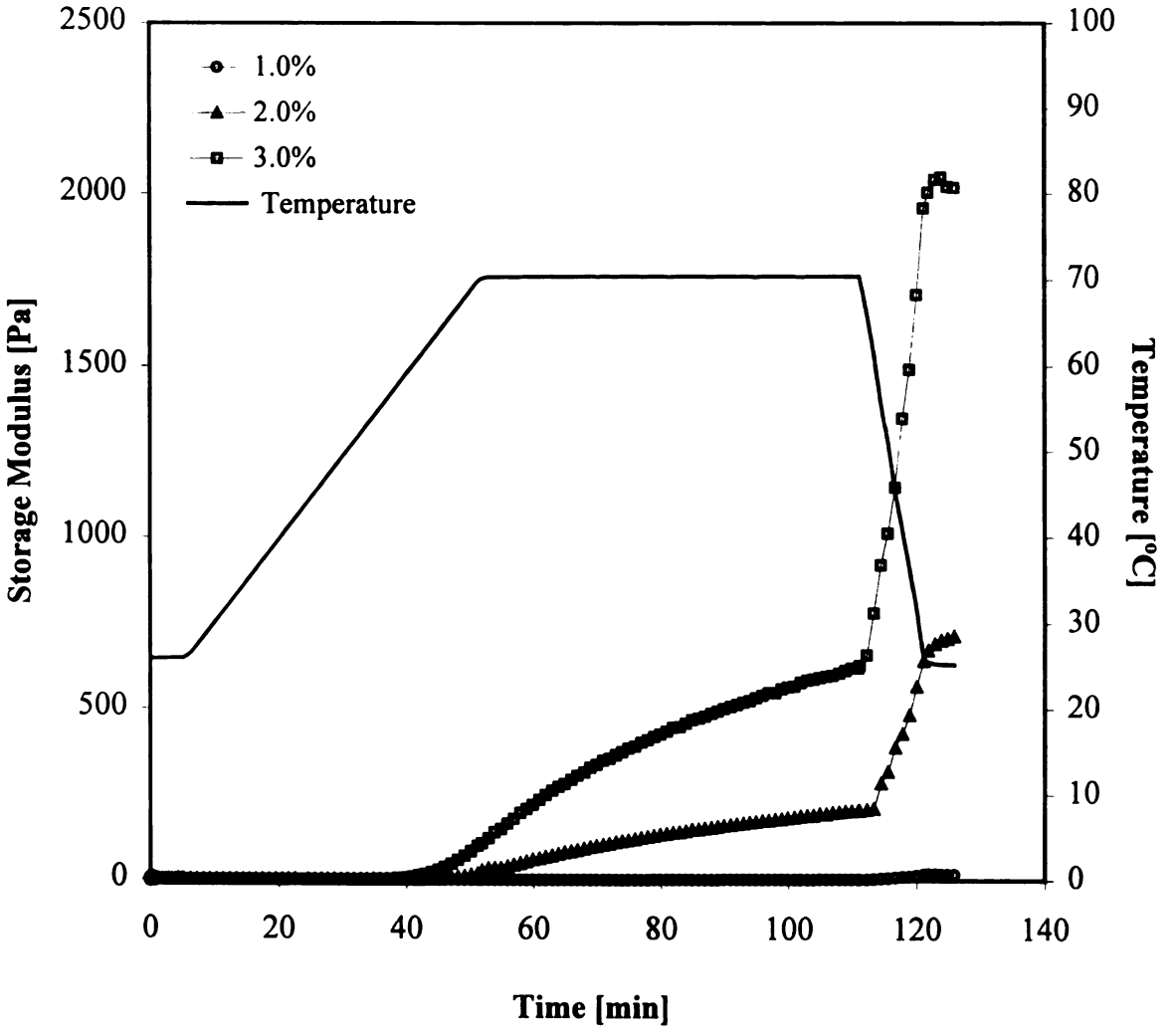


Figure 6.5. Storage moduli of 1-3% heat-denatured β-lactoglobulin in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 7.0, during heating at 1 °C/min from 25 to 71°C, holding at 71°C for 60 min and cooling to 25°C.

Table 6.1. Gel point, storage moduli (G') and tan delta (tan δ) after cooling of 1-3% heat-denatured β-lactoglobulin (HDLG) heated to 71°C in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 6.0, 6.5 and 7.0[1]

Parameter	Protein Concentration	PH		
		6.0	6.5	7.0
Gel point (°C)	1	63.0 ± 0.5^{a,x}	71°C^{a,y}	71°C^{a,y}
	2	49.0 ± 0.9^{b,x}	58.5 ± 0.5^{b,y}	62.0 ± 0.5^{b,z}
	3	42.0 ± 0.5^{c,x}	50.0 ± 1.0^{c,y}	52.5 ± 1.0^{c,z}
G' (Pa)	1	100 ± 14^{a,x}	40 ± 11^{a,y}	16 ± 2^{a,y}
	2	860 ± 50^{b,x}	700 ± 108^{b,x}	585 ± 58^{b,x}
	3	3,100 ± 57^{c,x}	2,700 ± 150^{c,x}	2,200 ± 140^{c,x}
Tan δ	1	0.12 ± 0.01^{ax}	0.14 ± 0.02^{ax}	0.13 ± 0.01^{ax}
	2	0.10 ± 0.02^{bx}	0.10 ± 0.00^{bx}	0.10 ± 0.01^{bx}
	3	0.10 ± 0.00^{bx}	0.09 ± 0.01^{bx}	0.09 ± 0.00^{bx}

[1] Values are means of three observations ± standard deviation.
[a, b, c] Means with different superscripts in each column are significantly different ($p<0.05$).
[x, y, z] Means with different superscripts in each row are significantly different ($p<0.05$).

1% HDLG were very weak (16 Pa at pH 7.0 and 100 Pa at pH 6.0), suggesting that more than 1% HDLG is needed for the formation of a strong gel network.

The results show HDLG formed a gel at concentrations higher than 1% whereas, higher than 4% of β-LG was needed to form gel when heated to 71°C in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 7.0. A lower gelling temperature and greater gel rigidity have been previously reported for a preheated WPI when compared to unheated whey protein (McClements and Keogh, 1995). In 0.2M NaCl, pH 7.0, 9% preheated WPI gelled at 48°C, whereas the unheated WPI formed a gel at 77°C or higher. The complex modulus after cooling of the preheated WPI was much higher than that of untreated WPI. Other globular proteins, such as ovalbumin, bovine serum albumin and egg lysozyme have been reported to form more transparent and harder gels by reheating the proteins which have been preheated without addition of salts (Doi, 1993).

Effects of pH on gelling properties of HDLG were studied (Figure 6.6). When the pH was decreased from 7.0 to 6.0, the gel point of HDLG at all concentrations decreased about 10-12°C (Table 6.1). The decrease in gel point at pH 6.0 may be due to an acceleration of aggregation resulting from the neutralization of the more negatively charged molecule at pH 7.0. Melko and Foegeding (1999a) reported that a weak gel was formed from preheated WPI when pH was decreased from 8.0 to 6.0 without addition of salt. The rigidity of the gels was increased with an increase of temperature from 7 to 25°C, suggesting that both electrostatic and hydrophobic interactions were involved in gel network formation.

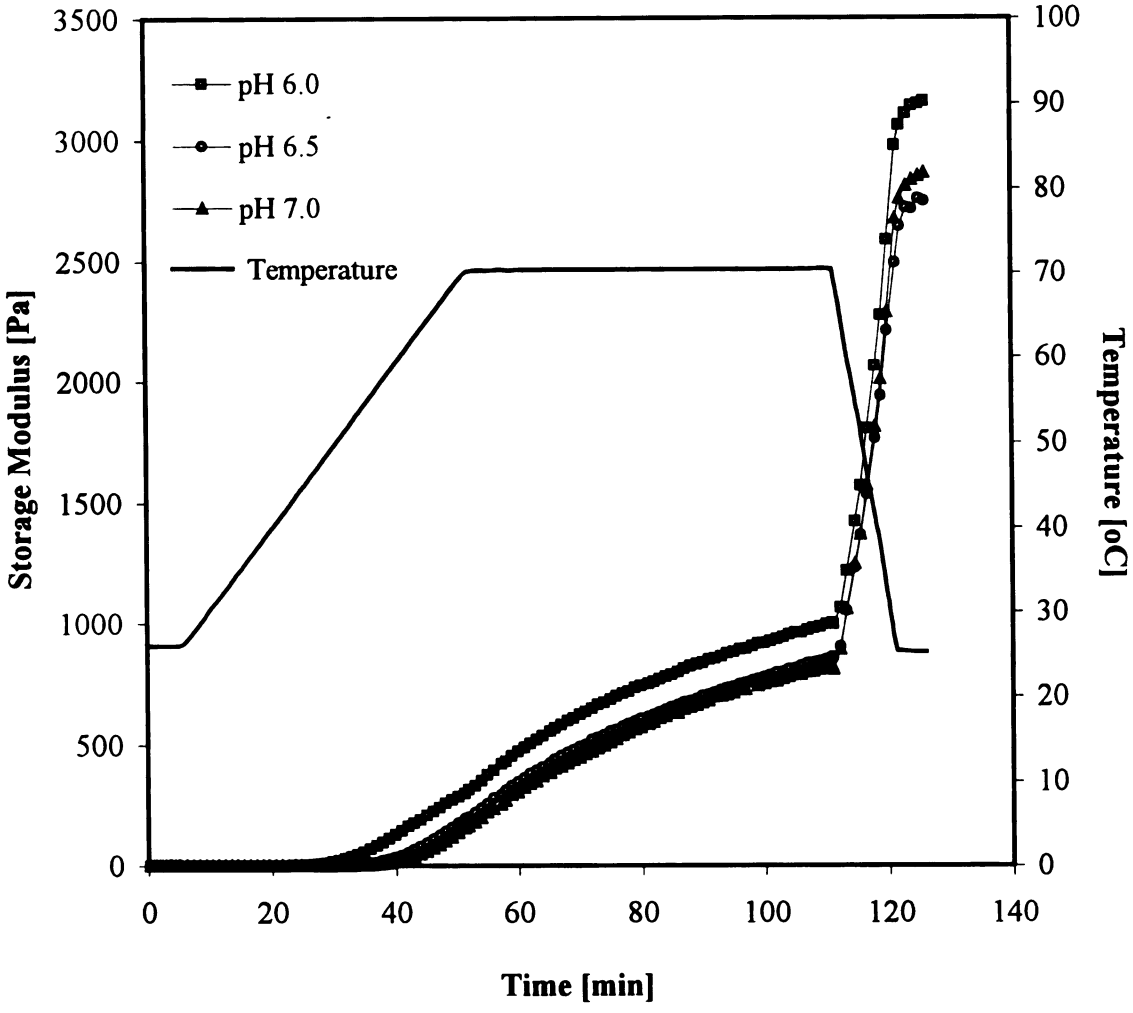


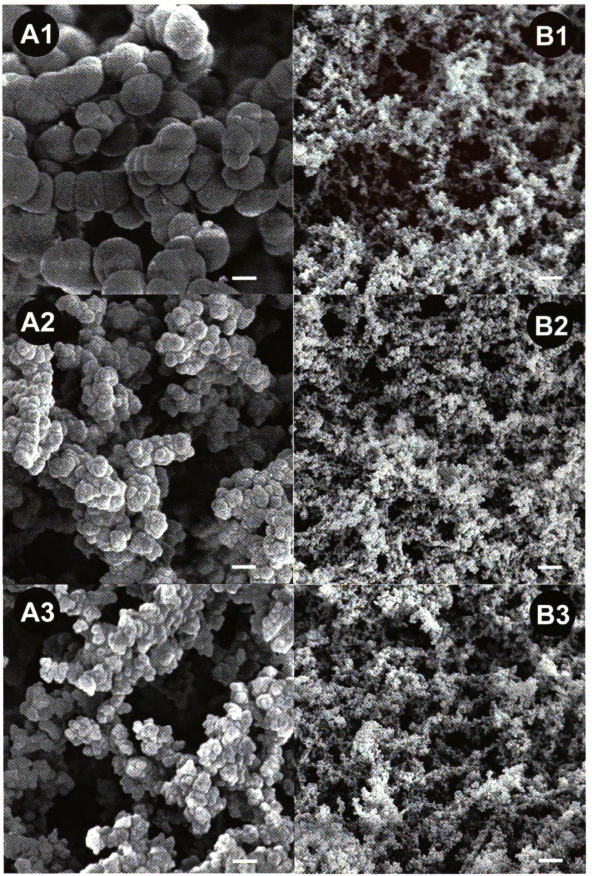
Figure 6.6. Storage moduli of heat-denatured β-lactoglobulin in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 6.0, 6.5 and 7.0, during heating at 1 °C/min from 25 to 71°C, holding at 71°C for 60 min and cooling to 25°C.

Tan δ after cooling of 2.0-3.0% HDLG gels were low (≤0.1) at pH 6.0, 6.5 and 7.0 (Table 6.1), indicating a typical viscoelastic of gel (Steffe, 1996). The G' of HDLG after cooling were the same ($p>0.05$) at all pHs (Table 6.1). The results suggested that electrostatic interactions caused by pH changes affected the aggregation step, while hydrophobic interactions during heating contributed to the formation and strengthening of the HDLG gel network. However, the ultimate gel structure and strength also depend on salt type and concentration. The combined effect of 0.6M NaCl, pH, and temperature contributed to HDLG gel properties observed.

6.4.5. Microstructure of β-lactoglobulin and heat-denatured β-lactoglobulin gels

Microstructures of 4% β-LG gels prepared at 90°C for 30 min and those of 2% HDLG gels prepared by heating at 71°C for 60 min were compared (Figure 6.7). β-LG formed particulate gels comprised of grape-like compacted globular aggregates. Globule diameter decreased from 1-3 μm at pH 6.0 to 0.2-0.4 μm at pH 7.0. These globule sizes were similar to those of β-LG gels prepared by heating 0.9% β-LG in 0.5M NaCl, pH 7.0, at 68.5°C for 1.5h (Verheul et al., 1998). HDLG formed a protein network comprised of small globules of 30-70 nm diameter (determined by comparing with bar size in enlarged images) arranged in clumps and strands. Based on the size and shape of the HDLG soluble aggregates (Figure 6.7), the HDLG gel networks appeared to form via cross-linking of the primary soluble aggregates. When compared to β-LG gels, the HDLG gel structure was much finer with smaller void spaces.

Figure 6.7. Scanning electron microscopic images at 5,000 x magnification of (A) 4% β-lactglobulin gels heated to 90°C for 30 min and (B) heat-denatured β-lactoglobulin gels heated to 71°C for 60 min in 0.6M NaCl, 0.05M sodium phosphate buffer, (1) pH 6.0, (2) 6.5, and (3) 7.0. Bar = 1.0 μm


A1
B1
A2
B2
A3
B3

No change in the microstructure of HDLG gels was observed when the pH was decreased from 7.0 to 6.0. The G' of HDLG gels also did not change with pH (Table 6.1). The HDLG gels were visually less opaque than β-LG gels corresponding to the differences in microstructures. β-LG gel networks were comprised of 50 fold bigger aggregates than those of HDLG gels, resulting in more opacity. Many studies have shown a relationship between the network microstructure and the opacity of the gels. Cold-set gels with larger aggregates and bigger pore sizes were more opaque than gels with finer structure (Hongsprabhus and Barbut, 1998). Bigger aggregates form a gel network with the higher opacity due to increased light scattering (Barbut and Foegeding, 1993; Foegeding et al., 1998).

6.5. CONCLUSIONS

Pre-heating at 80°C for 30 min in distilled water at pH 7.0 changed the molecular characteristics of β-LG leading to alteration of its gelling properties. The results from this experiment indicated that β-LG formed primary aggregates from denatured molecules via disulfide bonding during the preheat treatment. When reheated at 71°C in 0.6M NaCl, networks of HDLG gels were formed through the secondary association of the primary aggregates. The two-step aggregation process allowed β-LG to form a finer gel structure at a lower protein concentration and lower temperature when compared to a one-step aggregation process using untreated β-LG. The results are similar to those obtained using preheated WPC and WPI (Barbut and Foegeding, 1993; McClements and Keogh, 1995; Hongsprabhus and Barbut, 1996; Ju and Kilara, 1998b, 1998e). The cold-

set whey proteins form gels at lower temperatures than traditional WPC or WPI and may allow increased use of whey proteins in a variety of applications where protein gelation at low temperature is desirable. Cold-set whey protein could also be used to prepare food with functional properties different from those of traditional whey ingredients.

This experiment was carried out at temperatures and pH conditions favorable for myosin gelation, confirming the potential use of whey protein aggregates in meat systems. More experiments are needed to understand interactions between HDLG and myosin in mixed systems. An understanding of factors influencing the characteristics of the primary aggregates, which ultimately govern the textural properties of the final gels, will provide direction for achieving desirable functional properties of whey products.

CHAPTER 7: INTERACTIONS OF HEAT-DENATURED β-LACTOGLOBULIN WITH MYOSIN DURING HEAT-INDUCED GELATION

7.1. ABSTRACT

Effectiveness of whey proteins to enhance texture of processed meat products is often variable as the major component, β-lactoglobulin (β-LG), does not form a gel at typical processing temperatures (ca. 68-71°C). Pre-heated whey protein isolates (WPI) gel upon addition of salt at temperatures well below those for the native proteins. To understand the functions of preheated whey proteins in meat systems, heat-induced gelation mechanisms of a model system using myosin and heat-denatured β-LG (HDLG), were studied.

The HDLG was prepared by heating 4% (w/v) β-LG solutions in deionized water, pH 7.0 at 80°C for 30 min. Thermal denaturation patterns of myosin and myosin/HDLG at pH 6.0 and 7.0 were similar, except for the appearance of an endothermic peak at 56°C and 54°C, respectively, in the mixed system. When heated to 71°C at pH 7.0, 2% myosin began to gel at 48°C and had a storage modulus (G') of 500 Pa after cooling. Mixed solution of 1% myosin/1% HDLG gelled at 48°C and G' was 650 Pa after cooling, whereas myosin/β-LG gelled at 49°C but the G' was lower (180 Pa). As the pH was decreased, the gel points of myosin and myosin/HDLG decreased and G' increased. When compared to 2% myosin gels, myosin/β-LG gel structures were comprised of

thicker stranded networks with larger void spaces, whereas myosin/HDLG gels had similar fine stranded networks with additional clumps of small globules. In the co-gel systems heated to 71°C, β-LG remained soluble, whereas HDLG was incorporated within the myosin network. Co-gel formation between preheated whey and meat proteins when heated at 71°C led to enhanced gel stiffness. Lowering the gelling temperatures of whey protein may lead to their increased use in meat products.

7.2. INTRODUCTION

Whey protein has been widely used as a functional food ingredient. In meat products, whey protein is used to improve color, yield and textural quality. Heat induced gelation of whey protein was reported to be responsible for texture enhancement of some meat products. However, the effect of whey protein on textural attributes of meat products is often variable. Commercial whey proteins do not form gels at typical meat processing temperatures (ca. 68-71°C), which limit their potential for textural attributes.

Many studies have looked at the cold-set gel properties of pre-denatured whey protein aggregates (Roff and Foegeding, 1996; McClements and Keogh, 1995; Hongsprabhus and Barbut, 1996; 1998, Ju and Kilara, 1998b). Heating whey protein solution without addition of salt leads to formation of denatured soluble aggregates or polymers. Cold-set gels of the aggregates can be induced by increasing the ionic strength through the addition of salts, lowering the pH or by proteolytic digestion. The gels formed from pre-heated whey proteins were reported to be clearer and harder than those from untreated whey protein gels. The preheating of protein solutions before heat-induced

gelation (two-step heating process) also enhanced the gel properties (harder and clearer gels, and able to gel at broader range of NaCl concentrations) of other globular proteins, such as bovine serum albumin, ovalbumin and egg lysozyme (Doi, 1993).

Recently, Hongsprabhus and Barbut (1999) studied the textural properties and water-holding capacity of comminuted chicken mixed with preheated whey protein isolate (WPI). They found that addition of 2% pre-heated WPI aggregates (prepared by heating at 80°C for 30 min, pH 7.0) in conjunction with cold set gelation (6h at 1°C) lowered cook loss and improved hardness of the comminuted chicken after cooking to 78°C at salt concentrations of 1.5% or below, when compared to unheated WPI. More work is needed to understand interactions between whey protein aggregates and meat proteins during heating to optimize the use of whey protein in meat products.

We have previously studied heat-induced gelation of β-LG and myosin, the major functional components of meat and whey protein. The synergistic effect expected from addition of β-LG to myosin solutions was not achieved until the protein mixture was heated to the gelling point of β-LG (over 75°C). At 55-75°C, β-LG interfered with network formation of myosin. We have also studied gelling properties of β-LG compared to heat-denatured soluble aggregates of β-LG (HDLG). The HDLG was prepared by preheating β-LG solutions at 80°C for 30 min at pH 7.0. At pH 6.0-7.0 and 0.6M NaCl (optimum for myosin gelation), HDLG formed a gel with a finer microstructure and with much higher gel stiffness at lower protein concentration and temperature than β-LG.

The salt-induced gelation of heat-denatured soluble aggregates of β-LG at low temperature indicates a potential benefit for the use of the whey aggregates in meat

products. Salt is commonly added to meat products to extract salt soluble protein and for flavor. The objective of this experiment was to characterize interactions between myosin and β-LG or HDLG during heat-induced gelation in 0.6M NaCl at pH 6.0-7.0.

7.3. MATERIALS AND METHODS

7.3.1. Protein solution preparation

Myosin from breast muscle (*M. pectoralis*) was extracted immediately after sacrifice from 9 week-old commercial type broilers, and stored in 48% saturation of $(NH_4)_2SO_4$ and 30% glycerol at –20°C (Wang and Smith, 1994b). Immediately prior to use, myosin was suspended in 0.6M NaCl, 50 mM sodium phosphate buffer (PBS) at the desired pH, dialyzed against three changes of the same buffer for 48h and centrifuged at 78,000 x g to remove denatured proteins. Solutions of 1% and 2% myosin were prepared by dilution of this stock solution with PBS at the desired pH.

Bovine milk β-LG, containing valiants A and B, was purchased from Sigma Chemical Co. (St. Louis, MO). β-LG solutions were prepared by dissolving the protein in PBS, pH 7.0, overnight at 4°C. Heat-denatured β-LG was prepared by dissolving 4% (w/v) β-LG in deionized water and pH was adjusted to 7.0 with 0.1M HCl. The solution was heated in a water bath at 80°C for 30 min, then cooled to 4°C in an ice bath. To prepare 2.0% HDLG solutions, HDLG was diluted 1:1 with 1.2M NaCl, 0.1M sodium phosphate buffer.

Mixtures of 1% (w/v) myosin and 1% (w/v) β-LG or HDLG were prepared by mixing together equal amounts of 2% myosin and 2% β-LG or 2% HDLG. Mixed

protein solutions were prepared immediately before use. Protein concentrations were determined by absorption using extinction coefficients ($E^{1\%}$) of 5.5 at 280 nm for myosin (Smyth et al, 1996) and 9.55 at 278 nm for β-LG (Foegeding et al, 1992).

7.3.2. Differential scanning calorimetry

The thermal denaturation patterns of 1% myosin, 1% myosin/1% β-LG (myosin/β-LG) and 1% myosin/1% HDLG (myosin/HDLG) in PBS, pH 6.0 and 7.0, were investigated using a differential scanning calorimeter (DSC) (MC-2, Microcal Inc., Amherst, MA). The protein and buffer solutions (PBS) were degassed in a vacuum chamber before loading into 1.24 mL capacity cells of the DSC. Experiments were conducted at a scan rate of 1°C/min from 25°C to 90°C. Heat capacity profiles (endotherm; C_p vs temperature), calorimetric enthalpy (ΔH_{cal}) and endothermic peak or melting temperature (T_m) were obtained by using the software (DA-2 Data Acquisition and Analysis system) provided by the manufacturer. DSC scans were performed at least in triplicate using samples from two separate preparations.

7.3.3. Dynamic rheological measurement

A controlled stress rheometer (RS 100, Haake,Karlsruhe, Germany) equipped with a 35 mm diameter stainless steel parallel plate was used to monitor storage modulus (G') and loss modulus (G") during heat-induced gelation of 1% and 2% myosin, myosin/β-LG and myosin/HDLG solutions. The tests were performed at a fixed frequency of 0.464 Hz. Using constant stresses (producing strains from 0.1-0.3%) within

the range of linear viscoelastic behavior determined from stress sweeps performed for each protein at 71°C and after cooling to 25°C. About 1.0 mL of each protein solution was loaded between the plate and base with a gap of 1.0-1.1 mm. A few drops of maize oil (Mazola, Best Food, CPC International, Inc., Englewood Cliffs, NJ) were used to cover the edge of the plate to prevent evaporation. The solutions were equilibrated at 25°C for 5 min, heated to 71°C or to 90°C at 1°C /min, held for 60 min at 71°C or 30 min at 90°C, cooled to 25°C within 10 or 15 min, and held at 25°C for 5 min. The frequency (0.01-100 radian/sec) sweep tests were conducted at the end of the cooling step using stress which was controlled to produce a strain of 0.1-0.2%. The gel point was defined as the temperature at which G' and G" crossed over on the rheogram. All experiments were performed in triplicate.

7.3.4. Scanning electron microscopy (SEM)

To prepare protein gels, 1.0 mL of each protein solution (myosin, myosin/β-LG or myosin/HDLG) was transferred into 12 mm x 75 mm glass tubes and sealed with Teflon™ tape. Tubes were placed in a water bath (PolyScience, model 9510, Niles, IL) programmed to heat from 25°C to 71°C or to 90 at 1°C /min, and hold for 60 at 71°C or 30 min at 90°C. The samples were cooled in an ice bath. Protein gels were cut into 1 x 2 x 2 mm pieces. Specimens were prefixed for 3h in 2.0% glutaldehyde buffered with 0.1M sodium phosphate pH 7.0, and postfixed overnight in 0.1% osmium tetraoxide. Fixed gels were rinsed thoroughly with 0.1M sodium phosphate buffer, pH 7.0, and dehydrated in a graded series of ethanol (25, 50, 75, and 95%) for 20 min each followed by three 20 min

changes in 100% ethanol. Gels were then dried using a Balzers carbon dioxide-critical point dryer (Balzers CPD, FL-9496, Balzers, Liechtenstein) and coated with a 25-30 nm gold layer in an Emscope ion-sputter coater (Emscope Laboratories Ltd., Ashford, Kent, UK). Protein gel microstructures was observed with a JEOL scanning microscope (Model JSM-6400V, version 96-2, Tokyo, Japan) using an accelerating voltage of 12 kV at a magnification of 5000x. Gels were prepared in duplicate and three pieces from each gel were examined by SEM.

7.3.5. Soluble proteins expressed from co-gels

Proteins remaining in the liquid phase of the protein gel networks were quantified by Bradford protein assay and identified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). A half milliliter of 2% myosin, 4% β-LG, 2% HDLG, myosin/β-LG, and myosin/HDLG solutions were heated in 1.5 mL micro-centrifuge tubes using the heating schedule previously described for SEM gel preparation. The caps of all tubes were punctured with a needle to prevent them from popping up during heating. After cooling, the protein gels were centrifuged at 10,000 x g for 30 min (Sorvall RC 5B/Plus, Sorvall Products, Newtown, Connecticut). The supernatants were collected and weighed.

Protein concentration in the supernatants was determined following the method of Bradford (1976) using bovine serum albumin as the protein standard. Absorbance was measured at 595 nm in Minireader II (Thermomax, Molecular Devices Corp, Menlo Park,

CA), and the protein concentration was estimated by regression analysis (Softmax program version 231, Molecular Devices).

SDS-PAGE was used to identify soluble protein expressed from gels by centrifugation. Electrophoresis was run in a Mini-Protein II Dual Slab Cell (Bio Rad Laboratories, Hercules, CA) using a Tris (hydroxymethyl) aminomethane (Tris)-glycine electrode buffer, pH 8.3, containing 0.1% sodium dodecyl sulfate (SDS) as described by Laemmli (1970). The acrylamide concentrations of stacking and resolving gels were 4% and 12%, respectively. Protein solutions were diluted 1:2 with sample buffer (0.0625M Tris pH 6.8, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, and 0.2% bromophenol blue), mixed well using a vortex mixer and heated in boiling water for 5 min. About 10 μL of each protein sample were loaded into the sample wells and the gels were run at 80 mA current and 200 volts constant voltage for 40 min. Gels were stained for at least 20 min with Coomassie Brilliant Blue R250 solution (0.25%) in acetic acid:methanol:water (9:45:45 v/v/v) and were destained overnight in acetic acid-methanol-water (6:4:7, v/v/v) solution. Molecular weight of the proteins was estimated from their relative mobilities compared to those of molecular weight standard (Bio-Rad Laboratories) run under the same electrophoretic conditions (Weber and Osborn, 1969).

7.4. RESULTS AND DISCUSSION

7.4.1. Thermal denaturation of mixed proteins

Denaturation patterns of myosin showed two T_m at 45°C and 66°C at pH 6.0 and three T_m at 48.5, 53.2 and 57°C at pH 7.0 (Figure 7.1A and B). The calorimetric

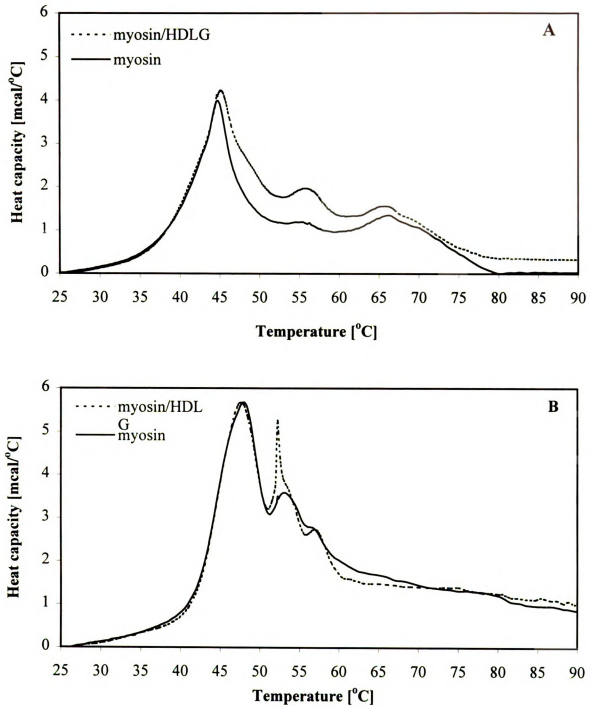


Figure 7.1. Heat capacity profiles of 1.0% myosin and 1% myosin/1% heat-denatured β-lactoglobulin (HDLG) in 0.6M NaCl, 0.05M sodium phosphate buffer, (A) pH 6.0 and (B) pH 7.0.

enthalpies (ΔH_{cal}) of myosin at pH 6.0 and 7.0 were 2138 ± 74 and 2310 ± 80 Kcal/mol, respectively. Two T_m at 48°C and 67°C with ΔH_{cal} of 2140 Kcal/mol at pH 6.0 (Lui et al., 1996), while four T_m at 49.2, 50.2, 57.2 and 66.8°C with ΔH_{cal} of 2215.8 Kcal/mol at pH 6.5 (Wang and Smith, 1994b) have been previously reported for chicken breast myosin (*pectoralis*). Myosin is a multi-domain protein and has a complex thermal denaturation pattern (Smyth et al., 1996). Many studies have previously indicated that transition peaks (T_m) may vary due to differences in ionic strength and pH of the myosin solutions. At pH 6.0 unfolding of chicken breast myosin domains occurred over a broader temperature range (45-66°C) than at pH 7.0 (48.5-57°C).

The thermograms of myosin/HDLG mixtures were similar to those of pure myosin at the same pH, except for the increase in transition peak of myosin at 56°C (pH 6.0) and the appearance of an additional endothermic peak at 54°C (pH 7.0) (Figure 7.1A and B). The source of this signal is unclear. Since HDLG was irreversibly denatured and had no transition peak (data from section 6.4.3), the new peak might indicate interaction between the two proteins.

7.4.2. Rheological properties

The G' of 1% and 2% myosin in 0.6M NaCl, pH 7.0, increased during heating and cooling (Figure 7.2). Myosin at 2% gelled at a lower temperature (47.8°C) than 1% myosin which reached its gel point at 49.0°C. The G' began to increase after the gel point, formed a transition peak at 53°C and then increased rapidly between 58 and 71°C before reaching a plateau when held at 71°C. The G' decreased during the first 5 min of

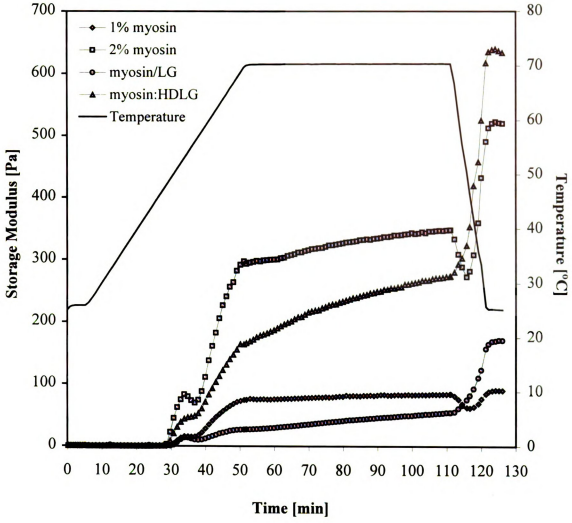


Figure 7.2. Storage moduli of myosin, 1% myosin/1% β-lactoglobulin (β-LG) and 1% myosin/1% heat-denatured β-LG (HDLG) in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 7.0, during heating at 1°C/min from 25 to 71°C, holding at 71°C for 60 min and cooling to 25°C.

cooling and then increased rapidly throughout the cooling step. The G' reached a minimum during cooling at 46-47°C, regardless of the protein concentration (1.0 or 2.0%). A similar G' curve was reported during heating of chicken myosin in 0.6M NaCl, pH 6.0 (Lui and Foegeding, 1996). The authors noticed that the temperatures corresponding to the minimum G' during cooling were close to the onset temperatures of gelation, agreeing with our observation. During heating, hydrogen bonds decrease while the hydrophobic interactions of protein increase leading to gel network formation (Lui and Foegeding, 1996). The decrease of G' at the beginning of the cooling step may reflect relaxation of the gel networks before reformation of hydrogen bonds which lead to an increase of gel stiffness during further cooling.

Differences in G' during heating of myosin, myosin/β-LG and myosin/HDLG in 0.6M NaCl, pH 7.0 can be compared in Figure7.2. When compared to 1% myosin, myosin/β-LG had lower G' above 55°C, but G' of the gel was greater during cooling. In contrast, the G' of myosin/HDLG was greater than myosin throughout the heat treatment. The addition of β-LG or HDLG had no effect on the gel point, but altered the G' above the gel point of 1% myosin (Table 7.1). The results suggest that β-LG may interfere with myosin gel network formation during heating above 55°C and holding at 71°C, while HDLG enhanced network formation of the mixed protein gels.

In previous experiments, we found that HDLG had a lower gel point and gelled at a lower concentration than β-LG. However, 1% HDLG did not form a gel at pH 7.0. The increase in G' of myosin/HDLG co-gels may be due to formation of a "coupled gel

Table 7.1. Gel point, storage moduli (G') and tan delta (tan δ) after cooling of myosin, 1% myosin/1% β-lactoglobulin (myosin/β-LG) and 1% myosin/1% heat-denatured β-LG (myosin/HDLG) in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 7.0, heated to 71°C for 60 min or 90°C for 30min[a]

Protein	Temperature (°C)	Total protein concentration (%)	Gel point (°C)	G' after cooling (Pa)	Tan δ after cooling
Myosin	71	1	49.0 ± 0.30	120 ± 6	0.08 ± 0.00
	71	2	47.8 ± 0.10	500 ± 37	0.08 ± 0.00
Myosin/HDLG	71	2	48.0± 0.20	650 ± 27	0.08 ± 0.00
Myosin/β-LG	71	2	49.0 ± 0.30	180 ± 16	0.11 ± 0.02
Myosin/β-LG	90	2	49.2 ± 0.20	500 ± 29	0.10 ± 0.01

[a] Values are means of three observations ± standard deviation.

network" rather than "phase separated gel network" as described by Brownsey and Morris (1988). Besides intermolecular hydrophobic interactions, which normally occur during heating, disulfide bonds might be involved in the protein network formation. Shiga et al. (1985) suggested that availability of sulfhydryl-groups of soy protein increased with an increase in preheating time (0-4 min) and enhanced the gel strength of a soy and meat protein system. Chicken breast muscle myosin possess 43 thiol groups which formed intermolecular disulfide bonds, contributing to myosin gel network development during heating at 45-54°C (Smyth et al., 1998b). More work is needed to understand the mechanism of network formation of HDLG and myosin co-gels.

The G' did not decrease during cooling when myosin was mixed with either β-LG or HDLG, suggesting that the gel matrices of mixed protein were formed differently from myosin gel networks. The G' after cooling of myosin/HDLG gels heated to 71°C was 650 Pa or 3.6 fold greater than the myosin/β-LG gel and 1.3 fold greater than the 2% myosin gel (Table 7.1). The G' of myosin/HDLG gels heated to 71°C was also greater than that of myosin/β-LG gel or 2% myosin (500Pa) heated to 90°C. Results indicated that HDLG enhanced gel stiffness of myosin at temperatures lower than that needed for β-LG. This could be attributed to a lower gel point and partially unfolded state of HDLG, allowing for co-aggregation and network formation of myosin and HDLG during heating. A similar finding has been previously reported by Hongsprabhus and Barbut (1999). Addition of 2% pre-heated WPI aggregates (prepared at 80°C for 30 min, pH 7.0) in conjunction with cold set gelation (6h at 1°C) improved hardness of comminuted chicken after cooking to 78°C when compared to the use of unheated WPI. Hardness and

deformability of co-gels formed with 4% SSP and 12% WPC in 0.6M NaCl heated at 65°C were enhanced by low solubility WPC, whereas a higher temperature (90°C) was needed for mixtures with highly soluble WPC to enhance the hardness of the co-gels (Beuschel et al., 1992). Hung and Smith (1993) found that when heated at 65°C, the elasticity of a combination gel of SSP and low solubility WPC was higher than that of a combination gel containing highly soluble WPC. The WPC of low solubility was prepared using higher temperatures and longer holding times than highly soluble WPC. Therefore, this low solubility WPC may in fact, have similar properties as whey protein aggregates or HDLG.

Two percent myosin and myosin/HDLG exhibited viscoelastic gel characteristic as indicated by their tan delta (tan δ) values after cooling (Table 7.1) and frequency dependence curves of G' and G" (Figure 7.3). Polymer systems show high tan δ for dilute solutions, 0.2-0.3 for amorphous polymers, and low (near 0.01) for glassy crystalline polymers and gels (Steffe, 1996). The G' and G" were independent of frequency and G' was 8-10 times greater than G" throughout the frequency range. Frequency independence (low slope) describes a strong cross-linked gel network, whereas an entangled weak gel is strongly dependent on frequency (Stading et al., 1992). The G" of myosin/β-LG was increased at high frequency resulting in a smaller difference between G' and G". A difference between moduli indicates a lower recovery of stored energy which is a characteristic of weak gels (Rao, 1999). The results suggested that myosin/β-LG formed a weaker gel than 2% myosin and myosin/HDLG.

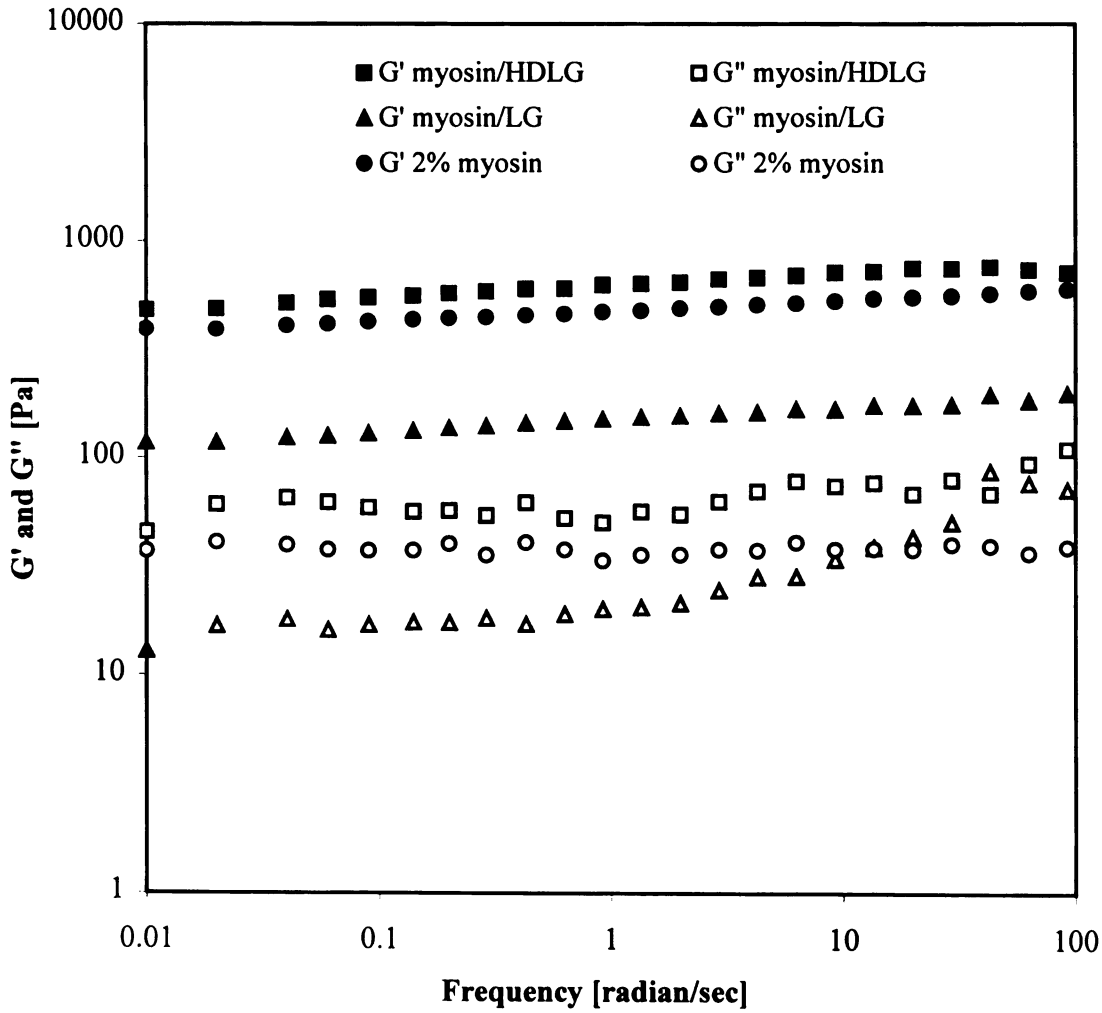


Figure 7.3. Frequency dependence of storage (G') and loss (G") moduli of 2% myosin, 1% myosin/1% β-lactoglobulin (β-LG) and 1% myosin/1% heat-denatured β-LG (HDLG) gels in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 7.0, measured at 25°C. Gels were prepared by heating at 1°C/min from 25 to 71°C, holding at 71°C for 60 min and cooling to 25°C.

The effects of pH on rheological properties of myosin and myosin/HDLG were examined (Figure 7.4). As the pH was decreased from 7.0 to 6.0, the gel points of both myosin and myosin/HDLG decreased. The gel point of 1% myosin decreased from 49°C at pH 7.0 to 44°C at pH 6.0. Meanwhile the gel point of myosin/HDLG decreased from 48°C at pH 7.0 to 43.5°C at pH 6.0 (Table 7.2). Since the gel points of 1% myosin and myosin/HDLG were similar at the same pH, it can be concluded that pH mainly affected the gel point of myosin in the mixed protein system. Conformation of myosin and charge distribution on its surface is altered by pH leading to changes in protein-protein interactions (Xiong, 1992). Wang et al. (1990) reported that chicken salt soluble protein reached the first G' transition at a lower temperature at pH 5.5 than at pH 6.5 and 7.5. The G' after cooling increased for both myosin and myosin/HDLG gels when pH was decreased. The G' after cooling of 1% myosin at pH 6.0 (510 Pa) was 4.25 times greater when compared to that at pH 7.0 (120 Pa) (Table 7.2). Maximum gel rigidity of myosin from chicken breast was found at pH 5.4 to 5.9 in 0.6M KCl (Asghar et al., 1984; Morita et al., 1987). The G' after cooling of myosin/HDLG gels at pH 6.0 was 2.3 times greater than that of the gel at pH 7.0. The effects of pH on G' was less in the mixed system than in myosin gels. However, the final G' of myosin/HDLG gels at pH 6.0-7.0 remained 3-5 times greater than that of 1% myosin gel at the same pH.

7.4.3. Microstructure of mixed protein gels

Microstructures of myosin gel networks were dependent on ionic strength and pH. When heated at 71°C, 2% myosin formed a fine stranded protein network comprised of small globular aggregates at pH 6.5 and 7.0 (Figure 7.5). A similar structure has been

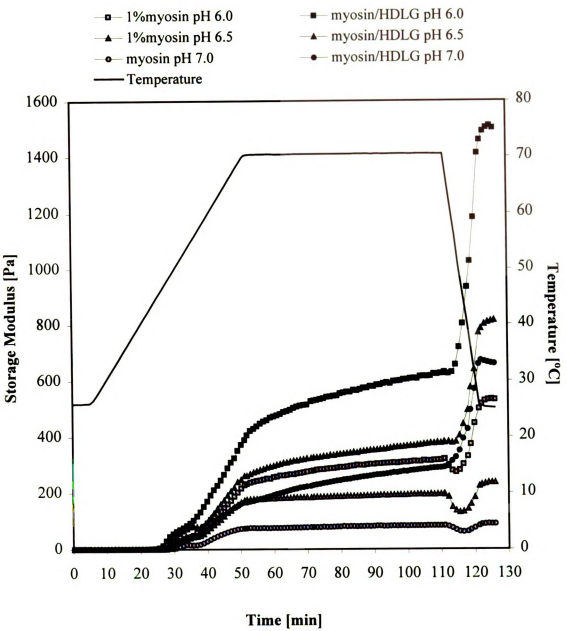


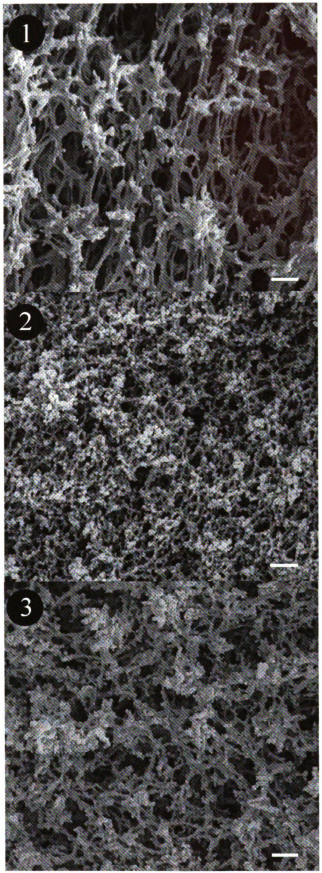
Figure 7.4. Storage modulus of 1% myosin and 1% myosin/1% heat-denatured β-lactoglobulin (HDLG) in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 6.0, 6.5 and 7.0, during heating at 1 °C/min from 25 to 71°C, holding for 60 min and cooling to 25°C.

Table 7.2. Gel point, storage moduli and tan δ after cooling of myosin and 1% myosin/1% heat-denatured soluble aggregates of β-LG (myosin/HDLG) heated at 71°C for 60 min in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 6.0, 6.5 and 7.0[a]

Property	pH	1% Myosin	2% Myosin	Myosin/HDLG
Gel point	6.0	44.2 ± 0.3	40.8 ± 0.3	43.5 ± 0.1
(°C)	6.5	47.2 ± 0.3	46.3 ± 0.3	46.8 ± 0.3
	7.0	49.0 ± 0.3	47.8 ± 0.1	48.0 ± 0.1
Storage moduli	6.0	510 ± 23	2,350 ± 240	1,482 ± 30
after cooling (Pa)	6.5	235 ± 4	580 ± 30	781 ± 29
	7.0	120 ± 6	500 ± 37	650 ± 16
Tan δ after cooling	6.0	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01
	6.5	0.1 ± 0.02	0.07 ± 0.01	0.09 ± 0.01
	7.0	0.12 ± 0.01	0.08 ± 0.01	0.08 ± 0.01

[a] Values are means of three observations ± standard deviation.

Figure 7.5. Scanning electron microscopic images of 2% myosin gels heated to 71°C in 0.6M NaCl, 0.06M sodium phosphate buffer, (1) pH 6.0; (2) 6.5; and (3) 7.0 at 5,000 x magnification. Bar = 1 μm.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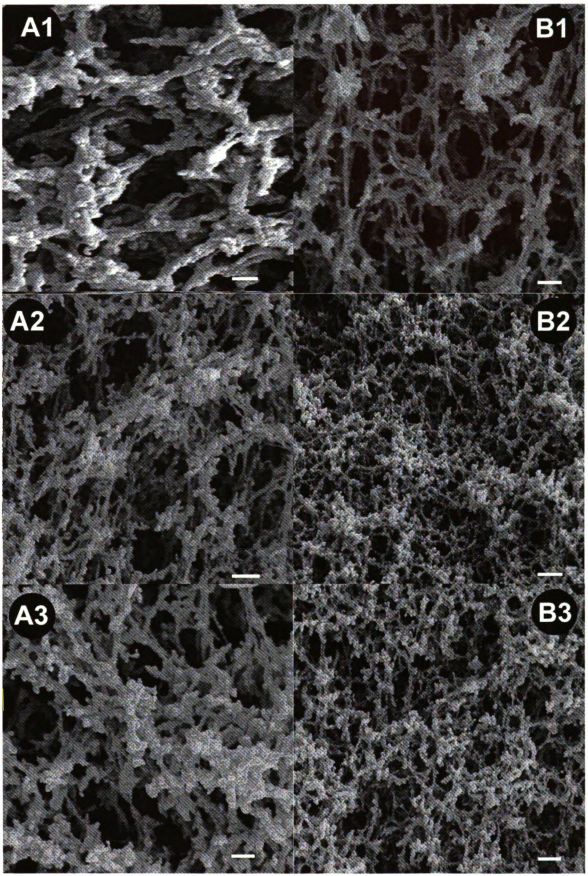
previously reported in rabbit myosin gels formed in 0.6M KCl at pH 7.0 (Haga and Ohashi, 1984). The myosin network at pH 6.0 contained thicker, smoother strands than those formed at a higher pH. Rabbit *Psoas major* myosin formed at pH 6.0 in 0.6 KCl had a very similar gel ultrastructure (Boyer et al., 1996).

Myosin/β-LG gels heated to 71°C at all three pHs contained coarser, thicker protein strands with larger voids when compared to 2% myosin gels at the same pH (Figure 7.6). The amount of myosin in myosin/β-LG gels was one half of that in 2% myosin gels. Moreover, some of soluble proteins were washed out during fixing as indicated by the dark color of the fixing solution, resulting from interactions between protein and osmiun tetraoxide. Rheological experiments showed a decrease in G' during heating of myosin/β-LG, indicative of interruption in network formation of the proteins. Thus, the large void spaces observed in myosin/β-LG co-gels could be a result of a lower functional protein concentration and/or caused by disruption of the myosin network.

Myosin/HDLG gels had a finer protein networks and smaller voids in the gel network when compared to myosin/β-LG gels at all pHs (Figure 7.6). The protein networks in myosin/HDLG gels were denser than myosin/β-LG gel networks, indicating that more proteins have been incorporated within the co-gel networks. This resulted in a higher G' of myosin/HDLG gels when compared to myosin/β-LG gels.

The microstructures of myosin/HDLG gel networks contained a homogeneous granular structure at pH 6.5 and 7.0, similar to that of 2% myosin gels at the same pH (Figure 7.5 and 7.6). At pH 6.0, myosin/HDLG gels had larger voids and a more uneven network when compared to 2% myosin gels, suggesting that interaction of myosin and

Figure 7.6. Scanning electron microscopic images of (A) 1% myosin/1% β -lactoglobulin and (B) 1% myosin/1% heat-denatured β-lactoglobulin co-gels heated to 71°C in 0.6M NaCl, 0.06M sodium phosphate buffer, (1) pH 6.0; (2) 6.5; and (3) 7.0 at 5,000 x magnification. Bar = 1 μm.


A1
B1
A2
B2
A3
B3

HDLG at pH 6.0 resulted in a more disordered network structure. There was no distinct phase separated regions in the gel structure and the gel matrix of myosin/HDLG gels as dense as that of 2% myosin gels. Heat-denatured β-LG did not form a gel at 1% concentration, suggesting that HDLG and myosin possibly formed a co-gel in which both proteins participated in network formation (coupled gel) (Brownsey and Morris, 1988), especially at pH 6.5 and 7.0.

7.4.4. Soluble proteins expressed from co-gels

Myosin light chains (LCs) were observed in the supernatants expressed from myosin gels heated at 71°C (lane 9) and 90°C (lane 5) (Figure 7.7). The LC-1 and LC-3 was detected in supernatant expelled from myosin gels heated to 71°C, while LC-2 was also found when myosin gels were heated to 90°C. These proteins were present in very low concentration in unheated myosin (lane 2). The dissociation of LC from myosin by heat treatment has been previously reported (Dreizen and Richards, 1972; Sharp and Offer, 1992). The LC fractions were also observed in supernatant of both myosin/HDLG (lane 7) and myosin/β-LG gels (lane 4 and 8). Since the molecular mass of LC-2 (18.6 KDa) and β-LG (18.4 KDa) are similar, it was difficult to determine which protein was responsible for the middle band in SDS-PAGE (lane 4) of soluble protein from myosin/β-LG gel heated to 90°C. Myosin light chains have been identified in supernatant expressed from mixed protein gels of myosin and 11S soy protein (Peng et al., 1982), chicken breast muscle salt soluble protein and WPI, WPC, or soy protein isolate (McCord et al., 1998), and salt soluble protein and β-LG (Smyth et al., 1998a). Myosin heavy chain was not

Figure 7.7. Electrophoretogram of proteins expressed by centrifugation at 10,000 x g from myosin, β-lactoglobulin (β-LG), heat-denatured β-LG (HDLG), myosin/β-LG, and myosin/HDLG gels heated to 71°C and 90°C. Each lane was loaded with 10 μL of sample: Lan 1, unheated β-LG; Lane 2, unheated myosin; Lane 3, β-LG heated to 90°C; Lane 4, myosin/β-LG gels heated to 90°C; Lane 5, myosin gels heated to 90°C; Lane 6, HDLG heated to 71°C; Lane 7, myosin/HDLG heated to 71°C; Lane 8, myosin/β-LG heated to 71°C; Lane 9, myosin heated to 71°C; Lane 10, broad range standard molecular weight markers.

detected in the supernatants of myosin and mixed protein gels, suggesting that it was a principal contributor to the network formation of both myosin and mixed protein gels.

β-LG remained soluble in myosin/β-LG gels heated to 71°C as indicated by an additional band (lane 8) when compared to myosin (lane 9). More β-LG was expressed from myosin/β-LG gels heated to 71°C (lane 8) when compared to that from the mixed gels heated to 90°C (lane 4). This agreed with results from total soluble protein analysis (Figure 7.8). When heated to 90°C, the amount of total protein in the supernatant expelled from myosin/β-LG gels decreased regardless of the increase of the light chain fraction of myosin. The results suggested that more β-LG aggregated or was incorporated into the network of the mixed protein gels when heated to 90°C than at 71°C. Hung and Smith (1993) studied microstructures of co-gels formed with 4% chicken breast muscle salt soluble protein and 12% WPC in 0.6M NaCl, pH 7.0. They reported that the co-gels showed the typical fibrous structure of salt soluble protein at 65°C, whereas the globular structure of WPC was observed in the co-gel networks heated at 90°C.

A small amount of β-LG was found in the supernatant expressed from both HDLG and myosin/HDLG gels heated to 71°C (lane 6 and 7), indicating that some β-LG monomers remained in HDLG. This fraction did not participate in network formation of either HDLG or myosin/HDLG gels heated to 71°C. The total amount of soluble protein expressed from myosin/HDLG gel at 71°C was less than that of myosin/β-LG gels heated to 71 and 90°C, indicating more protein was incorporated within the gel network. This contributed to a denser protein network (Figure 7.5) and a greater G' of myosin/HDLG

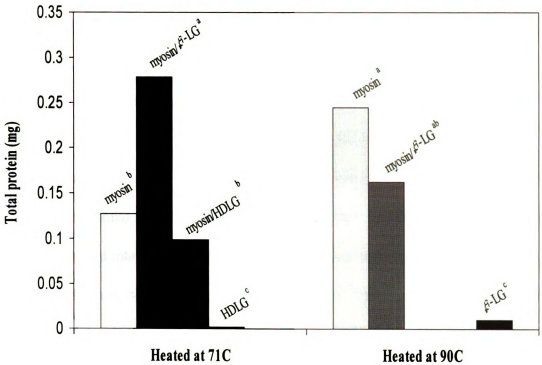


Figure 7.8. Total protein in supernatant expressed from myosin, β-lactoglobulin (β-LG), heat-denatured β-LG (HDLG), myosin/β-LG, and myosin/HDLG gels prepared in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 7.0, heated at 71°C for 60 min or at 90°C for 30 min and cooled to 25°C. Values are means of three observations. Means with different superscripts are different ($p<0.05$).

gels heated to 71°C, when compared to myosin/β-LG gels heated to 71 and 90°C (Table 7.1).

7.5. CONCLUSIONS

To begin to understand how whey protein polymers function in meat products, we used a model system to study thermal denaturation, rheological properties, and microstructures of the gels of mixtures of heat-denatured polymers of β-LG and myosin, the major functional whey and meat protein. When heated to 71°C, β-LG did not interact with myosin and interfered with the network formation in mixed gels, whereas HDLG interacted with myosin during heating to enhance gel formation. Myosin heavy chains played a major role in network formation while the LC fractions dissociated and remained soluble.

Myosin/HDLG co-gels in 0.6M NaCl solution at pH 6.5 and 7.0 had similar stiffness and microstructure as 2% myosin gels. HDLG/myosin co-gel heated to 71°C had greater G' than myosin alone and myosin/β-LG gels heated to 90°C, suggesting the HDLG aggregates could have a texture enhancing effect in meat products processed at their typical processing temperatures (≤ 71°C). This work indicates co-gel formation between preheated whey and meat proteins which enhance texture parameter of the mixed proteins when heated at 71°C and may contribute to enhanced applications for whey protein in meat products. The next step would be to optimize the functionality of whey protein polymers for their use in value-added meat and poultry products. A better understanding of interactions between whey and meat protein will allow increased use of

whey protein in meat products, and in a variety of other applications where protein gelation at low temperature is desirable.

here protein

CHAPTER 8: CONCLUSIONS

The purpose of this research was to understand gelation mechanisms of whey in meat products by using myosin and β-lactoglobulin (β-LG), the major functional components of meat and whey protein, in a model system. β-LG interfered with aggregation and network formation of myosin when heated between 55 and 75°C. However, β-LG enhanced stiffness of myosin/β-LG gels when heated above 80°C, which is the gelling temperature of β-LG. The gelling temperature of whey protein could be one factor limiting its ability to enhance texture in food products processed at temperatures below 80°C.

Soluble protein aggregates were formed by heating β-LG solutions in deionized water, pH 7.0 at 80°C for 30min. Gels were prepared using heating and buffer conditions used for myosin (71°C for 60 min in 0.6M NaCl, 0.05M sodium phosphate buffer, pH 6.0-7.0) to determine if heat-denatured β-LG aggregates (HDLG) could enhance gel formation in meat systems. The HDLG formed stiffer gels at lower temperature and lower concentration when compared to unheated β-LG.

When heated to 71°C, HDLG was incorporated within networks of the co-gels, whereas β-LG remained soluble. Microstructures of 1% myosin/1% HDLG co-gels were similar to those of 2% myosin gels, while coarser gel networks with bigger voids were observed in 1% myosin/1% β-LG gels. The stiffness of myosin/HDLG co-gels heated to

71°C was greater than that of myosin gels at the same total concentration. The stiffness of myosin/HDLG gels was also higher that that of myosin/β-LG gels heated to 90°C. The results suggested that HDLG and myosin formed a co-gel network with greater stiffness than those formed from myosin alone or myosin/β-LG.

Results of this research indicate that preheat treatment improved gelling properties of β-LG by lowering its gelling temperature and allowing the formation of a stronger gel at lower concentration than the unheated protein. The HDLG might be used to improve textural qualities of meat products processed at 71°C or lower. Pre-aggregation by heating could be used as a modifying process to optimize properties of whey products needed by meat processors and may contribute to broader applications of whey where protein gelation at low temperature is preferred.

CHAPTER 9: FUTURE RESEARCH

In this research, we prepared heat-denatured β–lactoglobulin (HDLG) aggregates by heating at 80°C for 30 min in deionized water, pH 7.0. Protein and salt concentration, heating time and temperature, and pH were important factors affecting properties of whey protein polymers (Ju and Kilara, 1998b; Mleko and Foegeding, 1999b). The characteristics and properties of aggregates or polymers influence the texture quality of the gels. Thus, more research is needed to study influence of these factors on the characteristics of the aggregates, such as size, shape, viscosity, hydrophobicity and number of active sulhydryl group.

The size and shape of HDLG aggregates were examined after heating using scanning electron microscopy. In situ dynamic light scattering allows detection of sample size during heating, therefore this technique could be very useful for investigating aggregate formation during heating under different conditions. Understanding the mechanisms and factors affecting aggregate formation could be greatly beneficial for manipulating properties and characteristics of the aggregates.

Investigation into the relationship between aggregate characteristics and its gelling properties is essential in selecting the optimal conditions for the production of the protein aggregates with the desired functionality. To study the gelling properties of the protein aggregates, environment conditions, such as pH and salt concentration, should be

varied to include real conditions found in meat products. Reducing reagents could be used to elucidate the role of disulfide bonds in the co-gel formation.

In this study, pure proteins were used in a model system to avoid confusion in interpretation of results and allow greater understanding of heat-induced gelation mechanisms. However, in commercial food systems, whey and meat products contain many other components which could affect their gelling properties. Therefore, in future research, whey products should be used to produce the protein aggregates. The effectiveness of texture enhancing properties of the aggregates in meat products must be validated in a real meat system. Knowledge gained from studies of β-LG aggregate formation could be used as directions for the production of whey protein aggregates. The relationship between aggregate characteristics and the gel properties could be used to screen protein aggregates to find the most promising properties in meat products. Finally their efficacy in enhancing textural properties should be verified with meat product in a pilot scale production.

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