Structure-Directing Properties of Na^+ in the Solution Ordering of Guanosine 5'-Monophosphate. Stoichiometry of Aggregation, Binding to Ethidium, and Modes of Na^+ Complexation

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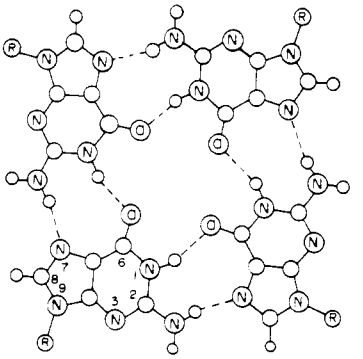
Abstract: Tetramethylammonium has been found to be a structure-inert cation in the solution ordering of guanosine 5'-monophosphate (5'-GMP) at neutral or slightly basic pH. This finding has made it possible to study quantitatively by ^1H NMR spectroscopy the stoichiometry of nucleotide ordering in the presence of Na^+ as a structure-directing counterion. The dependence of the ordered structures on total nucleotide concentration is consistent with the formation of octamers. Independent evidence for octamer formation is provided by the binding of ethidium to the ordered nucleotide. Two octamer–ethidium complexes are observed with octamer to ethidium ratios of 1:1 and 1:2. The dependence of ordered structure formation on Na^+ concentration indicates the binding of four Na^+ ions per octamer unit. Two types of Na^+ binding sites are inferred from mixed Na^+–K^+ experiments. One binding site is a highly Na^+-specific structure-directing site. The second site is less Na^+ specific, but it plays an important role in stabilizing the ordered structures. The replacement of Na^+ by K^+ at the second site dramatically stabilizes the Na^+-directed self-structures. A model involving coaxial stacking of planar hydrogen-bonded tetramer units is proposed for the ordered structures. Normal and inverted tetramer stacking arrangements account for the presence of three NMR-observable isomers, provided that twisting about the C_4 symmetry axis is rapid. The hole defined by the four carbonyl oxygens of a tetramer unit is believed to be the highly Na^+-specific binding site. Chelation of Na^+ (or structure-stabilizing K^+) by phosphate oxygens on adjacent tetramer plates is proposed as the less specific binding site. Model building studies suggest that interplate hydrogen bonding also may be involved in stabilizing the stacked tetramers.

Introduction

Guanine nucleotides are unique among the nucleic acids in their ability to form regular ordered structures in aqueous media. In the case of guanosine 5'-monophosphate (5'-GMP) the ordering phenomenon at pH <5.5 is sufficiently extensive to form anisotropic gels.[1] Under neutral or slightly basic conditions where the phosphate moiety is not protonated, the more negatively charged nucleotide aggregates less extensively, and ordered *solution* structures can be observed by IR,[2] NMR,[3,4] and Raman[5,6] spectroscopy and by calorimetry.[7,8]

Recent studies[9,10] have demonstrated that the solution ordering of the 5'-GMP dianion is dramatically dependent on the nature of the alkali metal counterion. The Na^+, K^+, and Rb^+ salts all form ordered structures that can best be observed by the appearance of nonequivalent H(8) resonances in the proton NMR spectra.[9] The Li^+ and Cs^+ salt have little or no tendency to form ordered structures. These observations indicate that size-selective alkali metal complexation reactions are involved in the self-assembly process. In contrast, the normal role of alkali metal ions in the structural chemistry of nucleic acids is to shield phosphate charge through nonspecific ion pairing interactions.[11-13]

Although several models have been proposed for the solution ordering of 5'-GMP, the model that remains most attractive to us is based on the limited coaxial stacking of tetramer units formed by interbase hydrogen bonding between N(1)–H and N(2)–H as donors and O(6) and N(7) as acceptors (I). The evidence favoring



I

the presence of tetramer units is based in part on anologies between the C=O, C=N, and C=C stretching frequencies of the ordered 5'-GMP aggregates and 3'-GMP gel,[2] wherein extensive tetramer stacking is known to occur from X-ray diffraction studies.[14] The stacking of tetramers is verified by the large upfield shifts that are observed for certain H(8) resonances of the ordered nucleotide.[3,9] Very recent ^{13}C NMR studies[15] indicate that the T_1, T_2, and NOE data for the C(8) lines of one ordered component of Na_2(5'-GMP) can be explained by an octamer formed by the head to tail stacking of two tetramer units.

The coaxial stacking of two tetramer units can also account for the relative structure-directing power of the alkali metal ions ($K^+ > Na^+$, $Rb^+ \gg Cs^+ > Li^+$). The central cavity defined by the eight carbonyl oxygens of two tetramers at a normal stacking distance of 3.4 Å is ideally suited[16] for complexation of K^+ (K–O

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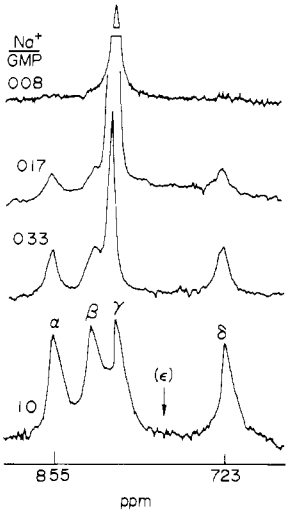

Figure 1. Effect of added NaCl on the H(8) resonances (180 MHz) of 0.76 M $(TMA)_2$(5'-GMP) in D_2O at 2.8 °C. The three lines labeled α, β, and γ are due to ordered forms of the nucleotide and appear at the expense of the disordered nucleotide (line γ). ϵ marks the position of a fourth resonance component of the ordered nucleotide, which is too weak (<4% abundance) to resolve under the conditions defined here.

distance 2.8 Å). Since Rb^+ is similar in size to K^+, Rb^+ may also occupy this eight-coordinate position. The distance between the center of the tetramer unit and the four carbonyl oxygens (2.2–2.3 Å) is highly compatible with the values observed for sodium to carbonyl oxygen bonds.[17] Li^+ and Cs^+, however, have ionic radii that do not fit the "hole" hypothesis and are not capable of acting as structure-directing cations.

Despite all of the attractive features of the tetramer stacking model, there is insufficient stoichiometric evidence to support the model. Also, the model has not been interpreted in sufficient detail to account for all the ordered species present in solution. One of the objectives of the present work is to determine by proton NMR spectroscopy the number of nucleotide and alkali metal ions present in the ordered aggregates of 5'-GMP when Na^+ is the structure-directing ion. To observe the dependence of ordered structure formation on 5'-GMP and Na^+ concentration, we required a structure-inert cation with good solubility properties. Tetramethylammonium (TMA^+) is shown to be such an ion. We also find that the binding of ethidium to the ordered nucleotide strongly supports octamer formation. Model-building studies and symmetry considerations demonstrate that all of the NMR-observable structures can be explained by the coaxial stacking of two tetramer units. In addition, two different types of Na^+ binding sites per octamer unit are revealed through mixed Na^+–K^+ binding studies.

Results

Structure Inertness of TMA^+. ^1H NMR studies of $(TMA)_2$(5'-GMP) in D_2O solution indicate that the salt does not form ordered regular structures even at high concentration (1.0 M) and low temperature (0.1 °C). Normally, the presence of ordered 5'-GMP species is indicated best by the appearance of nonequivalent H(8) resonances 7.0–9.0 ppm downfield from 3-(trimethylsilyl)propionate (TSP) as an internal reference. However, $(TMA)_2$(5'-GMP) solutions show only a single relatively sharp resonance ($\nu_{1/2} \simeq 6$ Hz) near 8.17 ppm, which is characteristic of the disordered nucleotide.

A comparison of NMR spectra for an ordered solution of 0.78 M Na_2(5'-GMP) at 0.3 °C before and after the addition of (TMA)Cl indicates that no change occurs in the fraction of ordered nucleotide. The structure inertness of TMA^+ is further indicated by the spectra in Figure 1 in which NaCl is added in incremental amounts to a solution of disordered $(TMA)_2$(5'-GMP). The three H(8) resonances (H_α, H_β, and H_δ) which appear at the expense of the disordered nucleotide (H_γ) are identical with those observed for an ordered solution of pure Na_2(5'-GMP). A fourth resonance due to ordered nucleotide (H_ϵ) can also be observed under certain conditions of temperature and concentration,[15] but the line is too weak (<4% relative abundance) to be resolved under the conditions employed in the present work. Analogous spectral results are obtained when $(TMA)_2$(5'-GMP) and Na_2(5'-GMP) solutions are mixed in the presence of (TMA)Cl at constant ionic strength.

Table I. Dependence of 5'-GMP Ordering on Total Nucleotide Concentration in 0.85 M NaCl at 15 °C

5'-GMP concn,[a] mol/L	ordered GMP, %[b]	5'-GMP concn,[a] mol/L	ordered GMP, %[b]
0.13	7.8 ± 4.8[c]	0.19	27.2 ± 1.9
0.14	13.4 ± 4.9	0.20	28.7 ± 2.0
0.15	16.4 ± 1.8	0.21	29.9 ± 2.5
0.16	18.0 ± 2.0	0.22	32.2 ± 1.9
0.17	22.0 ± 1.8	0.23	32.8 ± 2.4
0.18	23.6 ± 1.7		

[a] The 5'-GMP was introduced as the TMA^+ salt. [b] The fraction of ordered nucleotide was determined by integration of the H_α, H_β, and H_δ resonances. [c] Average values of two independent H(8) integrations; errors are estimates of systematic and random contributions.

The only significant difference between 5'-GMP solutions containing only Na^+ cations and mixed Na^+/TMA^+ cations is that the H(8) chemical shift of the disordered nucleotide shifts to higher field with increasing structure formation in the homocationic Na^+ system, whereas little or no upfield shift occurs in the mixed cation system. The upfield shifts of the disordered nucleotide line presumably is caused by diamagnetic ring current effects that result from nonregular base stacking of the 5'-GMP anions.[18] The suppression of nonregular base-stacking interactions in the presence of TMA^+ may arise from differences in the ability of Na^+ and TMA^+ to ion pair with the nucleotide dianion.

Dependence on 5'-GMP Concentration. To observe the dependence of ordered structure formation on 5'-GMP concentration, we investigated over a wide temperature region (0–35 °C) a series of $(TMA)_2$(5'-GMP)–NaCl mixtures in which the metal ion concentration was large relative to the concentration of ordered nucleotide. In general, precipitation of the nucleotide severely limited the observable range of ordered structure formation. The best experimental conditions were observed at 15 °C for a 0.85 M NaCl solution containing between 0.13 and 0.23 M $(TMA)_2$(5'-GMP). As illustrated by the data in Table I, the fraction of ordered nucleotide varied from 7.8 ± 4.8% to 32.8 ± 2.3%, as judged by the intensities of the H_α, H_β, and H_δ resonances. Under all conditions the intensity of the H_ϵ resonance was less than the experimental uncertainty in the intensity of the three major structure lines. Consequently, the contribution due to the ϵ line was disregarded.

The formation of ordered polymers from disordered 5'-GMP dianions in the presence of Na^+ as the structure-directing cation can be represented by the mass action expression

$$x\mathrm{Na}^+ + n(5'\text{-GMP})^{2-} \rightleftharpoons \mathrm{Na}_x(5'\text{-GMP})_n^{x-2n} \quad (1)$$

Since the concentration of Na^+ remains nearly constant when the total concentration of Na^+ is large with respect to the Na^+ complexed by the ordered nucleotide, the mass action expression can be simplified to define an apparent equilibrium constant

$$n\mathrm{M} \rightleftharpoons \mathrm{M}_n \quad (2)$$

$$K = [\mathrm{M}_n]/[\mathrm{M}]^n \quad (3)$$

where M represents the disordered monomer and M_n is the ordered polymer. Letting C_M and C_S represent the concentration of nu-

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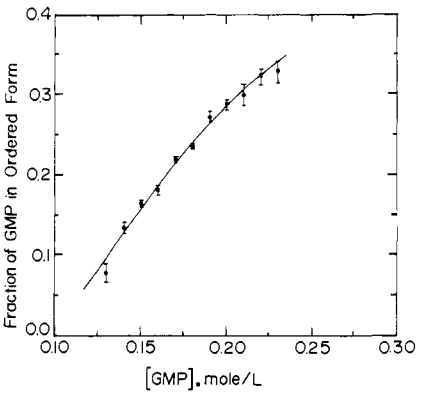


Figure 2. Best least-squares fit of the data in Table I to the octamer model $8M \rightleftharpoons (M_4)_2$. The solid line is the computed curve ($K = (4.0 \pm 0.6) \times 10^4$), and the points represent the experimental data.

cleotide present in the disordered and order forms, respectively, we may rewrite eq 3 as

$$C_M = (C_S/nK)^{1/n} \quad (4)$$

The best least-squares fit of the data in Table I to eq 4 gives $n = 6.5 \pm 1.6$ and $K = (2.7 \pm 9.1) \times 10^3$, where the errors are estimated at the 95% confidence level.

The above treatment disregards ion pairing and base-stacking interactions of the disordered nucleotide. Even if such interactions were to be considered, an accurate value of K would not be expected from a least-squares technique because of the large uncertainties in the NMR integrations over the rather narrow range of concentrations for which data are accessible. Although these uncertainties do not permit an unequivocal determination of the stoichiometry for nucleotide aggregation, the data at least are compatible with octamer formation. As will be shown later, the binding of ethidium to the ordered nucleotide provided independent evidence for the ordered aggregates containing eight nucleotide units. It also will be shown that only octamers constructed from two stacked, planar tetramer units are capable of explaining all of the NMR data.

To illustrate that the data in Table I are consistent with tetramer stacking, we have fit the data to the mass action expression

$$8M \rightleftharpoons (M_4)_2 \quad (5)$$

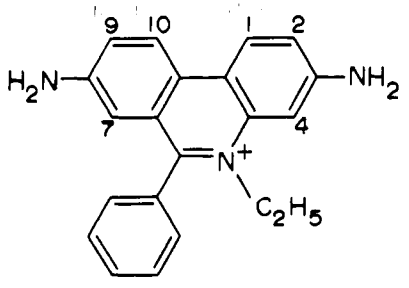
$$K = [(M_4)_2]/[M]^8 \quad (6)$$

The expression for the apparent equilibrium constant can be rearranged and expressed in terms of C_M and C_S as

$$C_M = (C_S/8K)^{1/8} \quad (7)$$

The best least-squares fit, which is illustrated in Figure 2, gives $K = (4.0 \pm 0.6) \times 10^4$.

Ethidium Binding to Ordered 5′-GMP. The drug ethidium (II)



II

is known to bind to DNA by intercalation between stacks of hydrogen-bonded base pairs.[19] Similar binding occurs with helical oligonucleotides and dinucleotides.[20-22] In the present study II has been found to bind also to the ordered forms of 5′-GMP when Na^+ is the structure director.

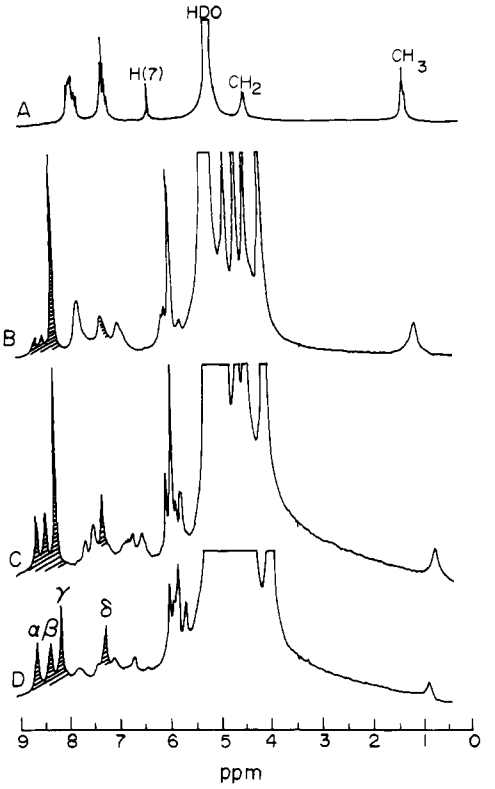


Figure 3. Proton NMR spectra (250 MHz) for ethidium and ethidium–Na_2(5′-GMP) mixtures in D_2O at 5 °C. Spectrum A is for 0.034 M ethidium bromide. Spectra B–D are for 0.034 M ethidium bromide containing 0.18, 0.29, and 0.60 M Na_2(5′-GMP), respectively. The H(8) resonances of the nucleotide are shaded.

The addition of Na_2(5′-GMP) at a concentration where all of the nulceotide is disordered (0.10 M) to a dilute solution of ethidium bromide (0.034 M) results in a relatively small upfield shift (~0.05 ppm) in the drug methyl resonance near 1.40 ppm. However, at Na_2(5′-GMP) concentrations where ordered nucleotide is present, dramatic shifts occur in the drug methyl resonances. Figure 3A illustrates the NMR spectrum of 0.032 M ethidium bromide. As the concentration of Na_2(5′-GMP) is increased to 0.18 M, ordered nucleotide begins to appear (Figure 3B) and the drug methyl resonance broadens and shifts markedly upfield. The methyl resonance continues to shift upfield until the nucleotide concentration reaches 0.29 M (Figure 3C), and then the line begins to move downfield with further increases in nucleotide concentration (Figure 3D). Eventually, the chemical shift levels off at 0.85 ppm. The maximum upfield shift observed for the drug methyl proton resonance is 0.71 ppm. Upfield shifts as high as 0.50 ppm have been observed previously for the binding of ethidium to helical dinucleotides.[20-22]

The aromatic proton resonances of ethidium near 7.7, 7.1, and 6.3 ppm also shift upfield and broaden with increasing concentration of ordered nucleotide (cf. Figure 3). The changes in the aromatic proton lines are similar to those reported earlier for ethidium binding to dinucleotides.[22] The H(8) resonances of ordered 5′-GMP shift upfield by as much as 0.07 ppm in the presence of ethidium, but their relative intensities are essentially the same as those observed in the absence of drug. The absence of new H(8) resonances in the presence of ethidium indicates rapid drug exchange. This latter result is verified by the existence of only a single time-averaged drug methyl proton line in the presence of ordered nucleotide. Since the chemical shift difference between

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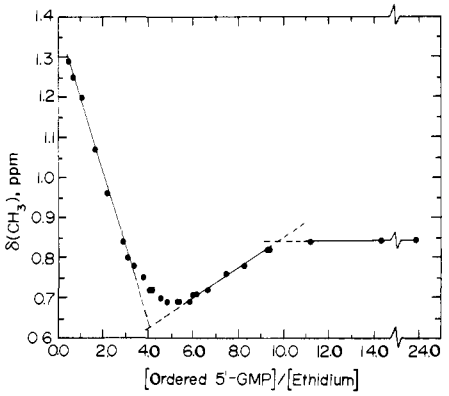


Figure 4. Dependence of the methyl proton chemical shift of ethidium on ordered Na_2(5'-GMP) to ethidium ratio. The data were obtained in D_2O at 5 °C and an ethidium concentration of 0.034 M.

bound and unbound drug is at least 0.55 ppm, the mean lifetime is $<1.5 \times 10^{-4}$ s.

The NMR observations indicate that ethidium binds to the ordered forms of Na_2(5'-GMP) without significantly altering the structures and relative abundances of the aggregated units. In this case the drug may be bonded to external positions of the ordered nucleotide aggregates, instead of being intercalated between 5'-GMP units. Whatever the mode of binding may be, the stoichiometry of drug binding provides valuable information on the composition of the ordered 5'-GMP aggregates. Figure 4 illustrates the dependence of the drug methyl proton chemical shift on the ordered 5'-GMP to ethidium ratio. Strong complexation of the drug is revealed by the nearly linear upfield shift in the methyl proton resonance over the ordered 5'-GMP to ethidium ratios between 0.5 and 3.5. Weaker drug binding in a second complex is revealed by the subsequent upfield shifts at higher ordered 5'-GMP to ethidium ratios. Linear extrapolations of portions of the curve shown in Figure 4 indicate the ordered nucleotide to drug ratio to be 4:1 for the strong complex and between 8:1 and 10:1 for the weaker complex.

On the basis of the above observations, an attempt was made to fit the drug chemical shift dependence to an octamer binding model as defined by eq 8–11, where D represents the drug ethidium

$$M_8 + 2D \overset{K_1}{\rightleftharpoons} M_8{\cdot}D_2 \tag{8}$$

$$K_1 = [M_8{\cdot}D_2]/[M_8][D]^2 \tag{9}$$

$$M_8{\cdot}D_2 + M_8 \overset{K_2}{\rightleftharpoons} 2M_8{\cdot}D \tag{10}$$

$$K_2 = [M_8{\cdot}D]^2/[M_8{\cdot}D_2][M_8] \tag{11}$$

and K_1 and K_2 are apparent equilibrium constants. The total concentration of drug (C_D^t) and the total concentration of octamer ($C_{M_8}^t$) may be expressed as in eq 12 and 13.

$$C_D^t = [D] + 2K_1[M_8][D]^2 + (K_1K_2)^{1/2}[M_8][D] \tag{12}$$

$$C_{M_8}^t = [M_8] + K_1[M_8][D]^2 + (K_1K_2)^{1/2}[M_8][D] \tag{13}$$

The observed drug chemical shift (δ_{obsd}) is given by

$$\delta_{obsd} = \delta_f X_f + \delta_1 X_1 + \delta_2 X_2 \tag{14}$$

where X_f is the mole fraction of drug not bound to ordered nucleotide, X_1 is the mole fraction of drug as $M_8{\cdot}D_2$, X_2 is the mole fraction of drug as $M_8{\cdot}D$, and δ_f, δ_1, and δ_2 are the corresponding chemical shifts. The mole fractions are defined by eq 15–17.

$$X_f = [D]/C_D^t \tag{15}$$

$$X_1 = 2K_1[M_8][D]^2/C_D^t \tag{16}$$

$$X_2 = (K_1K_2)^{1/2}[M_8][D]/C_D^t \tag{17}$$

The set of equations for the octamer–drug binding model was solved by a nonlinear least-squares iteration technique. An initial estimate of [D] was used in eq 13 to solve for $[M_8]$. The value of $[M_8]$ then was used in eq 12 to calculate a more accurate value of [D]. The procedure was repeated until convergence occurred. The final values of $[M_8]$ and [D] were used to calculate the mole fractions given by eq 15–17, and then eq 14 was solved by fitting the calculated δ_{obsd} values to the experimental values. Figure 5 shows the fit of the chemical shift–mole ratio data to the octamer–drug binding model. Although the uncertainties in K_1, K_2, and δ_1 are relatively large because of the near linearity of the data up to $[M_8]/[D] = 0.5$ and the uncertainties in the integrations for the H(8) resonances of the ordered nucleotide, the fit provides additional independent support for the presence of octamers.

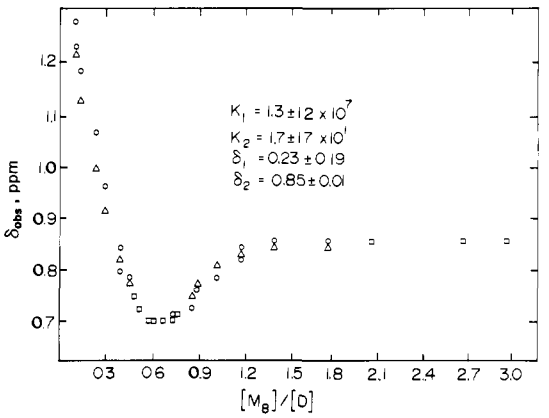


Figure 5. Best least-squares fit of the chemical shift–mole ratio data to the drug–octamer binding model defined by eq 8–17: (○) an experimental point; (△) a calculated point; (□) the experimental and calculated points are equal.

Table II. Dependence of 5'-GMP Ordered Forms on 5'-GMP to Na^+ Ratio at 0.3 °C

5'-GMP/Na^+[a]	ordered 5'-GMP, %	5'-GMP/Na^+[a]	ordered 5'-GMP, %
2.0	80.0 ± 3.5	6.0	35.8 ± 3.1
3.0	70.0 ± 3.5	7.0	24.9 ± 2.7
4.0	53.5 ± 3.1	9.0	20.2 ± 2.2
5.0	45.2 ± 4.3		

[a] The ratios of 5'-GMP/Na^+ were obtained by mixing 0.78 M solutions of Na_2(5'-GMP) and $[TMA]_2$(5'-GMP) containing 0.5 M [TMA]Cl.

Effect of Na^+ and K^+ on 5'-GMP Ordering. The number of complexed Na^+ ions per octamer unit was determined by observing the dependence of 5'-GMP ordering on GMP to Na^+ ratio. The relevant mass action expression is

$$xNa^+ + 8GMP^2 \rightleftharpoons Na_x(GMP)_8^{x-16} \tag{18}$$

with the apparent equilibrium constant defined as

$$K = \frac{[Na_x(GMP)_8^{x-16}]}{[Na^+]^x[GMP^{2-}]^8} \tag{19}$$

In the procedure for determining K and x in eq 19, two criteria were met. First, the total 5'-GMP concentration was held constant by mixing incremental amounts of 0.78 M Na_2(5'-GMP) and 0.78 M TMA_2(5'-GMP). Second, changes in ionic strength were minimized by the presence of 0.5 M [TMA]Cl so that $\mu = 2.8 \pm 0.1$ M over the range of GMP/Na^+ ratios investigated.

Table II lists the fraction of 5'-GMP in ordered form at different 5'-GMP to Na^+ ratios. These data were fit to eq 19 to obtain $x = 4.1 \pm 0.1$ and $K = 1.7 \pm 0.7 \times 10^{10}$. Thus an average of four Na^+ ions are complexed per octamer unit.

The behavior of ordered Na_2(5'-GMP) in the presence of excess Na^+ indicates that multiple equilibria are involved in the overall complexation of the alkali metal ion. Although the mass action expression given in eq 18 requires an increase in ordered nucleotide with increasing Na^+ concentration, the fraction of ordered 5'-GMP in a mixed TMA^+–Na^+ nucleotide solution actually decreases by a small but perceptible amount when 5'-GMP/$Na^+ < 1.0$. The

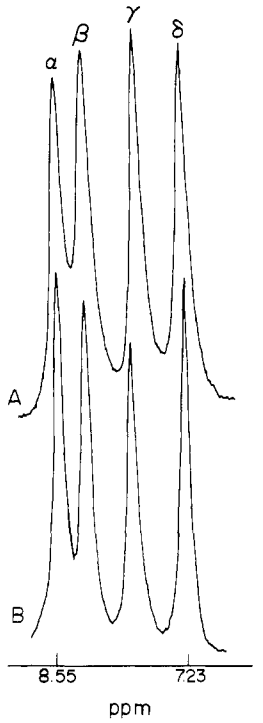


Figure 6. H(8) resonance lines (180 MHz) of 0.78 M Na_2(5′-GMP) in the presence of 0.38 M NaCl (spectrum A) and in the absence of added NaCl (spectrum B). The spectra were recorded at 0.3 °C in D_2O.

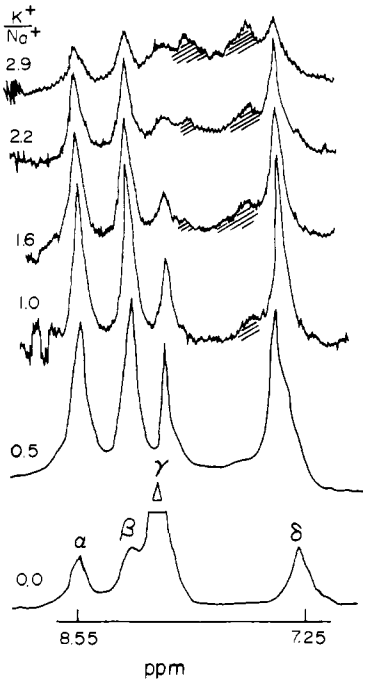


Figure 7. Effect of KCl on the H(8) resonance (180 MHz) of 0.63 M $[TMA]_2$[5′-GMP] containing 0.32 M NaCl. The shaded H(8) resonance lines that appear at $K^+/Na^+ \geq 1.0$ are due to potassium-directed self-structures (see text). The spectra were recorded in D_2O at 2.9 °C.

destabilizing effect of excess Na^+ is illustrated qualitatively by the spectra in Figure 6 for 0.78 M Na_2GMP in the presence and absence of 0.38 M NaCl. It can be seen that the relative intensity of the H_γ resonance characteristic of disordered nucleotide is larger in the spectrum containing NaCl. The destabilization of ordered 5′-GMP by excess Na^+ suggests that some fraction of the complexed Na^+ is chelated by two or more nucleotide donor sites. At higher Na^+ concentration monodentate binding of the metal competes with chelation, and the ordered structure is destabilized.

The addition of K^+ to solutions of $(TMA)_2$(5′-GMP) leads to the formation of K^+-directed self-structures that differ from the Na^+-directed structures and give rise to more complex H(8) NMR patterns.[9] Although K^+ is capable of uniquely ordering 5′-GMP on its own, the ion also exhibits a remarkable ability to stabilize the Na^+-directed self-structures. Figure 7 illustrates that the addition of KCl to a 0.63 M 5′-GMP solution containing 2.0 5′-GMP/Na^+ leads initially to a dramatic increase in the intensities of the ordered H(8) resonance lines and a concomitant decrease in the intensity of the H_γ resonance of the disordered nucleotide. The changes in equilibrium concentrations caused by the added K^+ ions are much greater than would be observed by the addition of an equivalent amount of Na^+. The further addition of KCl beyond a K^+/Na^+ ratio of 1.0 leads eventually to the appearance of new H(8) lines that can be assigned to K^+-directed self-structures.

The stabilization of the Na^+-directed 5′-GMP self-structure by K^+ is also illustrated by the NMR melting experiments shown in Figure 8. The mixed metal ion solution (solution A) containing 1.0:0.32 K^+:Na^+ (total M^+:5′-GMP = 0.66:1) is not entirely melted out to disordered nucleotide at 41 °C. In contrast, an analogous solution (solution B) containing only Na^+ ions is completely melted out by 26.5 °C.

The stabilization of the Na^+-directed self-structures by K^+ may occur in one of two ways. The K^+ ion may *add* to the ordered aggregates or it may *replace* bound Na^+ at non-structure-directing binding sites. The "addition" mechanism would lead to a decrease in net negative charge on the aggregates, whereas no change in aggregate charge would occur in the "replacement" mechanism. The addition mechanism seems unlikely, because the extent of nucleotide aggregation and structure formation is sensitive to net charge. The protonation of 5′-GMP at pH <5, for example, leads

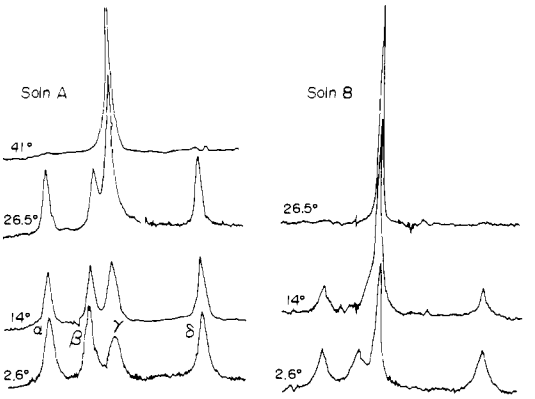


Figure 8. Temperature dependence of the H(8) resonances (180 MHz) for two D_2O solutions containing the sodium-directed 5′-GMP self-structure at a total alkali metal ion to 5′-GMP ratio of 0.66:1. Solution A is K^+ stabilized, K^+:Na^+ = 1.0:0.32. Solution B contains only Na^+. For both solutions $[(TMA)_2$(5′-GMP)] = 0.57 *M*.

to extensive aggregation and gelation. Also, the more highly charged GDP and GTP derivatives at neutral pH do not aggregate as readily as GMP. Since the structural nature of the ordered GMP aggregates is not altered in the K^+-stabilization process, the net charge on the aggregates apparently remains unchanged upon K^+ binding. Therefore, the mechanism of K^+ binding most likely involves displacement of some Na^+ from less-specific binding sites, while the remaining Na^+ is held at Na^+-specific structure-directing positions.

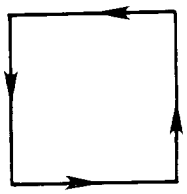
Discussion

The stoichiometric studies along with the ethidium and metal ion binding results permit two general conclusions regarding the solution ordering of 5′-GMP in the presence of Na^+ as a structure director. First, the ordered aggregates that give rise to the H_α, H_β, and H_δ resonances most likely are octamers. Second, at least two types of binding sites are involved in the complexation of Na^+ by the ordered octamers. One site is highly Na^+ specific and plays an essential role in directing nucleotide structure. The second site is important in stabilizing the structure, but it is less Na^+ specific.

Although the H_α, H_β, and H_δ resonances have comparable intensities, it is unlikely that all three lines belong to a single octamer aggregate. Earlier studies have shown that the H_α and H_δ lines have T_1 values that are larger than the value observed for the H_β resonance.[3,15] Moreover, the addition of Mn^{2+} or Cu^{2+} to ordered solutions of Na_2(5'-GMP) preferentially shortens the relaxation time of the H_β resonance, as well as the H_γ resonance of the disordered nucleotide.[3] Significantly, the ^{13}C NOE, T_1, and T_2 values for the C(8) resonances associated with the H_α H_δ species also differ from those for the C(8) resonance of the H_β and H_γ species.[15] The differences in the H(8) resonances are interpreted in terms of two structures. One structure gives rise to the H_α and H_δ lines, and the other is responsible for the β line. The recent observation[15] of a fourth but very weak H_ϵ line for the ordered nucleotide brings to three the total number of NMR-observable ordered structures present in solution.

All three ordered structures can be explained by the coaxial stacking of two hydrogen-bonded tetramer units (I). Such stacking may occur in two ways, either normal (head to tail) or inverted (head to head, tail to tail) arrangements. In addition, the two tetramers probably twist relative to one another, in part, to minimize nonbonding repulsions and to optimize hydrophobic base-stacking interactions. A precedent for coaxial stacking of 5'-GMP tetramers has been provided by X-ray diffraction studies of microcrystalline Na_2(5'-GMP) fibers grown from neutral solution.[23] In this structure tetramer units are coaxially stacked in a head to tail fashion with a 30° twist angle between adjacent units.

To illustrate the symmetry properties of two stacked tetramers, we find it useful first to disregard the presence of the chiral ribose groups. A planar tetramer of guanine units represents a prochiral entity with C_{4h} symmetry. The unit can be represented schematically by III, wherein each bent arrow is a guanine unit with



III

the head of the arrow representing the NH(1) and NH(2) hydrogen donors and the tails representing the O(6) and N(7) hydrogen acceptors. Normal stacking of two units of III with a twist angles of ±30° generates a pair of enantiomers with C_4 symmetry. The inverted stacking of two units of III with ±30° or, equivalently, ±60° twist angles gives two pairs of enantiomers with D_4 symmetry. Therefore, when the chiral ribophosphate groups are added to the three pairs of enantiomers, a total of *six* diastereomers are generated.

The six possible diastereomers are illustrated in Figure 9. Here the twist angle is defined as the angle formed by the projection of a line joining the center of a tetramer unit and O(6) onto an analogous line for an overlapping base on the tetramer plate below. The angle is arbitrarily defined as being positive when the bottom plate twists clockwise with respect to the top plate. The notation in parentheses for the D_4 diastereomers (e.g., D_4(CW, CCW, +60°)) provides the clockwise (CW) or counterclockwise (CCW) sense of the hydrogen bonding for the upper and lower tetramer plates, respectively, and the twist angle.

Table III summarizes the expected number of isomers and H(8) resonances. In the absence of chemical-exchange processes, the two C_4 isomers with normal base stacking each should exhibit two H(8) resonance lines of equal intensity. The reversed-stacked D_4 isomers should exhibit a single H(8) resonance. Thus if all six isomers were present under slow chemical exchange conditions, a total of eight H(8) lines would be observed. However, twisting the two stacked tetramers about the C_4 axis should be a relatively facile process. Rapid back and forth twisting of the two tetramer plates averages the two C_4 isomers and the two isomers in each of the D_4 sets. The twisting motion requires only 30 and 60° displacements in twist angle, respectively, for C_4 and D_4 isomer interchange. Such displacements are possible without eclipsing the ribophosphate groups on the upper and lower tetramer plates. It is noteworthy that complete $C_4 \rightleftharpoons D_4$ isomer interchange is possible only through rapid separation of the tetramer stacks. However, if rapid stack separation does occur, the process must be accompanied by rapid tetramer dissociation and time averaging with disordered nucleotide. Separate resonances for disordered and completely time-averaged ordered forms of the nucleotide are not observed under a variety of conditions of temperature and concentration. The stoichiometric results, however, suggest that the fraction of nucleotide present as unstacked tetramer probably is not very large.

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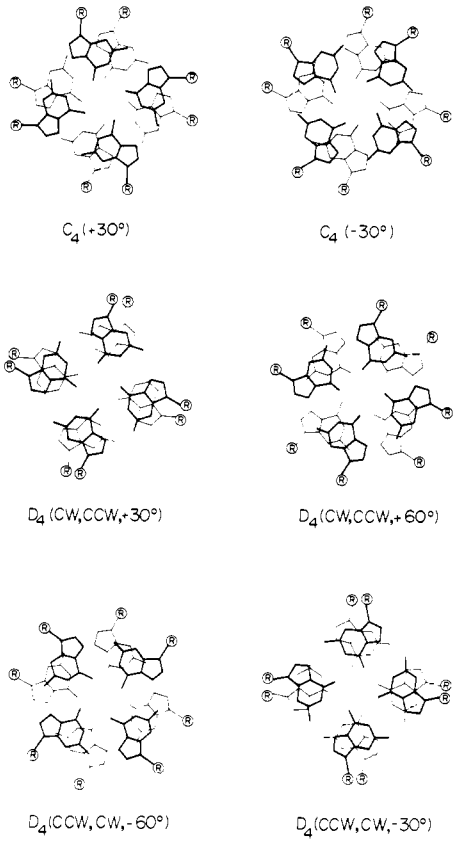

Figure 9. The six possible diastereomers formed by coaxial stacking of 5'-GMP tetramer units in normal (C_4 isomers) and inverted (D_4 isomers) stacking arrangements. The heavier lines represent the upper tetramer unit, and the lighter lines represent the lower tetramers. R represents the chiral ribophosphate group. The CW and CCW notation for the D_4 isomers designates the clockwise or counterclockwise sense of the hydrogen bonding for the upper and lower tetramer units.

Table III. Isomers and H(8) Lines Expected for Stacked 5'-GMP Tetramers under Conditions of Slow and Fast Tetramer Twisting

conditions	normal stacking	inverted stacking
slow twisting	isomers: 2 H(8) lines: 4 C_4(+30°) C_4(−30°)	isomers: 4 H(8) lines: 4 D_4(CW, CCW, +30°) D_4(CW, CCW, +60°) D_4(CCW, CW, −30°) D_4(CCW, CW, −60°)
fast twisting	isomers: 1 H(8) lines: 2 C_4(+30°) ⇅ C_4(−30°)	isomers: 2 H(8) lines: 2 D_4(CW, CCW, +30°) ↓↑ D_4(CW, CCW, +60°) D_4(CCW, CW, −30°) ↓↑ D_4(CCW, CW, −60°)

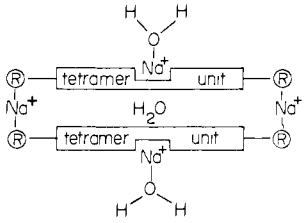

Figure 10. Proposed binding sites for Na^+ in ordered 5′-GMP octamers. The internal sites defined by the four O(6) oxygens at the center of the tetramer units are believed to be highly Na^+-specific structure-directing sites. The external sites involving chelation by interplate phosphate oxygens are structure stabilizing but less Na^+ specific. The proposed encapsulated water molecule is also illustrated.

On the basis of the above symmetry analysis, the H_α and H_δ resonances are assigned to the time-averaged C_4 isomer pair. Similarly, the two time-averaged sets of D_4 isomers are assigned as the species responsible for the H_β and H_ϵ resonances, though it is not possible to say which isomer set is present in minor amount. Another possibility, of course, is that only one isomer from each time-averaged isomer pair is actually present. It could be argued that since base-stacking interactions are important, probably essential, for 5′-GMP aggregation, differences in base overlap for the D_4 isomer pairs will cause one isomer to be favored over the other. However, the complexation of Na^+ also is essential for stabilization of stacked tetramer units. The Na^+ complexation reactions, along with possible intertetramer hydrogen-bonding interactions (see below), could compensate for differences in base overlap. Indeed, there is no difference in base overlap for C_4 isomer pair, and arguments favoring only one of these isomers are even less compelling. It should be noted here that rapid rotation of the tetramer units may occur on a time scale that is longer than the lifetime of intratetramer H bonds and complexed Na^+. Thus rapid twisting is not necessarily incompatible with octamer stabilization by intratetramer H bonding and Na^+ complexation.

As noted earlier, the hole defined by the four carbonyl oxygens of a tetramer unit has a radius (2.2–2.3 Å) compatible with known Na^+–carbonyl oxygen bond distances.[17] A fifth coordination position may be defined by a water molecule at an apical position on the C_4 symmetry axis. The placement of Na^+ at the centers of both tetramer units at a distance of 3.4 Å might seem prohibitive, because of the repulsive interaction between them. Inspection of CPK molecular models reveals that the Na^+–Na^+ repulsive interaction can be reduced by the encapsulation of a water molecule in the central cavity between the two tetramer plates. For twist angles near ±30 or ±60° the encapsulated water molecule can hydrogen bond to carbonyl oxygens in the upper and lower plates, though H bonding is not essential when Na^+ occupies the tetramer cavities. An analogous role for water has been considered in discussions of the helical, four-stranded structure of poly-G and poly-I, which also contain stacked tetramer units.[24,25]

The less-specific binding of Na^+, as inferred from the K^+ stabilization and Na^+ destabilization of the ordered aggregates, most likely involves chelation by phosphate oxygens from adjacent tetramer units. Apparently, the larger K^+ ion is preferred over Na^+ at these chelation sites, and this preference leads to K^+ stabilization. At sufficiently high Na^+ concentration monodentate phosphate–sodium ion pairing may compete with Na^+ chelation and lead to destabilization of the ordered structures. The binding of Na^+ to two or more phosphate oxygens is not uncommon. In NaGpC,[26] the Na^+ ions are bound by phosphate oxygens of two different double-helical fragments. In NaApU,[27] one Na^+ is bound to two oxygens of one phosphate and to two oxygens of another phosphate from different fragments. Also, the ATP dimers in Na_2ATP[28] are linked together by Na^+ bridging phosphate oxygens. Since the Na^+–O distances are in the range 2.3–2.4 Å, the interphosphate distances in the ordered 5′-GMP system should be 4.8 Å or less for Na^+ chelation to be feasible. Inspection of molecular models indicates that for a twist angle of ±30°, interphosphate distances can range from 2 to 12 Å, depending on the stacking pattern, glycosidic bond angle, and the ribose O(5′)–C(5′)–C(4′)–C(3′) dihedral angle. Thus interplate Na^+ chelation is possible within the constraints of the porposed model.

Figure 10 schematically illustrates the highly specific and less-specific Na^+ binding sites being proposed in the present work. Alternative models for Na^+ complexation are possible but less likely. One alternative is to place all four Na^+ ions *exclusively* at chelated phosphate positions. This model is not appealing in part because Na^+ and other alkali metals are known to be essential for the ordering of guanosine gels.[29] In these anisotropic gel structures ordered tetramer stacking is achieved without the benefit of phosphate oxygens. Also, alkali metal ions are essential for the helical ordering of four-stranded poly-I[30] and poly-X[31] in which stacked tetramer units are formed despite the constraints placed on the phosphate oxygens in the phosphodiester backbone. Thus, the structure-directing influence of the metal ions in the poly-I, poly-X, and guanosine gel systems is almost certainly a consequence of complexation by O(6) donor sites on the base. In the Na^+-directed 5′-GMP solution structures, therefore, it is reasonable to identify the Na^+-specific structure-directing binding site with the O(6) donor groups and to regard Na^+ chelation by phosphate oxygens as structure-stabilizing interactions.

Still another alternative to our proposed model for Na^+ binding is one in which the structure-directing Na^+ is placed at the eight-coordinate position defined by the O(6) oxygens of adjacent tetramer plates. In this case the structure stabilizing effect caused by the addition of K^+ to an ordered TMA^+–Na^+–5′-GMP solution could be explained by the replacement of the eight-coordinate Na^+ by the larger and better suited K^+. However, in addition to the ionic radii considerations presented earlier, there is strong experimental evidence against Na^+ and K^+ occupying the same structure-directing site, namely, under no conditions does K^+ alone form ordered 5′-GMP solution structures identical with those formed by Na^+ alone. Although the eight-coordinate site may well be occupied when K^+ is *exclusively* the structure-directing cation,[32] Na^+ binding at the centers of the stacked tetramers competes favorably with K^+ binding at the eight-coordinate site, at least under conditions where the K^+ to Na^+ ratio is <3.0 (cf. Figure 7).

In addition to interplate Na^+ chelation, CPK model building studies suggest that interplate hydrogen bonding may also contribute to the stability of two stacked tetramers. The results of the model building studies are summarized in Table IV. In each case the twist angle was held near ±30 or ±60°, and the ribose conformation (3′-endo), the glycosidic bond angle (36 ± 19°), and the ribose dihedral angles (ψ = 53 ± 7°, ϕ = 187 ± 24°, ω = 69 ± 4°) were maintained within the range of their commonly observed values.[33,34] The number of possible hydrogen bonds quoted in Table IV disregards competition for donor sites by Na^+. The least attractive interplate hydrogen bonding scheme occurs for the D_4(CW,CCW, +30°) isomer, wherein a ribose OH serves as an acceptor. In all the other isomers, however, a phosphate oxygen serves as the acceptor. Future ^1H NMR studies of 5′-GMP

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Table IV. Interplate Hydrogen Bonds Suggested by Model-Building Studies

isomers	type of hydrogen bonds	no. of hydrogen bonds[a]
$C_4(+30°)$	OH(3') → OP	4
$C_4(-30°)$	OH(2') → OP	4
D_4(CCW, CW, −30°)	NH(2) → OP	8
D_4(CCW, CW, −60°)	NH(2) → OP	8
D_4(CW, CCW, +30°)	OH(2') → O(3')	4
D_4(CW, CCW, +60°)	OH(2') → OP	4

[a] Maximum number of interplate hydrogen bonds, assuming no competion for phosphate oxygens by interplate Na^+ chelation.

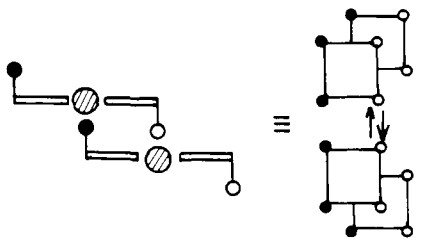

Figure 11. Laszlo's slipped stacked tetramer model for ordered 5'-GMP octamers, adapted from ref 10b.

ordering in H_2O solution may provide direct experimental evidence for interplate hydrogen bonding.

Laszlo and his co-workers have reported several ^{23}Na NMR studies[4,10a,35-37] of ordered Na_2(5'-GMP) solutions that show that exchange of "free" and complexed Na^+ is fast, the mean lifetime of a complexed Na^+ being about 30 ns. Though the ^{23}Na NMR line broadening that accompanies Na_2(5'-GMP) ordering intially was rationalized in terms of micelle formation,[4] this model has been abandoned in subsequent studies in favor of various tetramer stacking models that have included the possibility of hexadecamers in addition to octamers.[37] The results obtained in the present work along with the recently reported ^{13}C NOE, T_1, and T_2 studies[15] do not support the existence of hexadecamers.

In one attempt to account for the H_α, H_β, and H_δ resonances of ordered Na_2(5'-GMP), Laszlo[10b] proposed an eqilibrium mixture of tetramers stacked in a coaxial and "slipped" fashion. The slipped stacking model is illustrated in Figure 11. The H_β resonance was attributed to coaxial stacking of two tetramers with a Na^+ complexed in the central cavity defined by the eight carbonyl oxygens of the two tetramers or, equivalently, with one Na^+ rapidly hopping between the centers of the tetramer units. The H_α and H_δ lines were attributed to a slipped stack of two tetramer units in which Na^+ occupies the center of each tetramer unit and a fifth coordination position is provided by a phosphate oxygen of the adjacent tetramer. In addition, rapid back and forth slippage along only one set of overlapping edges was invoked, as illustrated in Figure 11.

The very different tetramer stacking arrangements and coordination environments for the structure-directing sodium ion represent in our opinion strong arguments against the coexistence of slipped and coaxial tetramer stacking. It is interesting to note, however, that the slipped stacking model *alone* is capable of explaining the H(8) resonance pattern of ordered Na_2(5'-GMP), provided that normal and inverted stacking patterns and rapid slippage of one tetramer unit along all edges of the other are allowed. Nevertheless, even if these latter conditions are met, the slipped stacking model has several deficiencies, relative to the coaxial stacking model. Firstly, the slipped model requires equally probably syn and anti conformations about the glycosidic bond, whereas the coaxial model allows the nucleotide to adopt the preferred anti conformation.[33,34] Secondly, slipped stacking provides relatively little of the base-stacking interactions, which play an important role in the ordering of nucleotides in general[38] and which are known from X-ray fiber diffraction studies[14,23,24,39,40] and calorimetric data[7,8] to occur extensively in ordered forms of guanine nucleotides. Thirdly, only two Na^+ ions can be accommodated per octamer unit, instead of four. Model-building studies indicate that the distance between phosphate oxygens in a slipped stacked tetramer arrangement are too far apart to permit complexation of Na^+ at outer positions.

In the most recent studies by Laszlo and his co-workers,[37] a "phenomenological" treatment of the ^{23}Na NMR data allowed them to assume the presence of octamers. In this case, however, the slipped stacking model was abandoned in favor exclusively of the coaxial stacking model. On the basis of analogies to a mixed Na_2(5'-GMP)–K_2(5'-GMP) system,[41] they assumed a total of five complex Na^+ ions. One Na^+ ion was placed at the internal, eight-coordinate positions defined by the O(6) oxygens of the upper and lower tetramer units, and four Na^+ ions were placed at external positions, chelated by phosphate oxygens.[42] To the extent that coaxial tetramer stacking and Na^+ complexation to different O(6) and phosphate oxygen sites are involved in octamer formation, Laszlo's most recent interpretation of the ^{23}Na NMR data is compatible with our interpretation of the ^1H NMR data. However, we do not favor placement of Na^+ at the eight-coordinate position between tetramer plates for reasons discussed earlier.[47]

Experimental Section

Materials. Guanosine 5'-monophosphate was purchased as the disodium, monohydrate salt from Calbrochem, Inc., and as the free acid from Sigma Chemical Co. The free acid form was converted to $(TMA)_2$(5'-GMP) by titration with (TMA)OH. Dilute solutions of the free acid (~0.1 M) were titrated to pH 7.8 with the use of a pH meter. The distilled water used in the titration was passed through an Illinois Water Treatment Co. purification system to minimize paramagnetic metal ion and organic contaminants. All nucleotide solutions for NMR measurements were lyophilized three times from 99.8% D_2O (Stohler Isotope Chemicals). The D_2O solutions (pD ≃8.2) were prepared under a dry nitrogen atmosphere to minimize H_2O contamination. The concentrations of 5'-GMP solutions were determined spectrophotometrically by taking the molar absorptivity to be 13 700 M^{-1} cm^{-1} at 252 nm.[43]

Ethidium bromide (Sigma Chemical Co.) was lyophilized three times from 99.8% D_2O to remove ethanol and water of crystallization. Ethidium concentrations were determined spectrophotometrically by taking the molar absorptivity to be 5450 M^{-1} cm^{-1} at 480 nm.[44] Sodium trimethylsilylpropionate-2,2,3,3-d_4 was obtained from Merck and Co.

NMR Measurements. Proton NMR spectra were obtained on either a Bruker WH-180 spectrometer interfaced to a Nicolet 1180 computer with 16K memory or a Bruker WH-250 interfaced to an Aspect 2000 computer with 32K memory. Probe temperatures were maintained to ±1 °C by a Bruker temperature control unit coupled to a calibrated, probe-mounted thermocouple.

Integrations of the H(8) region of the spectra were accomplished by cutting and weighing photocopies of the spectra or by planimetry. Triplicate measurements were made, and the average was taken. Systematic errors were estimated by comparing the integrations obtained by two separate investigators. Under condition where the H_β resonance was masked by the H_γ resonance, the intensity of the H_β line was estimated from the intensities of the H_α and H_δ lines. At the temperatures and concentrations where all three structure lines are well resolved, their intensities are equal within experimental uncertainty.

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Computations. Least-squares curve fitting was accomplished on a Control Data Corp. Cyber 170 Model 750 computer using the KINFIT program developed by Dye and Nicely.[45] The procedures and subroutines used to fit the ethidium binding results were analogous to those developed by Mei et al.[46] for fitting multiple metal ion complexation equilibria to NMR chemical shift data.

Acknowledgment. The partial support of this research by NIH Grant GM-23516 is gratefully acknowledged.

Registry No. II, 3546-21-2; 5'-GMP, 85-32-5; Na, 7440-23-5; K, 7440-09-7.

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Structure and Stereochemical Nonrigidity in $Pd(F_6acac)_2P(aryl)_3$, a New Isomeric Class of Palladium Bis(hexafluoroacetylacetonate) Complexes

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Received November 30, 1981

Abstract: The reaction of palladium bis(hexafluoroacetylacetonate), $Pd(F_6acac)_2$, and $(aryl)_3P$ produces $Pd(F_6acac)_2P(aryl)_3$, a new class of 1:1 adducts isomeric with carbon-bonded $Pd(F_6acac\text{-}C)(F_6acac\text{-}O,O)$(ligand). $Pd(F_6acac)_2PPh_3$ crystallizes in the monoclinic space group $P2_1/n$ with $a = 20.106$ (3) Å, $b = 13.160$ (7) Å, $c = 23.185$ (3) Å, $\beta = 93.52$ (3)°, and $V = 6123$ (5) Å^3. The structure solution, which converged at $R = 0.071$ and $R_w = 0.086$ with 6536 reflections for which $F_o^2 \geq 2.0\sigma(F_o^2)$, showed that the palladium has a distorted, square-pyramidal coordination geometry. The base of the pyramid comprises a phosphorus atom, two oxygen atoms from a bidentate F_6acac, and one oxygen atom from a semichelating F_6acac. The Pd–O contact involving the apical oxygen, provided by the other terminus of the semichelating F_6acac, is long, 2.699 (6) Å. The $Pd(F_6acac)_2P(aryl)_3$ compounds are stereochemically nonrigid, and the barrier to rearrangement increases as the steric bulk of the $(aryl)_3P$ group increases. At low temperatures, the ^{19}F NMR spectrum of $Pd(F_6acac)_2P(o\text{-tolyl})_3$ displays four singlets of equal area. These simultaneously coalesce at ca. –40 °C, indicative of a single dynamic process involving a C_{2v} square-pyramidal transition state in which all four CF_3 groups are equivalent. Analysis of the DNMR spectra gave $\Delta H^{\ddagger} = 7.7 \pm 1$ kcal/mol and $\Delta S^{\ddagger} = -20 \pm 5$ eu. Comparison of the molecular structures of the Ph_3P and $(o\text{-tolyl})_3P$ adducts of $Pd(F_6acac)_2$ suggests that the less bulky triphenylphosphine ligand is associated with geometrical changes that may parallel those associated with formation of the transition state.

Introduction

We have previously reported that palladium bis(hexafluoroacetylacetonate), $Pd(F_6acac)_2$, behaves as a strong Lewis acid and forms an extensive series of crystalline complexes with molecular Lewis bases, L.[1,2] These can be grouped into four classes according to stoichiometry and have the general formula $Pd(F_6acac)_2L_n$, where n is 1, 2, 3, or 4. An investigation of the $n = 1$ class, along with an X-ray crystal structure of $Pd(F_6acac)_2(CH_3)_2NH$, showed that the 1:1 adducts contain four-coordinate palladium with one bidentate oxygen-bonded hexafluoroacetylacetonate ligand ($F_6acac\text{-}O,O$) and one carbon-bonded $-CH(COCF_3)_2$ group ($F_6acac\text{-}C$).[3] During the course of a systematic NMR study of the reactions of $Pd(F_6acac)_2$ with different Lewis bases, we found evidence for the presence of minor amounts of 1:1 complexes which had spectroscopic properties different from the previously characterized $Pd(F_6acac\text{-}O,O)(F_6acac\text{-}C)L$ materials.[4] Compounds of this new isomeric class are formed in high yield with phosphine donors. This paper describes the preparation of adducts with phosphorus bases and the delineation by ^{19}F DNMR spectroscopy and X-ray crystallography of fluxional behavior in representative members of this class, $Pd(F_6acac)_2PPh_3$ and $Pd(F_6acac)_2P(o\text{-tolyl})_3$.

Results

The reaction between $Pd(F_6acac)_2$ and triphenylphosphine in chloroform was monitored by ^{19}F NMR spectroscopy. Only 1 equiv of the phosphine is consumed and, when the P:Pd ratio is 1:1, $Pd(F_6acac\text{-}O,O)(F_6acac\text{-}C)PPh_3$, with $\delta(^{19}F)$ 76.14 (1 F), 76.17 (1 F), and 76.30 (2 F) and $\delta(^{31}P)$ 38.3, is produced along with the new isomeric compound $Pd(F_6acac)_2PPh_3$ (**1**). As will be shown below, **1** contains a monodentate oxygen-bonded F_6acac group and may be written as $Pd(F_6acac\text{-}O,O)(F_6acac\text{-}O)PPh_3$. The molar ratio of the carbon-bonded isomer to **1** is 0.2:1. Pure, crystalline samples of **1** may be obtained by cooling hexane solutions of $Pd(F_6acac)_2$ and triphenylphosphine. Titration experiments with tri-*o*-tolylphosphine showed that only $Pd(F_6acac\text{-}O,O)(F_6acac\text{-}O)P(o\text{-tolyl})_3$ (**2**), with $\delta(^{19}F)$ 76.22 and $\delta(^{31}P)$ 19.4, is formed in chloroform. In methanol, complexation of an additional 1 mol of triphenylphosphine occurs to form $Pd(F_6acac)_2(PPh_3)_2$, a member of the 2:1 adduct class, which was isolated as the hexafluorophosphate salt $[(Ph_3P)_2Pd(F_6acac)]PF_6$ (**3**). The reaction of $Pd(F_6acac)_2$ with 3 equiv or more of triphenylphosphine in methanol led to reduction of the metal and the formation of $Pd(PPh_3)_4$. There is a parallel between the chemistry of triphenylphosphine and triphenylarsine with the important exception that, in chloroform, the ratio of $Pd(F_6acac\text{-}O,O)(F_6acac\text{-}C)AsPh_3$ to $Pd(F_6acac\text{-}O,O)(F_6acac\text{-}O)AsPh_3$ is substantially larger, 2.2:1.

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0002-7863/82/1504-6584$01.25/0 © 1982 American Chemical Society