A New Model for the K^+-Induced Macromolecular Structure of Guanosine 5′-Monophosphate in Solution†

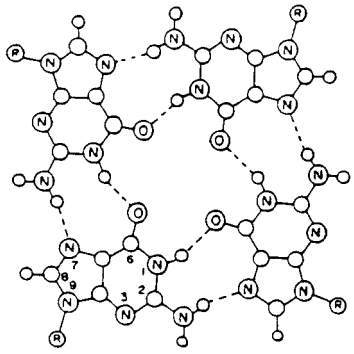
Judith A. Walmsley* and Jane F. Burnett

Division of Earth and Physical Science, The University of Texas at San Antonio, San Antonio, Texas 78249

Received January 7, 1999; Revised Manuscript Received August 23, 1999

ABSTRACT: The ^{31}P NMR spectra of $(TMA)_2$(5′-GMP), where TMA is $[(CH_3)_4N]^+$ and 5′-GMP is guanosine 5′-monophosphate, and K_2(5′-GMP), containing various amounts of KCl or TMACl, have been obtained at 2 °C. Variable-temperature spectra have also been obtained for K_2(5′-GMP). The TMA^+ ion serves to neutralize the charge on the dianionic 5′-GMP and permits the added K^+ to bond preferentially in structure-forming sites. ^{1}H NMR spectra (one- and two-dimensional) have been obtained for K_2(5′-GMP) and used to assign the proton resonances in the self-associated structures and determine that all residues have the anti glycosidic conformation. The ^{31}P and ^{1}H NMR spectra are very complex and indicate the presence of a large number of molecular environments and a structural variation dependent upon the mole ratio of 5′-GMP to K^+. A new model for the solution structure is proposed in which the 5′-GMP forms a pseudo-four-stranded helix with guanine−guanine hydrogen bonding forming a continuous helical strand, rather than the usual planar G-tetrad structure. The guanine−guanine hydrogen bonding sites are the same as that found in a G-tetrad. The K^+ ions would be located in the center of the helix and bonding to the carbonyl oxygens. They are interacting with the phosphates as well. Integration data from the largest sized species give an estimate of 14.3 ± 1.1 residues in a helical structure.

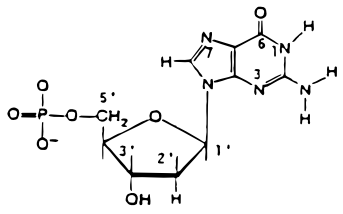
It has been known since 1962 that guanosine and guanosine monophosphates formed gels in the presence of certain monovalent cations at slightly acid pH (*1*). A G-tetrad stucture, I, was proposed to exist in the gels and was later



G-tetrad, I

shown to be consistent with fiber diffraction data *(2, 3)*. In the 1970s it was discovered that self-associated species also existed in neutral solutions and that much of the experimental data were consistent with species consisting of stacked G-tetrads (*4*−*9*). More recently, evidence has been found for the formation of G-tetrad structures in oligonucleotide sequences which model the G-rich telomeric sequences present in chromosomes, and numerous biological functions have been postulated for them (*10*−*13*). NMR, HPLC, X-ray diffraction, and other techniques have delineated the various types of G-tetrads (four-stranded or hairpin) and their conformations in the model telomeres (*4*, *5*, *14*, *15*). An X-ray crystal structure determination of $d(TG_4T)$ revealed a G-tetrad structure with Na^+ ions located in the center of the G-tetrad or between two adjacent G-tetrads, depending upon the position of the G-tetrad in the structure (*16*, *17*), but the K^+ ion has not been definitively located in a G-tetrad structure (*18*).

Although formation of macromolecular species was first observed in guanosine and its monophosphates, many questions remain regarding the solution structures of the species in these molecules. In part, this is a result of the larger number of structural and conformational possibilities in the guanine monophosphates compared to the oligonucleotides. The complexity of the self-associated guanosine 5′-monophosphate (5′-GMP),[1] II, system is illustrated by the



5′-GMP, II

† Supported by Grants GM08194 and GM07717 from the National Institutes of Health.

* To whom correspondence should be addressed at the Division of Earth and Physical Science, The University of Texas at San Antonio, 6900 N. Loop 1604 W., San Antonio, TX 78249-0663. Telephone: 210-458-5459. Fax: 210-458-4469. E-mail: jwalmsley@utsa.edu.

[1] Abbreviations: 5′-GMP, guanosine 5′-monophosphate; TMA, tetramethylammonium ion; EDTA, ethylenediaminetetraacetic acid.

10.1021/bi9900370 CCC: $18.00
Published on Web 10/01/1999

multiple ^{1}H resonances observed for each nonexchangeable proton in NMR, which are in slow exchange with the monomer resonances (*5*−*9*, *19*). Of the three alkali metal cations which promote self-association of 5′-GMP (Na^+, K^+, and Rb^+), the K^+ ion has the strongest effect, and its proton NMR spectrum is the most complex (*7*). Many aspects of the ^{1}H NMR spectra of the Na^+ and K^+ salts are consistent with the formation of G-tetrads (*19*), but the existence of other hydrogen-bonded motifs and/or a variety of isomeric tetrad structures is possible. The ^{31}P NMR spectra were initially examined by Laszlo (*20*) and Walmsley and Pinnavaia and their co-workers (*19*), and some structures were postulated.

On the basis of new data from ^{31}P and ^{1}H NMR experiments, we have proposed a new structure for 5′-GMP in solution in the presence of K^+. Rather than a structure composed of G-tetrads, it consists of a helical, stacked array of 5′-GMP, but still containing the same general hydrogen bonding scheme of a typical discrete G-tetrad. ^{31}P NMR has been used to observe the formation of the self-associated species in K_2(5′-GMP), in K_2(5′-GMP) with added KCl or tetramethylammonium chloride, and in the tetramethylammonium salt, [$(TMA)_2$(5′-GMP)], as small amounts of KCl were added. In the form of the TMA salt, 5′-GMP does not undergo self-association in aqueous solution at any concentration or temperature unless one of the structure-forming cations, such as Na^+, K^+, or Rb^+, is added to it (*21*). This provides a method of observing the formation of the self-associated species as they begin to form and gradually increase in size and complexity. 1-D and 2-D ^{1}H NMR measurements were used to assign most of the proton resonances and to determine the conformation about the glycosidic bond.

The reasons for the structural variation of 5′-GMP as a function of K^+ ion concentration are potentially of biological significance because K^+ is primarily an intracellular cation and is known to be important in nucleic acid functions. Based on the marked differences in the NMR spectra, the Na^+-induced structure in 5′-GMP solutions is quite different from that of the K^+ structure. It is also known that Na^+ and K^+ stabilize different G-tetrad structures in telomeric sequences (*22*−*24*).

MATERIALS AND METHODS

Materials. H_2(5′-GMP) was purchased from Sigma or Calbiochem. $(TMA)_2$(5′-GMP) and K_2(5′-GMP) were prepared as previously described (*25*). D_2O (99.9 atom %) was purchased from Norell or Aldrich. Ultrapure KCl was obtained from Alfa Chemical.

Sample solutions were prepared by dissolving the solid nucleotides in D_2O and adjusting the pD's with DCl or TMAOH dissolved in D_2O. For solutions containing added increments of KCl or TMACl, the salt was added to the NMR tube as the dried solid. The concentrations of the 5′-GMP solutions were determined by UV spectroscopy ($\lambda_{max} = 252$ nm, $\epsilon = 1.37 \times 10^4$ M^{-1} cm^{-1}). The K_2(5′-GMP) solution for ^{1}H NMR contained 0.1 mM TMA-EDTA.

Methods. The ^{31}P NMR spectra were run on a General Electric QE 300 spectrometer at 121.7 MHz. The samples were contained in 10 mm tubes except for the variable-temperature measurements which were done in 5 mm tubes; 85% H_3PO_4 was used as an external reference. The spectra were run with gated inverse broad band proton decoupling, a spectral width of 6000 or 15 000 Hz, a pulse width of 10 μs, and a relaxation delay time of 10 s. Constant low-temperature experiments were carried out at 2.0 ± 0.2 °C. T_1 relaxation experiments were done on a Varian INOVA 500 at 202.34 MHz at 2 °C.

The ^{1}H NMR 1-D and COSY spectra were run at 499.86 MHZ on a Varian INOVA 500 spectrometer, and the NOESY spectra were run at 300.65 MHZ on a General Electric QE 300. Spectra were run at 24 °C and were referenced to internal TMA^+ ion at 3.185 ppm. The mixing time for the NOESY was 200 ms, the data size was 1024, and the number of transients per increment was 16.

RESULTS

Considerably less research has been done on K_2(5′-GMP) than on Na_2(5′-GMP), largely because Na_2(5′-GMP) appears to be less complex, as indicated by both its ^{1}H and its ^{31}P NMR spectra (*7*). However, the solution structures and conformations of neither of the two salts have been completely determined. Since $(TMA)_2$(5′-GMP) by itself does not self-associate, the use of $(TMA)_2$(5′-GMP) with incremental addition of salt allows the observation of the associated species as they are formed. The TMA^+ serves to neutralize the charge on the dianionic 5′-GMP and permits the added cation to bond preferentially in structure-forming sites. Such experiments have provided substantially more information on the conformation and structure of K_2(5′-GMP).

$(TMA)_2$(5′-GMP) + KCl. The ^{31}P NMR spectra of 0.49 M $(TMA)_2$(5′-GMP) in D_2O at 2 °C with various amounts of added KCl are shown in Figure 1. The data show that the K^+ system is very complex and that numerous intermediate structured species are formed as KCl is added to $(TMA)_2$-(5′-GMP). The phosphate resonance in $(TMA)_2$(5′-GMP) is located at 4.198 ppm, and the majority of the new ^{31}P resonances that appear upon KCl addition are situated around 4−5 ppm, which is a typical value for a phosphate monoester dianion (*26*). Additional lines become prominent at 6.97 and 5.80 ppm as the K^+ concentration increases. These are most likely to result from phosphates that are found at the ends of stacks of guanine bases, in agreement with observations in RNA (*27, 28*). The multiplicity of the phosphate environments was rather surprising, but is consistent with the complex ^{1}H NMR spectra of this system (vide infra).

Examination of the spectra in Figure 1 reveals that there are rather dramatic changes at certain GMP:K^+ ratios. The first of these occurs at the 2:1 mole ratio, where the appearance and intensity of new lines suddenly increases and is taken to be the point at which a substantial amount of structured species are formed. At a ratio of 1:1, the intensities of the lines in the 5−7 ppm region increase, and again at 1:2 ratio, there is a further increase in intensity in this region, along with the virtual disappearance of the resonance at 6.3 ppm. It is clear that these changes are the result of K^+-induced structural changes, although it is not clear just what structures are forming at each ratio.

K_2(5′-GMP): ^{31}P NMR. The temperature-dependent ^{31}P NMR spectra of 0.54 M K_2(5′-GMP) are shown in Figure

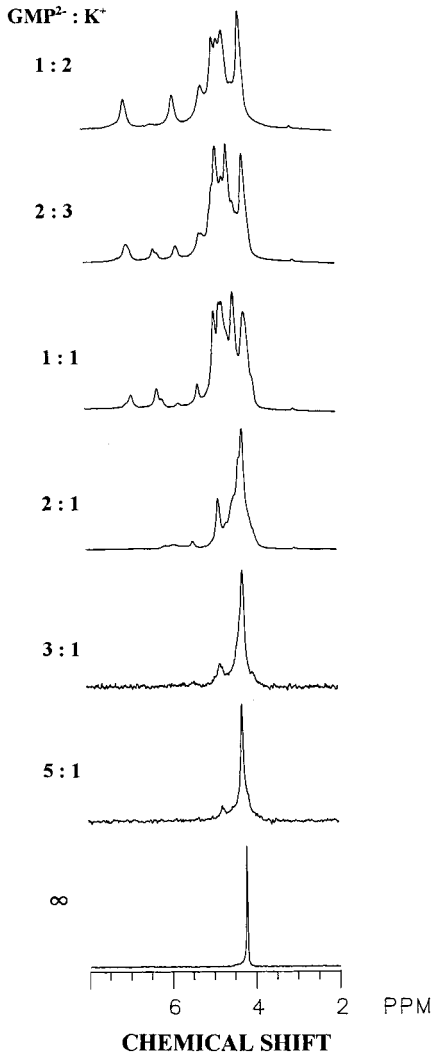


FIGURE 1: ^{31}P NMR spectra of 0.49 M $(TMA)_2$(5′-GMP) in D_2O with added KCl at 2 °C and pD 8.6 at various mole ratios of GMP:K^+.

2. Although there is similarity between these spectra and those in Figure 1, they appear to have fewer resonances than do the spectra of $(TMA)_2$(5′-GMP) + KCl. The lines at 5−7 ppm only appear below the 9−16 °C temperature range. Comparison of the 1:2 mole ratio of GMP:K^+ (Figure 1) with that of 0.54 M K_2(5′-GMP) at 2 °C reveals that the spectra are rather similar, but the latter is still less complex. It is thought that the difference in the two spectra is not only a result of the charge-neutralizing role of the TMA^+ ions, but also a result of the increased ionic strength from the presence of a greater amount of cations in the former case. This is shown to be the situation because the addition of solid KCl to the K_2(5′-GMP) solution leads to a spectrum which is very similar to that of the $(TMA)_2$(5′-GMP) + KCl solution (GMP:K^+ = 1:2) (Figure 3B). Although there are some differences in the relative intensities of the resonances in the main cluster, it is noted that the intensities of the 6 and 7 ppm signals have increased dramatically upon KCl addition to K_2(5′-GMP). With KCl additions greater than a GMP:K^+ ratio of 1:3, the spectra no longer are changing and appear to have reached a limiting structure.

Addition of TMACl to 0.54 M K_2(5′-GMP) has an effect similar to that of adding KCl to this solution (Figure 3C). At a K_2GMP:TMA^+ ratio of 1:3, the ^{31}P NMR spectrum is

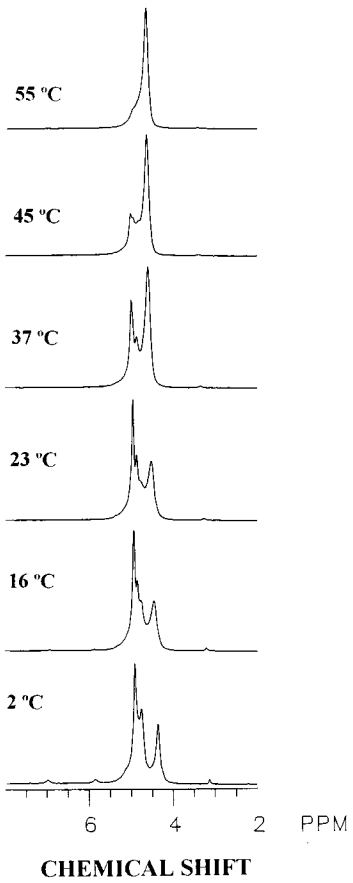


FIGURE 2: ^{31}P NMR spectra of 0.54 M K_2(5′-GMP) in D_2O at various temperatures, pD 8.6.

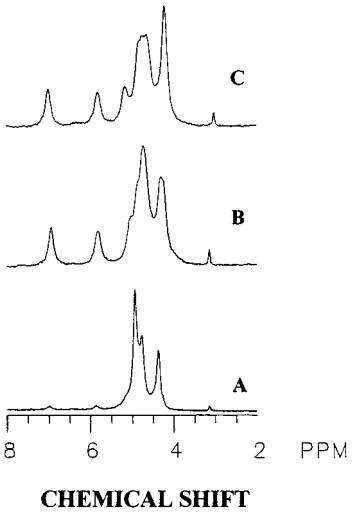


FIGURE 3: ^{31}P NMR spectra of 0.54 M K_2(5′-GMP) in D_2O at 2 °C. (A) No added salt; (B) with added KCl, GMP:K^+ = 1:4, total $[K^+]$ = 2.1 M; (C) with added TMACl, GMP:K^+ = 1:2, GMP:TMA^+ = 1:3, total cation concentration = 2.4 M.

the same as the limiting spectrum for K_2(5′-GMP) + KCl. It might have been thought that additional TMA^+ ion would compete with K^+ ion for structural cationic sites and reduce the amount of structured species, but clearly the structured species increase in quantity and complexity instead. It is concluded that ionic strength is an important factor in this case also. When there is sufficient K^+ ion present to give the structured species, the apparent effects of the added salts are to shift the equilibrium further toward that species. An

Table 1: T_1 Relaxation Times for K_2(5′-GMP) + TMACl (GMP:TMA^+ = 1:3)[a]

chemical shift (ppm)	T_1 (s)
6.974	13.75 ± 0.20
5.799	13.12 ± 0.40
5.145	7.70 ± 0.26
4.619	2.76 ± 0.04
4.182	2.83 ± 0.11

[a] ^{31}P at 2 °C.

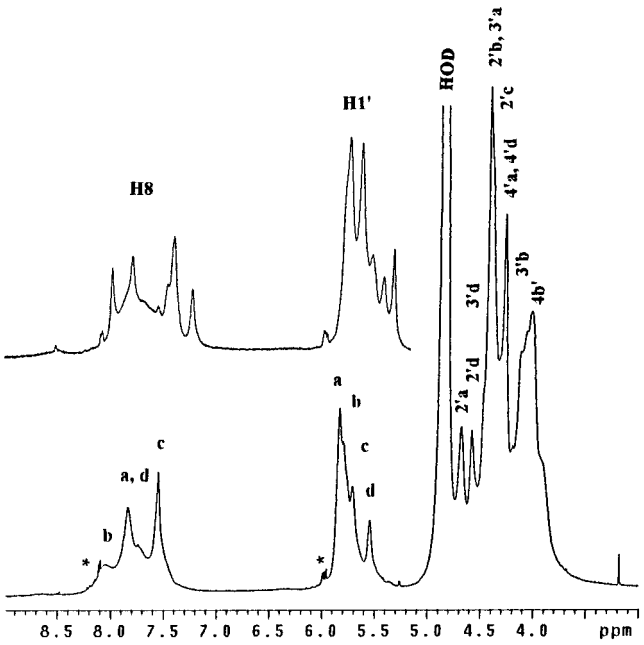


FIGURE 4: ^1H NMR spectrum of 0.49 M K_2(5′-GMP) in D_2O at 24 °C, pD 8.6. Ribose assignments are based on COSY spectra of the same sample at 24 °C. H1′ protons were arbitrarily assigned letters as a starting point for assignments. H8 proton assignments are based on NOESY data. TMA^+ is located at 3.185 ppm; * = unknown species. Inset: 0.54 M K_2(5′-GMP) in D_2O at 25 °C with added KCl, GMP:K^+ = 1:4.

anion effect can be ruled out because KNO_3 added to K_2-(5′-GMP) (*29*) has the same effect as KCl or TMACl.

A T_1 relaxation time experiment on the K_2(5′-GMP) + TMACl (GMP:TMA^+ = 1:3) solution at 2 °C shown in Figure 3C revealed that the lines at 6 and 7 ppm had very long times compared to those in the 4−5 ppm cluster (Table 1). The resonance at ∼5.1 ppm had an intermediate T_1. The 6 and 7 ppm lines have been proposed to arise from terminal phosphates and the long T_1's would be expected, while the phosphates belonging to residues located in the central part of the structure would have much shorter T_1's.

K_2(5′-GMP): ^1H NMR. The ^1H NMR spectrum of 0.49 M K_2(5′-GMP) at 24 °C is shown in Figure 4. All of the H8 resonances have chemical shifts in the ∼8.1−7.5 ppm region, and there is obvious overlapping of numerous lines representing different H8 environments (see also ref *7*). The assignments of the ribose protons (3.8−6.0 ppm) are based on COSY spectra, although it was not possible to assign all of the lines due to extensive overlap. The H1′ protons were arbitrarily assigned letters to serve as a starting point for the assignments. The H5′ and H5″ resonances could not be specifically assigned, but are located in the 3.8−4.1 ppm region. The H8 assignments are tentative and were based on the NOESY spectrum (see below).

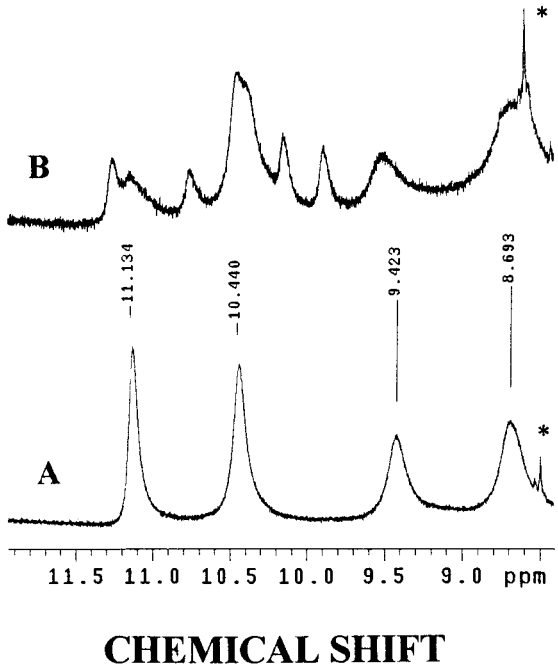


FIGURE 5: ^1H NMR spectrum of 0.47 M K_2(5′-GMP) in 90% H_2O/10% D_2O at 24 °C, pH 8.3. (A) No added salt; (B) with added KCl, GMP:K^+ = 1:4; * = unknown species.

In solutions to which either solid KCl or TMACl has been added, the H8 and the H1′ regions become even more complex with additional lines becoming visible (Figure 4, inset). Both salts have the same effect on the K_2(5′-GMP) spectrum and no effect on the H2′−H5′,5″ region.

It is significant that all of the H8 and H1′ resonances in the self-associated species are located upfield of those in the monomer (H8, 8.2; H1′, 5.9 ppm), indicating that considerable base stacking is occurring in all of the self-associated species. An upfield shift of NMR signals in nucleotides and nucleic acids is normally associated with ring current effects resulting from base stacking (*30*).

To examine the exchangeable protons and identify the hydrogen-bonding sites, the ^1H NMR spectrum of 0.47 M K_2(5′-GMP) was also obtained in 90% H_2O/10% D_2O at 24 °C (Figure 5A). As in Na_2(5′-GMP) (*19*), the imino proton resonance is located at 11.13 ppm, and the hydrogen-bonded NH_2 resonances are found at 10.44 and 9.42 ppm. The resonance at 8.69 ppm is likely to be a hydrogen-bonded ribose OH. The four exchangeable protons integrate at approximately one each. When KCl is added to the solution (Figure 5B), the number of resonances in this region increases, consistent with changes in the nonexchangeable proton resonances in the presence of KCl (Figure 4).

The NOESY spectrum of 0.49 M K_2(5′-GMP) in D_2O at 24 °C (data not shown) displayed strong dipolar interactions between the H8 protons and the 2′ and 3′ ribose protons, but no interactions between H8 and H1′. This indicates that all of the 5′-GMP units in the associated species have the anti conformation about the glycosidic bond. The NOESY data were used to assign the H8 resonances by assuming that the stronger NOE for each H8 arose from an intramolecular interaction. Although there are a few additional H8 to ribose NOE's, they cannot be used for a complete structural analysis because of the large uncertainty in the assignments and the multiple overlaps.

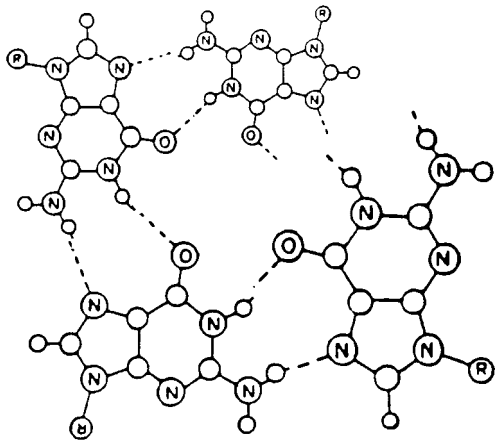


FIGURE 6: Proposed helical structure of K_2(5′-GMP) in aqueous solution at neutral to slightly basic pH.

DISCUSSION

The complexity and multiplicity of the phosphate environments in 5′-GMP in the presence of K^+ ion have a striking similarity in appearance to the ^{31}P NMR spectra of tRNA from yeast (*27*) and of 5S RNA from *E. coli* (*28*), both of which have many phosphodiester environments. However, the resonances of the RNA's occur in a different chemical shift range. The spectra consist of a main cluster of resonances with several downfield lines of weaker intensity and, in some cases, there are also a few weak upfield lines. The ^{31}P NMR assignments for 5S RNA were based on the spectra of its fragments of known structures (*28*). The most downfield lines (weak) were assigned to terminal phosphates, and the lines in the 5−7 ppm region of our spectra can be similarly assigned on the basis of their long relaxation times (Table 1). These would be phosphates at the ends of stacks of 5′-GMP and must have an unusual conformation since they are much further downfield than a typical phosphate monoester (*26*). This conformational change is induced by K^+ interactions because these resonances only appear at GMP:K^+ ratios of 1:2 or higher K^+ concentrations. Comparison of the other assignments in RNA with our spectra is not possible because it is difficult to imagine that 5′-GMP structures can have the loops and bulges found in the RNA structures. *Overall, it is the similarity in complexity between the RNA spectra and our spectra that seems to be important.*

To account for the ^{31}P and ^{1}H NMR spectra, a model is proposed which consists of a pseudo-four-stranded helix with guanine−guanine hydrogen bonding forming a continuous helical strand (Figure 6). This could be thought of as a G-tetrad in which two of the eight hydrogen bonds are broken and re-formed with another guanine from a similar G-tetrad to form the helix. This hydrogen-bonding arrangement in a helical, rather than planar, structure would lead to a large number of slightly different ^{1}H and ^{31}P environments when the number of nucleotides per turn of the helix is large, that is, when there is a small shift in angle between a nucleotide and the one closest above it. NOESY data show that all of the residues have the anti conformation about the glycosidic bond and, therefore, would all be stacked in the same direction (*5, 14*). In telomeric sequences in the presence of K^+, which are proposed to have a parallel four-stranded structure, all residues also have the anti conformation. On the other hand, the anitiparallel hairpin structures formed in the presence of Na^+ have both syn and anti conformations (*13, 15, 18*).

The hydrogen-bonding scheme between any two 5′-GMP moieties in the helix is the same as that in a discrete, planar G-tetrad. This is confirmed by ^{1}H NMR of K_2(5′-GMP) in H_2O (Figure 5, also ref *31*) which has the imino (11.13 ppm) and hydrogen-bonded amino (10.44, 9.42 ppm) resonances in the same chemical shift regions as Na_2(5′-GMP) (imino: 11.1−11.3; amino: 9.3−10.1 ppm) (*19*) and a number of telomeric oligonucleotides known to have the G-tetrad structure (*14, 24, 32, 33*). The location of the imino at 11.1 ppm results from an N(1)H···O═C bonding because an N(1)H···N(heterocyclic) hydrogen bond, such as in a Watson−Crick G−C base pair, is found at 13 ppm (*34, 35*).

Although a number of X-ray studies indicate the formation of essentially planar G-tetrad structures in guanine nucleotides and oligonucleotides (*2, 3, 16−18, 36*), there has also been one account of a helical structure such as the one proposed here for K_2(5′-GMP). Based on X-ray fiber diffraction data, a helical structure held together by hydrogen bonds between guanines was postulated for 5′-GMP fibers (Na:GMP < 1, no K^+) pulled from gels at pH 5 (*37*). Although the conditions were different from ours, this shows that a helical structure is possible in a 5′-GMP system.

However, the large number of resonances in the NMR spectra of K_2(5′-GMP) does not rule out a planar G-tetrad structure. If adjacent tetrads in a stacked array had a small rotation angle about the central axis, multiple environments are also probable. However, oligonucleotides (models of telomeric sequences) which are postulated to have planar G-tetrad structures and which have been studied by NMR lack the spectral complexity of K_2(5′-GMP); most or all of their imino and H8 resonances are distinctly separated (*14, 23, 24*). Also, Na_2(5′-GMP), for which a structure composed of discrete, stacked G-tetrads is quite well established (*6, 8, 9, 19, 21, 36*), has much simpler ^{1}H and ^{31}P spectra, with four separated resonances in the H8 region and four phosphate environments (*19, 21, 25*). Moreover, the presence of "terminal" phosphates at 6 and 7 ppm in K_2(5′-GMP) is inconsistent with a structure composed of planar G-tetrads.

The K^+ ions would be located in the central core of the helix and bonding to the C═O groups of the base. Evidence to support this comes from an X-ray crystal structure determination of d(TG_4T), which revealed a G-tetrad structure with Na^+ ions located in the center of the G-tetrad or between two adjacent G-tetrads, depending upon the position of the G-tetrad in the structure (*16, 17*). Kang and co-workers were not able to definitely locate the K^+ in an X-ray structural determination of d($G_4T_4G_4$), but they reported that a 'disturbance' in the center of the G-tetrad structure was thought to be due to the K^+ (*18*). An additional possibility for the role of K^+ in our structures is the bridging of a K^+ ion between the phosphate groups of two 5′-GMP residues which are vertically stacked. This latter interaction would considerably increase the stability of the self-associated structure.

An estimate of the size of the structured species can be obtained by integrating the ^{31}P NMR data from spectra in which the maximum size has been reached, as judged by the lack of further change in the spectra. This occurs for K_2(5′-GMP) with added KCl, with added TMACl, and for $(TMA)_2$(5′-GMP) + KCl. Combining the data from five

spectra, it was found that 14.0 ± 1.1% of the total GMP units was accounted for by the 6 and 7 ppm resonances. Assuming one of these terminal phosphates is found at each end of the helix, an estimate of 14.3 ± 1.1 residues per structured species is obtained. Although this is not a tremendously large molecule, a helix of this size could account for the multiple resonances observed in the NMR.

No one piece of data proves that the proposed structure is correct, but it is the sum of all the data which provides the best evidence for the structure. In light of the extreme difficulty or impossibility of obtaining crystals for a high-resolution X-ray determination, NMR solution data give the greatest amount of evidence for this structure. The helical model is consistent with the fact that the self-association of 5′-GMP in the presence of K^+ is much stronger than that in the presence of Na^+ (*7*, *9*). In our structure, the pseudo-G-tetrads would be held together by a continuous hydrogen-bond helical network, base stacking, and metal ion effects, whereas discrete G-tetrads composed of 5′-GMP will have fewer intermolecular interactions. It is also known that with G-rich telomeric sequences, K^+ leads to the formation of four-stranded G-tetrad structures while Na^+ favors hairpin structures (*22*).

The physiological relevance of the K^+-induced structure is uncertain, but could be related to the expansion of nucleic acid structures to facilitate the binding of proteins (*38*), such as the binding of DNA to thrombin (*39*), HIV-1 (*40*), and other proteins (*41)*.

ACKNOWLEDGMENT

J.A.W. thanks Dr. Thomas J. Pinnavaia, who suggested, some years ago, that the ^{31}P NMR of these systems might be of value.

REFERENCES

1. Gellert, M., Lipsett, M. N., and Davies, D. R. (1962) *Proc. Natl. Acad. Sci. U.S.A. 48*, 2013−2018.
2. Zimmerman, S. B., Cohen, G. H., and Davies, D. R. (1975) *J. Mol. Biol. 92*, 181−192.
3. Zimmerman, S. B. (1976) *J. Mol. Biol. 106*, 663−672.
4. Miles, H. T., and Becker, E. D. (1972) *Biochem. Biophys. Res. Commun. 49*, 199−204.
5. Guschlbauer, W., Chantot, J.-F., and Thiele, D. (1990) *J. Biomol. Struct. Dyn. 8*, 491−511.
6. Pinnavaia, T. J., Miles, H. T., and Becker, E. D. (1975) *J. Am. Chem. Soc. 97*, 7198−7200.
7. Pinnavaia, T. J., Marshall, C. L., Mettler, C. M., Fisk, C. L., Miles, H. T., and Becker, E. D. (1978) *J. Am. Chem. Soc. 100*, 3625−3627.
8. Borzo, M., Detellier, C., Laszlo, P., and Paris, A. (1980) *J. Am. Chem. Soc. 102*, 1124−1134.
9. Detellier, C., and Laszlo, P. (1980) *J. Am. Chem. Soc. 102*, 1135−1141.
10. Williamson, J. R. (1994) *Annu. Rev. Biophys. Biomol. Struct. 23*, 703−730.
11. Sen, D., and Gilbert, W. (1992) *Methods Enzymol. 211*, 191−199.
12. Kipling, D. (1995) *The Telomere*, pp 31−77, Oxford University Press, Oxford, U.K.
13. Henderson, E. (1995) in *Telomeres* (Blackburn, E. H., and Greider, C. W., Eds.) pp 11−34, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
14. Wang, Y., and Patel, D. J. (1992) *Biochemistry 31*, 8112−8119.
15. Smith F. W., and Feigon, J. (1993) *Biochemistry 32*, 8682−8692.
16. Laughlan, G., Murchie, A. I. H., Norman, D. G., Moore, M. H., Moody, P. C. E., Lilley, D. M. J., and Luisi, B. (1994) *Science 265*, 520−524.
17. Phillips, K., Dauter, Z., Murchie, A. I. H., Lilley, D. M. J., and Luisi, B. (1997) *J. Mol. Biol. 273*, 171−182.
18. Kang, C., Zhang, X., Ratliff, R., Moyzis, R., and Rich, A. (1992) *Nature 356*, 126−131.
19. Walmsley, J. A., Barr, R. G., Bouhoutsos-Brown, E., and Pinnavaia, T. J. (1984) *J. Phys. Chem. 88*, 2599−2605.
20. Borzo, M., and Laszlo, P. (1978) *Compt. Rend. 287*, 475−478.
21. Bouhoutsos-Brown, E., Marshall, C. L., and Pinnavaia, T. J. (1982) *J. Am. Chem. Soc. 104*, 6576−6584.
22. Miura, T., Benevides, J. M., and Thomas, G. J., Jr. (1995) *J. Mol. Biol. 248*, 233−238.
23. Hud, N. V., Smith, F W., Anet, F. A. L., and Feigon, J. (1996) *Biochemistry 35*, 15383−15390.
24. Bouaziz, S., Kettani, A., and Patel, D. J. (1998) *J. Mol. Biol. 282*, 637−652.
25. Walmsley, J. A., and Sagan, B. L. (1986) *Biopolymers 25*, 2149−2172.
26. Gorenstein, D. G. (1984) *Phosphorus-31 NMR, Principles and Applications*, Academic Press, New York.
27. Gueron, M., and Shulman, R. G. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 3482−3485.
28. Zhang, P., Reyna, R., and Moore, P. B. (1989) *Nucleic Acids Res. 17*, 7295−7302.
29. Bouhoutsos-Brown, E. (1980) Structure Directing Alkali and Alkaline Earth Metal Ions in the Solution Ordering of Guanosine Monophosphates, Ph.D. Dissertation, Michigan State University, pp 97−103.
30. Giessner-Prettre, C., Pullman, B., Borer, P. N., Kan, L.-S., and Ts'o, P. O. P. (1976) *Biopolymers 15*, 2277−2286.
31. Barr, R. G. (1981) The Solution Ordering of Alkali Metal Salts of Guanylic Acids, Ph.D. Dissertation, Michigan State University, pp 166−170.
32. Ghana, R., Walss, C., and Walmsley, J. A. (1996) *J. Biomol. Struct. Dyn. 14*, 101−110.
33. Deng, H., and Braunlin, W. H. (1995) *Biopolymers 35*, 677−681.
34. Gupta, G., Garcia, A. E., and Hiriyanna, K. T. (1993) *Biochemistry 32*, 948−960.
35. Boelens, R., Scheek, R. M., Dijkstra, K., and Kaptein, R. (1985) *J. Magn. Reson. 62*, 378−386.
36. Lipanov, A. A., Quintana, J., and Dickerson, R. E. (1990) *J. Biomol. Struct. Dyn. 8*, 483.
37. Sasisekharan, V., Zimmerman, S., and Davies, D. R. (1975) *J. Mol. Biol. 92*, 171−179.
38. Allen, S. H., and Wong, K.-P. (1986) *Arch. Biochem. Biophys. 249*, 137−147.
39. Macaya, R. F., Schultze, P., Smith, F. W., Roe, J. A., and Feigon, J. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 3745−3749.
40. Sundquist, W. I., and Heaphy, S. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 3393−3397.
41. Schierer, T., and Henderson, E. (1994) *Biochemistry 33*, 2240−2246.

BI9900370