SELF-ASSEMBLED POTASSIUM 5'-GUANOSINE MONOPHOSPHATE. NUCLEAR MAGNETIC RESONANCE EVIDENCE FOR STRUCTURE FORMATION IN AQUEOUS SOLUTION

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

CLAUDIA MARIE METTLER

1977

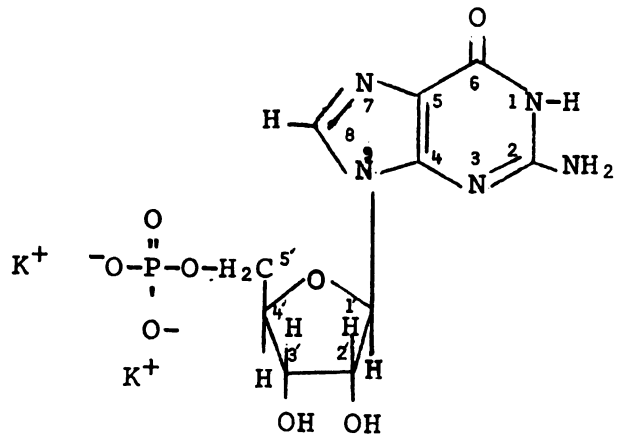
ABSTRACT

SELF-ASSEMBLED POTASSIUM 5'-GUANOSINE MONOPHOSPHATE. NUCLEAR MAGNETIC RESONANCE EVIDENCE FOR STRUCTURE FORMATION IN AQUEOUS SOLUTION

By

Claudia Marie Mettler

It has been shown by variable temperature proton NMR studies that the spontaneous intermolecular self-assembly of 5'-guanosine monophosphates (5'-GMP) in aqueous solution (pH = 7.0 - 8.0) is dramatically dependent on the nature of the alkali metal counterion. The potassium and sodium salts of the mononucleotide form self-structures which differ both in the number of H(8) resonance lines and in thermal stability. Moreover, the lithium salt fails to form any NMR detectable structure. These results show for the first time that selective alkali metal coordination by a nucleic acid does occur, and that the coordination interaction can be important in the formation of regular, order structures involving intermolecular association of nucleotides.



The proton spectra obtained at 60 MHz have limited utility in distinguishing the number of non-equivalent H(8) environments in self-assembled K_2 (5'-GMP). However, spectra obtained at 220 MHz indicate that at least two, and perhaps three, distinguishable structures are present. Previously reported[1] self-assembled Na_2 (5'-GMP) forms only one or two self-structures.[1] The chemical shift range of the H(8) resonances is smaller for the self-assembled K^+ salt (0.50 ppm) than for the self-assembled Na^+ salt (1.3 ppm). Also, the K_2 (5'-GMP) self-structures have higher solution melting temperatures than the Na_2 (5'-GMP) self-structures.

As in the case of self-structured Na_2 (5'-GMP), self-structured K_2 (5'-GMP) exhibits amino proton resonances.

The slow chemical exchange between amino protons and H_2O (solvent) protons indicates that hydrogen bonding as well as specific alkali metal ion coordination is also involved in the self-assembly processes. It is likely that the self-structures in both salts involves formation of associated nucleotide units through hydrogen bonding between position O(6) and N(7) as acceptors and N(1) and N(2) as donors. The hydrogen-bonded nucleotide units, perhaps tetramer units, then coordinate with selected alkali metal ions to form a regular, ordered structure that is slow to undergo chemical exchange. The results obtained in this work, however, do not allow identification of the coordination mechanism.

References

1. T. J. Pinnavaia, H. T. Miles, and E. D. Becker, J. Am. Chem. Soc., 97, 7198 (1975)

SELF-ASSEMBLED POTASSIUM 5'-GUANOSINE MONOPHOSPHATE. NUCLEAR MAGNETIC RESONANCE EVIDENCE FOR STRUCTURE FORMATION IN AQUEOUS SOLUTION

By

Claudia Marie Mettler

A THESIS

Submitted to

Michigan State University

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

Department of Chemistry

1977

DEDICATION

To John Michael Duranceau

ACKNOWLEDGEMENTS

I wish to thank Dr. Thomas J. Pinnavaia for suggesting this thesis problem and Dow Corning Corporation for permitting me to take a ten week leave of absence to complete the experimental work at Michigan State University.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

Mononucleotides are known as the building blocks of the nucleic acids. Nucleic acids participate in molecular mechanisms which store, replicate, and transcribe genetic information through the polynucleotide chains of DNA and RNA. Mononucleotides contain three components: 1. a nitrogenous base, 2. a five-carbon sugar, and 3. phosphoric acid.

The aromatic heterocyclic compounds of pyrimidine and purine are the two types of nitrogenous bases in nucleotides. The six-membered ring pyrimidines are cytosine, thymine, and uracil. The purines, pyrimidines with fused imidazole rings, are adenine and guanine. Nitrogenous bases have limited water solubility and exist in tautomeric forms.

Nucleosides are N-glycosides of pyrimidine and purine bases in which the N(1) of pyrimidines and the N(9) of purines are glycosidically linked to the C(1) of a five carbon sugar-usually ribose or deoxyribose. The nucleosides, cytidine, thiamine, uridine, adenosine, and guanosine, are more water soluble than their parent bases.

Nucleotides are the phosphoric acid esters of the nucleosides. Phosphoric acid is esterified to one of the free hydroxyl groups of the pentose sugar. Although 2' and 3' isomers exist, the nucleotides, in the free form in cells, generally have the phosphate group in the 5' position of the sugar. The mononucleotides are strong acids. The two dissociable protons of the phosphoric acid group have pK' values of about 1.0 and 6.2 At pH 7.0, the free nucleotides exist totally dissociated.[1]

Base-paired mononucleotides form hydrogen-bonded complexes in aqueous solution.[1,2,3,4] NMR evidence for hydrogen-bonding between complementary bases was based on the detection and measurement of chemical shifts for the amino protons involved in hydrogen-bonding. The amino protons involved in the hydrogen-bonding exhibited downfield chemical shifts from the unassociated monomers. These downfield chemical shifts are concentration and temperature dependent.

Guanylic acid (and its derivatives) Figure I is the only nucleic acid component which spontaneously forms a regular, ordered structure with itself in aqueous solution.

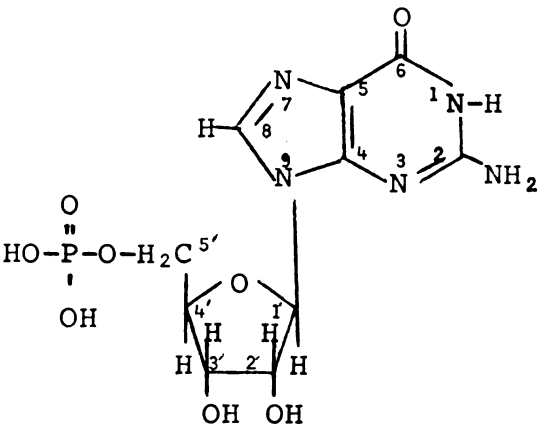


Figure I. Structure of Guanylic Acid

In 1910, Bang reported that guanylic acid formed a gel in concentrated solutions.[5] In 1962, Gellert, Lipsett, and Davies found that solutions of guanylic acid (GMP) at pH 5, where one proton is dissociated, were extremely viscous at room temperature and that both dilute and concentrated solutions formed a clear gel on cooling.[6] The authors investigated the optical properties of the gel and the fibral structure formed upon drying the gel. It was concluded that helix formation by guanylic acid was occurring. A hydrogen-bonded structure of the bases in the gel was proposed. The proposed structure consisted of continuous helixes (5'-isomer) or planar tetramer units (Figure II) stacked in a helical array (3'-isomer).

Figure II. Structure of Hydrogen-Bonded Tetramer of Guanylic Acid Monomers

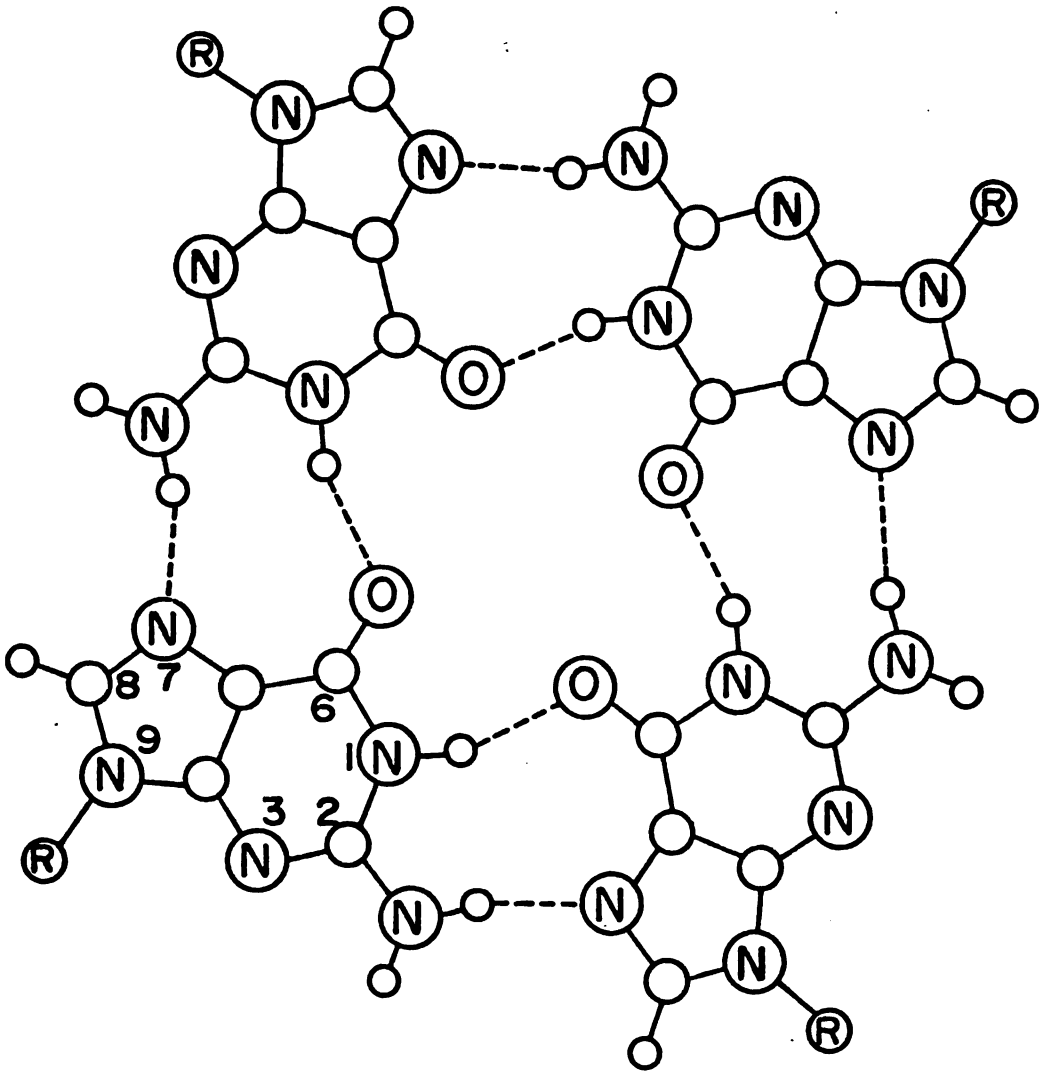


Figure II

In 1972, Miles and Frazier reported that guanosine 5'-monophosphate (5'-GMP) formed a regular, ordered structure in neutral solution. At this pH, both protons of guanylic acid are dissociated. The authors found that the ordered structure was not associated with the previously reported gel formation at pH 5. From infrared and chemical evidence, the proposed neutral structure consisted of planar tetramer units (Figure II). There was similarity in the pattern of these planar tetramer units to that of the acid gels. Nitrogen (1) and nitrogen (2) were proton donors, while oxygen (6) and nitrogen (7) were proton acceptors in the proposed planar tetramer units.

More recently, Pinnavaia, Miles, and Becker published nuclear magnetic resonance evidence for the formation of a regular, ordered structure and slow chemical exchange for self-assembled Na_2 (5'-GMP) (Figure III) in neutral solution.[8] Pinnavaia, et. al. showed that the structure formed involved hydrogen-bonding. In addition, the structure was stable enough to observe non-equivalent proton environments. In previous work with aqueous solution chemistry of base-paired mononucleotides, only rapid chemical exchange and time-average NMR spectra had been reported. The slow chemical exchange was unprecedented.

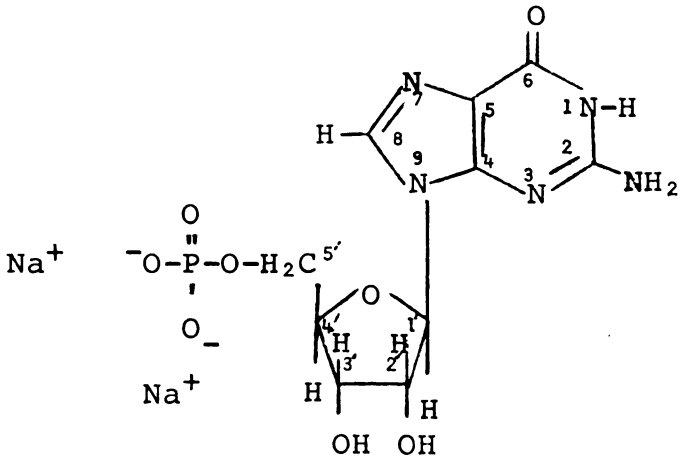


Figure III. Structure of Sodium Guanosine 5'-Monophosphate

The authors found that the NMR lines at 0.23M Na_2 (5'-GMP) were attributed to those previously reported for the disordered nucleotide in dilute aqueous solution. Specifically, the H(8) resonance was a sharp singlet at 8.1 ppm. The self-assembly of Na_2 (5'-GMP) in neutral aqueous solution was confirmed by proton NMR line broadening with increasing concentration or decreasing temperature. As the concentration of mononucleotide increased (0.42M), two H(8) lines were at 7.25 and 8.55 ppm. The chemical shifts of these new lines were independent of concentration, thus being indicative of slow chemical exchange. At 0.59M, a third H(8) line appeared on the low-field side of the central resonance (8.17 ppm) H(8) line (concentration dependence). The resolution of four H(8) lines was observed at 0.83M.

With decreasing temperature, similar changes were observed in the H(8) region of the Na_2 (5'-GMP). The upfield shift of one H(8) self-structure line was attributed to diamagnetic ring currents of overlapping bases. Isoshielding contours calculated for the ring currents in guanine by Giessner-Prettre and Pullman quantitatively agree with this upfield shift.[9,10] The bases stacked above and below guanine account for a large portion of the upfield chemical shift. The downfield shifts of the third and fourth highest field H(8) lines were presumed to be due to electric field effects caused by the nearness of the phosphate group (doubly ionized in Na_2 (5'-GMP)) to the H(8) protons. The H(8) lines were verified by their decrease in intensity upon exchange with D_2O. Low field lines observed in H_2O but not in D_2O were attributed to protons bonded to nitrogens. If these protons, specifically N1-H, were unassociated, i.e., not hydrogen-bonded, their exchange with H_2O would be too rapid to observe. The observation of these lines under complex formation conditions, but not at high temperatures or low 5'-GMP concentrations at which the ordered structure does not exist, provided evidence for hydrogen-bonding of these protons in the ordered structure of 5'-GMP.

Pinnavaia, Miles, and Becker worked exclusively with the disodium salt of guanosine 5'-monophosphate.

The present study investigates the effect of changing the metal ion on the self-assembly of guanosine 5'-monophosphate in aqueous solutions. The experiments were designed to determine whether changing the metal ion would: 1) simply affect the chemical shift in the H(8) region, or 2) directly alter the structure forming process. If the metal ion were present only as a counter-ion in solution, the former observation would be made. If a different structure was formed with a different metal ion, then direct metal ion coordination with the solution structure would be indicated.

II. EXPERIMENTAL

A. Materials, Techniques, and Preparation

Sodium guanosine 5'-monophosphate monohydrate (Na_2(5'GMP)) was purchased from P-L Biochemicals, Inc. Paramagnetic ion impurities were removed from distilled water by an electrolysis cell. The ion exchange column was prepared with Dowex 8, a 100-200 mesh cation exchange resin with a capacity of 1.7 meq/ml. The wet resin was washed with: 1) 3N hydrochloric acid - to remove gross amounts of dirt, 2) a dilute solution of disodium ethylenediaminetetraacetate- to remove paramagnetic ion impurities, and 3) an aqueous solution of the desired metal ion hydroxide or chloride (depending on the water solubility) - to exchange the cationic resin with the desired metal ion. An aqueous solution of Na_2 (5'-GMP) was passed through the ion exchange

column packed with the metal ion exchanged resin. Essentially total exchange occurred. The exchanged 5'-GMP salt solution was lyophilized (freeze-dried). Then, the exchanged 5'-GMP salt was recovered by subliming the water. Sodium 2,2,3,3,-d_4-3-trimethylsilyl-propionate (TSP) was purchased from Merck and Company, Inc. for the nuclear magnetic resonance reference standard. A 0.003M TSP solution had a pH of 7.90. A 0.63M solution of K_2 (5'GMP) made with the TSP solution had a pH of 8.85.

B. Instrumentation

1. Nuclear Magnetic Resonance Spectroscopy

Proton magnetic resonance spectra were obtained with a 60 MHz Varian A56/60D spectrometer. Probe temperature was regulated with a Varian V-6040 temperature controller. The probe temperatures were measured by the chemical shift differences between the proton resonances of methanol (0-40°C) and ethylene glycol (40-90°C). The instrument was calibrated by the chemical shift difference between tetramethylsilane and chloroform of 436 Hz. Maximum radio-frequency field strengths and spectrum amplitudes were used to maximize spectra resolution.

2. Ultra-violet Absorption Spectroscopy

Guanosine 5'-monophosphate solution U-V absorption spectra were determined with a Cary 17 Ultraviolet-Visible Absorption Spectrometer. Solution concentrations were obtained from Beer's Law:

$$\log (I_o/I)=\varepsilon lc=A$$

I_o - intensity of the incident light

ε - molar absorptivity (or decadic molar extinction coefficient)

l - cell length in centimeters

c - concentration in moles per liter

A - absorbance or optical density[11]

The molar absorptivity (ε) for Na_2 (5'-GMP) was taken to be 1.41 X 10^4 at 252 nm, as determined by P-L Biochemicals, Inc. This molar absorptivity value was assumed to remain constant while the metal ion was changed. The cell length was one centimeter.

III. RESULTS AND DISCUSSION

Preliminary experiments were carried out with the lithium, potassium, barium, and magnesium salts of guanosine 5'-monophosphate. The magnesium and barium salts have limited water solubility. The low solubility is attributed to the divalent charge and low ionic radius of the metal. No H(8) line is observed at 60 MHz for a saturated solution of magnesium or barium guanosine 5'-monophosphate at 5°C and 42°C.

The lithium salt of guanosine 5'-monophosphate is water soluble. At approximately 0.5M, a single H(8) line of the mononucleotide is observed at 7.1 ppm. Varying the temperature did not change the H(8) chemical shift, appreciably broaden the line, or cause extra H(8) lines to form. The lithium salt behaves differently

from the sodium salt; no evidence of self-assembly is apparent.

This observation was the first evidence for metal ion coordination in the formation of a self-structure by guanosine 5'-monophosphate. The lithium cations act only as counterions in solution for the mononucleotide. The sodium cations appear to be specifically coordinated with the guanosine 5'-monophosphate.

The potassium salt of guanosine 5'-monophosphate is water soluble. At approximately, 0.5M and 32°C two H(8) lines are observed at 7.8 and 7.5 ppm, respectively. Lowering the temperature alters these chemical shifts, appreciable line broadening is observed at 5°C. In H_2O solution, but not in D_2O, low-field lines are observed at 11.2, 10.8, 10.5 and 9.9 ppm. These latter are attributed to nitrogen-bonded protons. Two H(8) lines are observed with 60°C with 0.63M of the potassium salt. In contrast, the four H(8) lines of the sodium salt are melted out below 40°C.

These initial observations confirm: 1) the absence of a solution self-structure with the lithium salt of guanosine 5'-monophosphate, 2) the formation of a solution self-structure with the potassium salt, 3) a difference from the structure previously reported for the sodium salt, and 4) metal ion coordination in the formation of a solution self-structure of guanosine 5'-monophosphate. The solution self-assembly of

potassium guanosine 5'-monophosphate is investigated further to determine the behavior and the effect on structure of the potassium metal ion.

Proton nuclear magnetic resonance spectra at 60 MHz of potassium guanosine 5'-monophosphate (K_2(5'-GMP)) at various concentrations in water at 32°C are shown in Table I and Figure IV. The lines observed in dilute solution at 0.26M are assigned to the disordered mononucleotide. The single H(8) line is at 8.0 ppm. As the concentration increases to 0.30M, the lines broaden, spin-spin coupling of the C(1') ribose proton is obscured (5.6 ppm) and a second H(8) line grows out of the base line at 7.5 ppm. At 0.4M, both H(8) lines increased in intensity. At 0.50M and 0.63M, the H(8) lines increased little, if any, in intensity from 0.40M. At 1.13M, a saturated solution, there is extensive line broadening and upfield shifting of the H(8) lines. Four unresolved H(8) lines are observed at 7.6, 7.3, 7.1, and 6.5 ppm, respectively. This indicates rapid chemical exchange and time - averaging of two or more classes of protons.

Different changes in the H(8) region are observed with decreasing temperature in Table II and Figure V. The lines observed at 70°C at 0.63M in aqueous solution are attributed to the disordered mononucleotide. The single H(8) line is at 7.9 ppm. As the temperature

TABLE I

Concentration Dependence of H(8), H(1')
Chemical Shifts for K_2 (5'-GMP) in H_2O at 32°C

	δ, ppm from TSP					
	H(8)		H(8) Upfield Shoulder		H(1')	H_2O[a]
Concentration						
1.13M (saturated)[b]	7.60	7.30	7.10	6.50	-	-
0.63M	-	7.72	7.47	-	5.72	4.79
0.51M	-	7.76	7.47	-	5.71	4.76
0.40M	-	7.83	7.48	-	5.30	4.74
0.32M	-	7.96	7.50	-	5.70	4.72
0.26M	-	8.01	-	-	-	4.71
0.21M	-	8.04	-	-	-	4.69
0.17M	-	8.05	-	-	-	4.67
0.13M	-	8.06	-	-	-	4.66
0.11M	-	8.05	-	-	-	4.65

[a] Chemical shift of solvent H_2O

[b] In saturated solution, K_2 (5'-GMP) has four unresolved H(8) lines

Figure IV. Proton NMR Spectra (60 MHz) of K_2 (5'-GMP) in H_2O in the Concentration Range - 0.26M to 1.13M. Temperature is 32°C

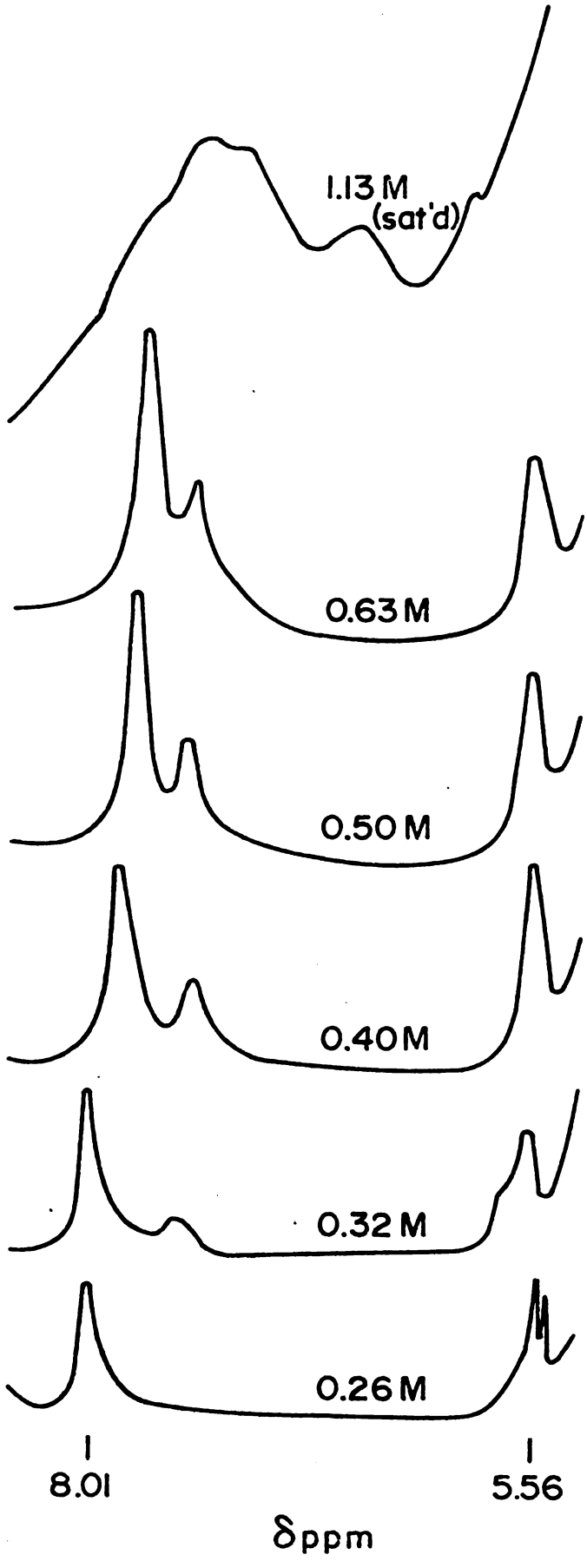


Figure IV

TABLE II

Temperature Dependence of H(8), H(1')
Chemical Shifts for K_2 (5'-GMP) in H_2O at 0.63M

Temperature (°C)	δ, ppm from TSP H(8)	H(8) Upfield Shoulder	H(1')	H_2O
32[c]	7.71	7.45	5.70	4.76
5	7.63	-	-	4.80
10	7.71	7.44	-	4.91
15	7.72	7.45	-	4.87
20	7.69	7.45	-	4.82
32	7.73	7.47	5.65	4.76
40	7.77	7.47	5.70	4.74
50	7.82	7.48	5.75	4.64
60	7.82	-	5.77	4.61
70	7.92	-	5.82	4.55
80	7.98	-	5.84	4.47
90	7.97	-	5.84	4.41
32[d]	7.67	7.47	5.74	4.76

[c]Before cooling to 5°C

[d]After heating at 90°C

Figure V. Proton NMR Spectra (60 MHz) of K_2 (5'-GMP) in H_2O in the Temperature Range - 5° to 70°C. Concentration is 0.63M

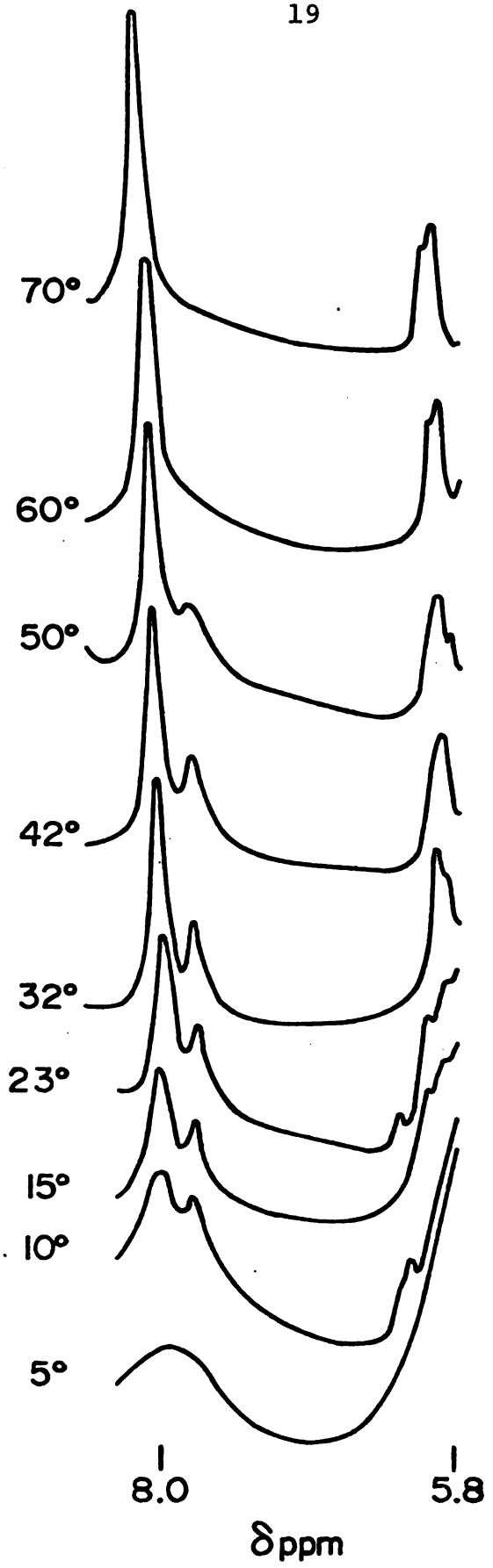


Figure V

decreases to 60°C, the lines broaden, and a second H(8) line grows out of the base line at 7.5 ppm. At 50°C, the H(8) line at 7.5 ppm grows in intensity and the spin-spin coupling of the C(1') ribose proton is obscured. Between 23°C and 10°C, the intensity of the H(8) peak attributed to the disordered nucleotide decreases (8.0 ppm). The H(8) line at 7.5 ppm does not change appreciably in intensity but moves slightly downfield as the temperature decreases. At 5°C, the two H(8) peaks merge into one unresolved peak, centered around 8.0 ppm.

Confirmation of the assignment of the H(8) lines is obtained by a decrease in their absolute intensities after exchange with deuterium oxide (D_2O). (Figure VI). The proton exchange conditions (heating at 70°C for two hours) are such that the H(8) protons (7.9-8.0 ppm) are replaced by deuterium but the ribose protons (specifically the H(1') proton at 5.7 ppm) are not. The low field lines which are observed in water but not in deuterium oxide are attributed to N-bonded protons (11.2, 10.8, 10.5, and 9.9 ppm; Figure VII). The lowest field lines are probably N1-H, as was reported by Pinnavaia, et. al.[8] The N1 proton exchange of unassociated guanylic acid is too rapid in water to permit observation of an NMR line.[2,13] However, N1-H is observed in hydrogen-bonded complexes.[13,14,15] The N-bonded proton lines are observed under conditions

Figure VI. Proton NMR Spectra (60 MHz) of Verification of H(8) Signals by D_2O Exchange

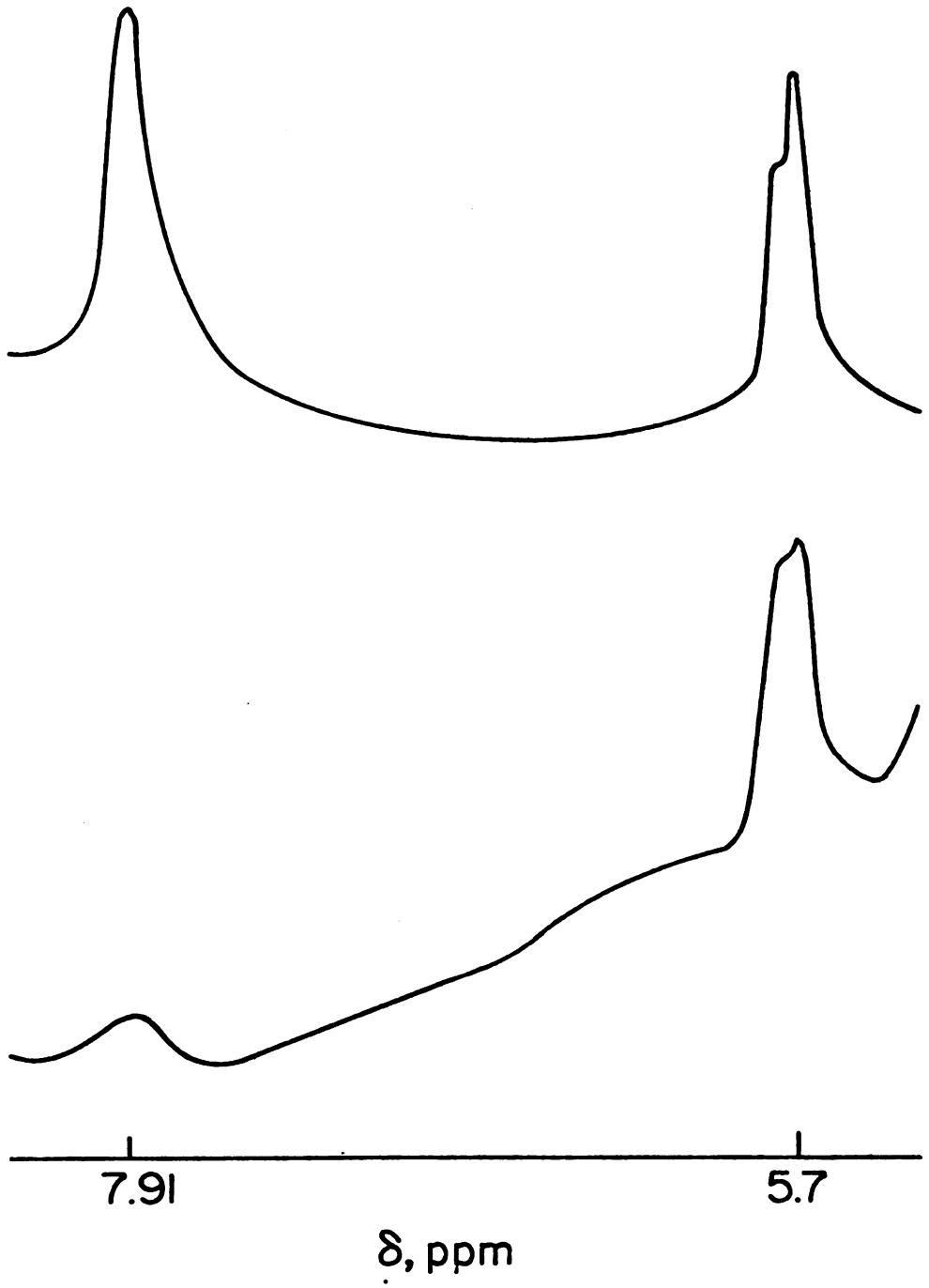


Figure VI

Figure VII. Proton NMR Spectra (60 MHz) of the Nitrogen-Bonded Protons of K_2 (5'-GMP) in H_2O at 0.63M

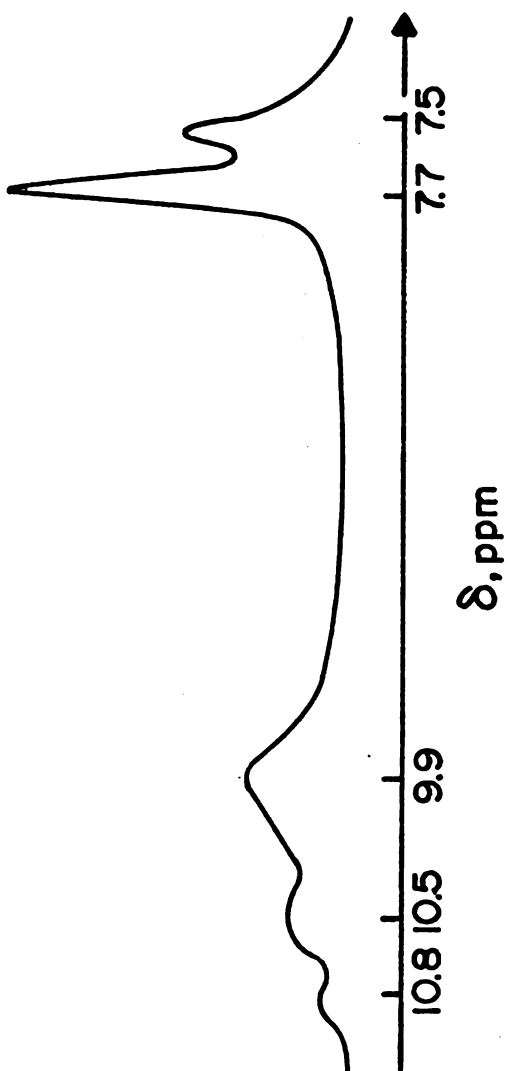


Figure VII

favorable for complex formation of K_2 (5'-GMP). These lines are not observed at temperatures and mononucleotide concentrations at which a solution structure is not found to exist. The appearance of the N1-H protons lines under conditions of complex formation provides evidence for the hydrogen-bonding of these protons in the ordered solution structure of K_2 (5'-GMP).

The H(8) resonance at 8.0 ppm is attributed to the unassociated mononucleotide. This line shifts somewhat to lower field with decreasing concentration and decreasing temperature (Figures IV and V). This is indicative of rapid exchange and time-averaging of two or more non-equivalent protons. This observation has previously denoted formation of non-regular stacked aggregates, perhaps in equilibrium with a regular structure containing rapidly exchanging classes of protons.[8,12,16]

The H(8) line upfield (7.5 ppm) from the unassociated mononucleotide signal did not shift with changes in concentration or temperature. This may have resulted from the formation of a regular structure in slow exchange with unassociated monomers or stacked aggregates. A limiting structure appears in both the concentration and temperature series (Figures VI and VII). There are small changes in the absolute intensities and chemical shifts of the H(8) protons between 0.40M and 0.63M and between 23°C and 50°C respectively. When

extreme conditions of either concentration (saturated solution) or temperature (less than 10°C) are applied, a new solution structure or structures may be formed. The structures formed at low temperatures and formed in saturated solutions of the mononucleotide are in rapid equilibrium - indicated by the broad, unresolved lines observed. The differences in these 60 MHz spectra at the extremes of temperature and concentration show that under different conditions, more than one solution structure is forming. This is in contrast with the similarity found between the concentration and temperature spectra of Na_2 (5'-GMP). (Pinnavaia, et. al.[8])

Since only one H(8) line is observed besides the monomer line at 60 MHz under structure forming conditions for K_2 (5'-GMP) (excluding extremes of temperature and concentration), a single solution structure with equivalent H(8) protons is hypothesized. The bonding scheme shown in Figure II - a tetramer of hydrogen-bonded guanine bases - is favored for the K_2 (5'-GMP) self-structure. The tetramer is formed by two hydrogen bonds per guanine base unit. All of the guanines and their H(8) regions are equivalent. Previous work shows that alternative bonding schemes are not reasonable, because none produce more than two hydrogen bonds per base.[7,17] This considerably stable tetramer could be the limiting K_2 (5'-GMP) structure indicated in the concentration range of 0.40M-0.63M and in the temperature

range of 32-50°C.

In the communication published by Pinnavaia, Miles and Becker, the H(8) non-equivalence (four H(8) lines were observed) in the solution structure formed by Na_2 (5'-GMP) was attributed to limited head-to-tail stacking of tetramer units. The new K_2 (5'-GMP) self-structures which form under extremes of concentration and temperature, could be stacking of the tetramer units. Further self-assembling would account for the upfield shift of H(8) lines in the concentration spectra. Such a variety of stacked tetramer units would account for the different H(8) environments observed with saturated solutions of K_2 (5'-GMP). It would not necessarily account for the single broad H(8) signal observed at 5°C with 0.63M. This could just have been a rapid equilibrium between the tetramer and the monomer.

The resolution obtained with the 60 MHz nuclear magnetic resonance spectrometer compared with the resolution obtained with the 220 MHz instrument used by Pinnavaia, Miles and Becker is mediocre. The 220 MHz spectra of K_2 (5'-GMP) were obtained from the National Institute of Health, compliments of Dr. C. Fisk. The 220 MHz spectra of the H(8) region of a 0.8M solution of K_2 (5'-GMP) are shown in Table III and Figure VIII. At 18°C, two lines are prominent at 7.85 and 7.53 ppm. The peak at 7.85 ppm has a low field shoulder. The peak at 7.53 ppm has an upfield shoulder. Another peak appears

TABLE III

H(8) Chemical Shifts of Self-Assembled K_2 (5'-GMP)[e] Obtained at 220 MHz

δ, ppm from TSP

Temperature (°C)	H(8)	
18	7.85	7.53
6.5	7.86	7.51
3	7.88	7.52

[e] 0.8M K_2 (5'-GMP)

Figure VIII. H(8) Resonances at 220 MHz for K_2 (5'-GMP)

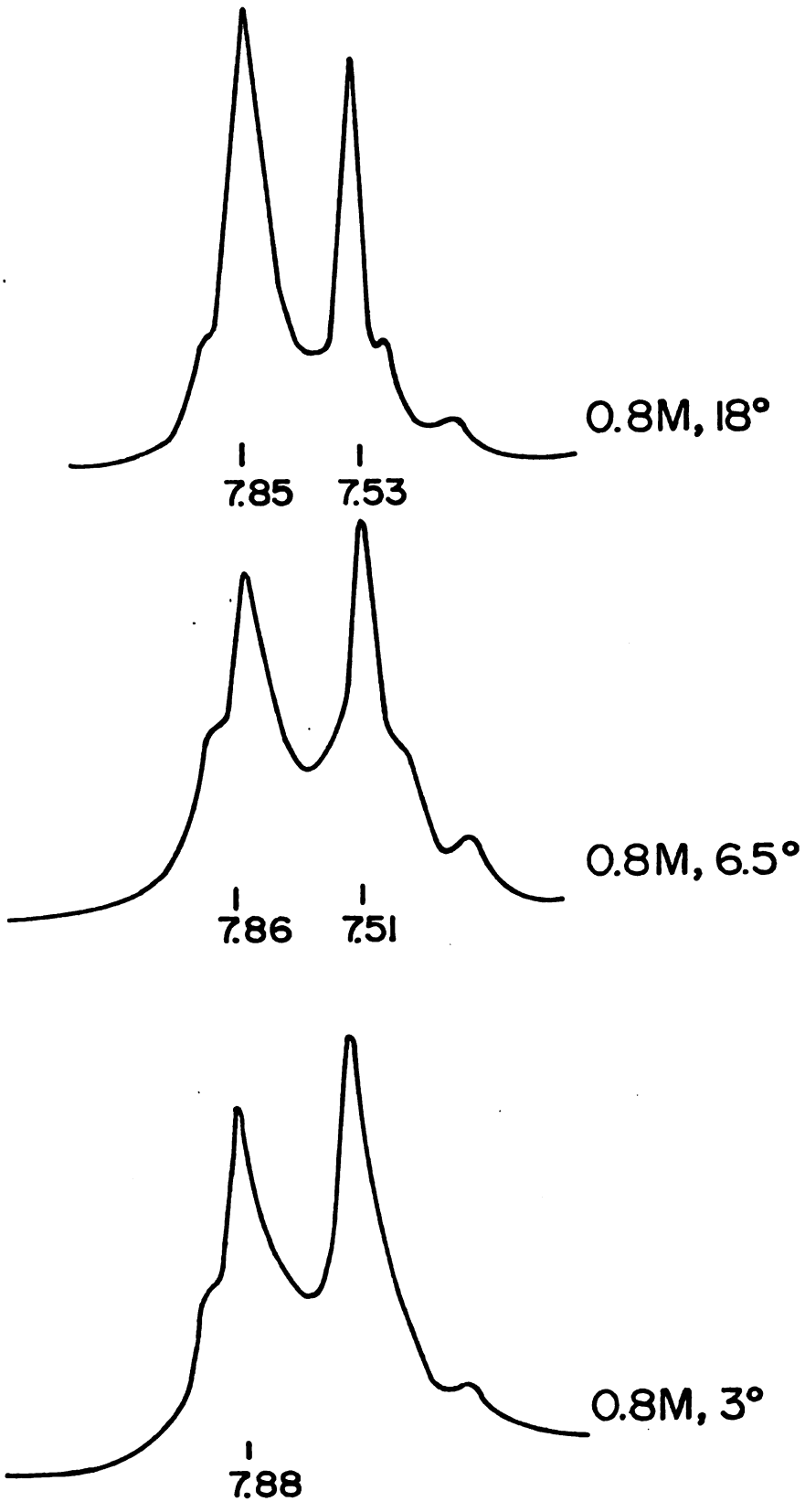


Figure VIII

at about 7.3 ppm. At 6.5°C, the H(8) region of 0.80M K_2 (5'-GMP) is broadened. The main H(8) peaks are at 7.86 and 7.51 ppm, respectively. All the shoulders, and the new peak at about 7.3 ppm are more pronounced. At 3°C, the H(8) region is broader than at 6.5°C. The main peaks are at 7.88 and 7.52 ppm. The shoulders are more prominent. Two new shoulders also appear - one on the high field side of the 7.88 ppm line and one on the low field side of the 7.52 ppm line. Both of the two main peaks move downfield with decreasing temperature (Table III).

Even though the resolution with the 220 MHz spectrometer is not complete for the K_2 (5'-GMP) solution, it is improved over the 60 MHz spectra. With the 220 MHz evidence, it is apparent that the K_2 (5'-GMP) solution structure is not as simple as the 60 MHz spectra portrays. In addition, the K_2 (5'-GMP) solution structures appear much more complicated than the previously reported Na_2 (5'-GMP) structures; with two prominent peaks, there are at least seven unresolved peaks.

The seven or more H(8) lines observed in the 220 MHz spectra indicate that more than one solution structure or more than one H(8) environment is present. The lack of resolution in part of the spectra indicates rapid equilibrium between H(8) environments. The predominance of a few peaks indicate that some structures (H(8) environments) are more stable than others. The

220 MHz spectra do not prove or disprove the presence of stacking of the tetramer units. The presence of tetramer units has been assumed. The existence of a stable upfield H(8) line upfield from the K_2 (5'-GMP) monomer line indicated a structure with equivalent H(8) environments was present. As mentioned previously, only the tetramer structure has been found to account for such H(8) equivalence. The tetramer is definitely not the only solution structure formed with K_2 (5'-GMP) but it certainly accounts for a major portion of the solution structure.

IV. CONCLUSION

Metal ion dependence has been proven in the solution self-assembly of guanosine 5'-monophosphate. In comparison, Li_2 (5'-GMP), Na_2 (5'-GMP), and K_2 (5'-GMP) each exhibit unique self-assembly behavior. The extent of self-association appears to increase with: 1) increasing ionic radius: Li^+- 0.68Å, Na^+- 0.97Å, and K^+- 1.33Å, or 2) decreasing charge-to-mass ratio.[10]

Potassium guanosine 5'-monophosphate forms different self-structures from those reported for Na_2 (5'-GMP); a limiting structure in slow exchange with the unassociated monomer forms. Under extremes of concentration or temperature, a new structure may form which is in rapid equilibrium with the monomer. If a new structure is not formed, the equilibrium state is shifted from that exhibited for the limiting case. The K_2 (5'-GMP)

self-structure exhibits more stability than Na_2 (5'-GMP). Self-structure H(8) lines appear in more dilute solutions of K_2 (5'-GMP) compared with Na_2 (5'-GMP). The self-structure of K_2 (5'-GMP) also forms at higher temperatures than Na_2 (5'-GMP).

The metal ion plays a structure forming role in the solution self-assembly of 5'-guanosine monophosphate. The location of the metal ion such that it can influence the solution self-assembly of 5'-GMP has yet to be discovered. The metal ion could be accommodated in one or more positions.

1) The tetramer structure of hydrogen-bonded guanine bases has a hole in the center (Figure II). The size of the hole would determine whether a metal ion could be complexed. 2) If stacking of the tetramers occurs, there may be complexing between the tetramer sheets. It is known that there is 3.4Å between stacked sheets.[1,19] 3) Each guanosine mononucleotide has a phosphate group containing two negative charges. In a tetramer structure, there are a total of eight negative charges. The positioning of these phosphates on one side of the tetramer would generate a region with high electron density which could attract positive metal ions in solution.

Since crystallization of 5'-guanosine monophosphate has not been achieved, X-ray crystallography can not be done to find out where the metal ions are. Metal ion specific nuclear magnetic resonance (Na^+ for example)

could be done to find out how many metal ion environments are present. Other metal ions with similar radii and/or charges, such as Tl^+, Ag^+, Rb^+, NH_4^+, and $(CH_3)_4N^+$, could also be exchanged for Na^+ in order to see if self-structures form. Perhaps a self-structure could be forced to form with the lithium salt (or other salt which forms no apparent self-structure) of 5'-guanosine monophosphate by the addition of an excess of a lithium halide to the lithium 5'-GMP solution. C-13 nuclear magnetic resonance could be done to observe which carbons are involved in the hydrogen-bonding or metal ion bonding.

In conclusion, the metal ion plays a structure forming role in the solution self-assembly of 5'-guanosine monophosphate. The metal ion dependence of a nucleic acid component in solution has significant biological relevance. However, the self-assembly occurs under conditions of such high mononucleotide concentration or at low temperatures, that the biological relevance has, at most, limited practical significance.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. A. L. Lehninger, Biochemistry: The Molecular Basis of Cell Structure and Function; Ch. 12, 25, 28; World Publishers, Inc., N. Y., N. Y., 1970.

2. M. Raszka and N. O. Kaplan, Proc. Nat. Acad. Sci. U. S. A., 69, 2025 (1972).

3. R. R. Shoup, H. T. Miles, and E. D. Becker, Biochem. Biophys. Res. Commun., 2, 194 (1966).

4. L. Katz and S. Penman, J. Mol. Biol., 15, 220 (1966).

5. I. Bang, Bioch. Ztschr., 26, 293 (1910).

6. M. Gellert, M. N. Lipsett, and D. R. Davies, Proc. Nat. Acad. Sci., 48, 2013 (1962).

7. H. T. Miles and J. Frazier, Biochem. Biophys. Res. Commun., 49, 199 (1972).

8. T. J. Pinnavaia, H. T. Miles, and E. D. Becker, J. Am. Chem. Soc., 97, 7198 (1975).

9. C. Giessner-Prettre and B. Pullman, J. Theor. Biol, 27, 87 (1970).

10. C. Giessner-Prettre and B. Pullman, Biochem. Biophys. Res. Commun., 70, 578 (1976).

11. W. L. Jolly, The Synthesis and Characterization of Inorganic Compounds; Prentice-Hall, Inc., Englewood Cliffs, N. J., p. 317.

12. H. T. Miles, F. B. Howard, and J. Frazier, Science, 142, 1458 (1963).

13. D. M. Crothers, C. W. Hilbers, and R. G. Shulman, Proc. Nat. Acad. Sci. U. S. A., 70, 2899 (1973).

14. D. B. Arter, G. C. Walker, O. C. Uhlenbeck, and P. G. Schmidt, Biochem. Biophys. Res. Commun., 61, 1089 (1974).

BIBLIOGRAPHY

(Cont'd.)

15. D. J. Patel and C. W. Hilbers, Biochemistry, 14, 2651 (1975).

16. M. P. Schweizer, A. D. Broom, P. O. P. T'so, and D. P. Hollis, J. Am. Chem. Soc., 90, 1042 (1968).

17. M. Hattori, J. Frazier and H. T. Miles, Biopolymers, 14, 2095 (1975).

18. Handbook of Chemistry and Physics, ed. Robert C. Weast, c. 1974, 1975.

19. J. D. Watson and F. H. C. Crick, Nature, 171, 738 (1958).