collision curve crossing" mechanism (ref 28 and references therein). However, the adiabatic channel model with the potential given in eq 16 extends the orbiting collision curve crossing model by incorporating the change in internal degrees of freedom during the reaction (the term in eq 14 involving W). It is this additional term that allows the adiabatic channel model to predict much smaller cross sections when the transition state occurs at smaller internuclear distance.

Equation 16 is a gross simplification of the real long-range potential of a neutral pair perturbed by a Coulomb curve. It neglects normal chemical bonding and van der Waals forces (this is a particularly serious failing for the slower reactions). The perturbation integral H is treated as a constant when it should be a function of the distance between the two molecules.[29] Attempts to calculate H as a function of r have been confined to diatomic interactions.[29,30] More realistic potential functions are needed for polyatomic systems of the type studied above. The success of eq 16 in fitting the R + O_3 and R + O_2 rate constants suggests that the perturbation between the neutral and Coulomb surfaces should be a significant factor in these long-range potentials, even for pairs of reactants that do not have large cross sections.

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Conclusions

In this paper we have reported the first measurements of rate constants for ozone reacting with alkyl radicals larger than methyl. The bimolecular R + O_3 reactions are closely analogous to the high-pressure R + O_2 reactions in that both show a definite trend of increasing rate constant with decreasing radical ionization potential. When ln (k) vs. IP – EA is plotted, the two sets of rate constants become close neighbors (Figure 1).

The adiabatic channel model with a simple long-range potential involving IP – EA is capable of reproducing the experimental rate constants for both R + O_3 and R + O_2. At low IP – EA this model merges with the orbiting collision curve crossing model, giving cross sections that are larger than those calculated from the classical harpoon model. At high IP – EA, the adiabatic channel model has the versatility to give smaller cross sections for those reactions with tight transition states. Clearly, more work is needed to develop suitable and realistic long-range potentials between polyatomic molecules.

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Ordered Forms of Dianionic Guanosine 5′-Monophosphate with Na^+ as the Structure Director. ^1H and ^{31}P NMR Studies of Hydrogen Bonding and Comparisons of Stacked Tetramer and Stacked Dimer Models

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The ordered forms of the guanosine 5′-monophosphate dianion in the presence of Na^+ as the structure-directing cation (Na^+/5′-GMP = 2.0) have been investigated in H_2O solution by ^1H NMR spectroscopy. The resonances assigned to H-bonded N(1)H (11.1–11.3 ppm) and N(2)H (8.8–10.4 ppm) in the ordered nucleotide have normalized intensities of 0.96 ± 0.12 and 1.1 ± 0.1 protons per ordered 5′-GMP, respectively. This results is compatible with the interbase H-bonding scheme expected for planar tetramer units (I) and supports the proposal that the ordered forms are isomeric octamers formed by stacking of tetramer units. An additional resonance at 7.69 ppm has been assigned on the basis of chemical shift, line width, and spin saturation transfer results to a ribose OH involved in extratetramer H bonding. The normalized intensity of the ribose OH proton (0.33 ± 0.06) is equal within experimental uncertainty to the normalized intensity of an unusually high-field line at 2.2 ppm in the ^{31}P NMR spectrum (0.30 ± 0.06), suggesting that a phosphte oxygen on an adjacent tetramer may be acting as the hydrogen acceptor. Several plausible alternatives for the extratetramer H bond also are discussed. Finally, the merits of the stacked tetramer model are shown to be superior to those of a recently proposed stacked asymmetric dimer model when the two models are compared in light of all the relevant data.

Introduction

Nucleosides and nucleotides containing guanine exhibit aggregation phenomena distinctly different from those containing other nucleic acid bases. One manifestation of these differences is the ability of guanosine and guanosine monophosphate (GMP) to form pH-dependent ordered structures.[1] In weakly acidic solution (pH ~5), guanosine monophosphates form anisotropic gels similar to those formed by guanine nucleosides in the presence of certain electrolytes.[2] However, in neutral or slightly basic solution where guanosine monophosphtes exist as dianions, *soluble* ordered aggregates have been observed.[3]

Several models have been proposed for the solution structures.[3-9] The favored models involve coaxial stacking of two or more tetramer units (I) formed by hydrogen bonding between donor positions N(1)H and N(2)H and the acceptor positions O(6) and N(7).[10-13] Extensive stacking of tetramer units is known to occur in the gel structures of guanine nucleosides[14] and nucleotides,[15]

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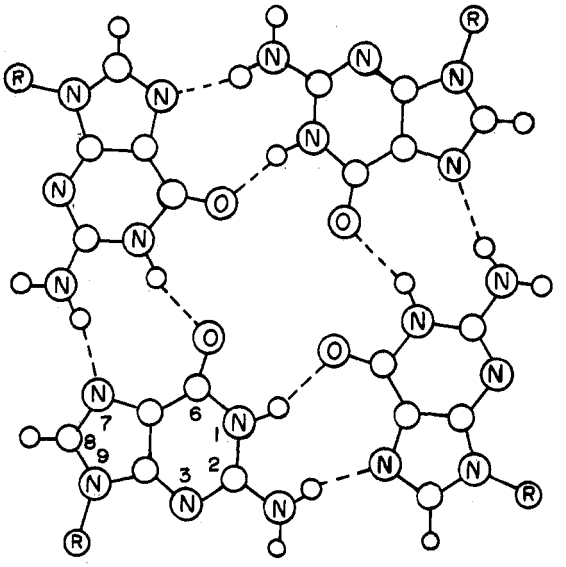

I

but in neutral solution, where the tetramer unit carries a formal charge of 8^-, the extent of stacking appears to be limited by repulsive forces to a small number of units.

The ordered forms of dianionic 5′-GMP are strongly cation dependent.[5-7] Na^+, K^+, and Rb^+ are good structure directors, whereas Li^+ and Cs^+ exhibit little or no tendency to promote structure formation. With Na^+ as the structure-directing cation, two major and one minor ordered species can be distinguished by ^1H and ^{13}C NMR spectroscopy. Our most recent studies[11] suggest that these ordered aggregates are isomeric octamers formed by coaxial stacking of tetramer units I in a normal (head to tail) and inverted (head to head, tail to tail) fashion. The stacked tetramer model is supported by the stoichiometry for ordered aggregation, ethidium binding to the ordered forms, symmetry considerations and model-building studies,[11] and estimates of the size of the ordered aggregates from ^{13}C NMR relaxation and Overhauser enhancement measurements.[10] Differences in the structure-directing properties of alkali metal ions are explained in terms of size-selective binding to the central O(6) cavity of a tetramer unit (Na^+ but not Li^+) or to the eight coordinate O(6) site between stacked tetramer units (K^+ and Rb^+ but not Cs^+).

The present work seeks verification of the stacked tetramer model for the ordered forms of Na_2(5′-GMP) by examining the ^1H resonances of exchangeable protons involved in H bonding. In addition to the *intra*tetramer H bonding expected for structural unit I, model-building studies[11] suggest *extra*tetramer H bonding, similar to the tetramer to tetramer interactions proposed for guanine nucleotide[16] and nucleoside[17] gel structures, may also play a role in stabilizing stacked tetramers in aqueous solutions. Since phosphate oxygens are reasonable candidates for participation in extratetramer H bonding, a ^{31}P NMR investigation of the ordered nucleotide is included in the present studies. Also, we consider the stacked asymmetric dimer model for ordered 5′-GMP as proposed recently by Petersen et al.[9] These workers criticize the stacked tetramer model as being incompatible with certain NMR results and say that the stacked dimer model is better able to account for these data. Thus, both models are compared here in light of all the relevant data.

Experimental Section

Materials. Ganosine 5′-monophosphate was purchased as the disodium salt from Calbiochem, Inc., and as the free acid from Sigma Chemical Co. The free acid form was converted to $(TMA)_2$(5′-GMP) by titration with tetramethylammonium hydroxide. Dilute solutions of the free acid (~0.1 M) were titrated to pH 7.8 with the use of a pH meter and the resulting salt was collected by freeze drying. The distilled water used in this study was passed through an Illinois Water Treatment Co. purification system to minimize paramagnetic metal ion and organic contaminants. ^1H NMR spectra of the nucleotide showed no differences in the presence or absence of EDTA as a complexing agent for paramagnetic impurities. Concentrations were determined spectrophotometrically by taking the molar absorptivity to be 13 700 M^{-1} cm^{-1} at 252 nm.[18] At the ambient pH values employed in this work (pH 8.00 ± 0.2), the nucleotide is mainly in the dianionic form. For instance, less than 3% of the phosphate is monoprotonated at pH 8.0, taking $pK_a = 6.4$. The other important protonic equilibrium for 5′-GMP, the ionization of N(1)H ($pK_a = 9.3$), predicts the extent of ionization to the trianion to be 5.9% at pH 8.0. By comparison, the extent of nucleotide ordering at 1 °C is 23% at a concentration of 0.22 M and 73% at a concentration of 0.75 M. Since no significant change in pH accompanies the ordering phenomenon, the ordered forms arise from the aggregation of the dianion.

NMR Spectra. Proton NMR spectra were obtained at 1–2 °C on a Bruker WM-250 spectrometer at 250 MHz. The pulse angles (30–90°) and recovery times (typically, 5–6 s) were sufficient to permit essentially complete magnetic relaxation between pulses. Sodium 3-trimethylsilypropionate-2,2,3,3-d_4 (NaTSP) was used as an internal proton reference.

^{31}P NMR spectra were obtained at 1 °C on a Jeol FX-900 spectrometer with inverse gated decoupling. The accumulation time was kept short (1.25 s) relative to the recovery time (25 s) to minimize temperature increases due to inductive heating during decoupling. Sample temperature was determined by inserting a thermocouple into the sample tube with the decoupler off. The samples were contained in 10-mm tubes with a concentric 3-mm capillary containing 85% H_3PO_4 as an external reference.

All NMR spectra were plotted on an expanded scale and integral intensities were determined with a planimeter or computer software.

Results and Discussion

The Current Stacked Tetramer Model. Earlier ^1H NMR studies[4,10,13] of Na_2(5′-GMP) in D_2O solution have demonstrated that the ordering phenomenon can best be monitored by observing the appearance of new lines in the H(8) region between 8.5 and 7.2 ppm. As the nucleotide becomes ordered with increasing concentration or decreasing temperature, the intensity of the H(8) resonance of the disordered nucleotide (H_γ) decreases and four new resonances appear near 8.54, 8.26, 7.69, and 7.24 ppm. The resonances due to the ordered nucleotide, which have been designated[10,13] H_α, H_β, H_ϵ, and H_δ, respectively, are illustrated in Figure 1 for a typical solution at a concentration of 0.46 M and 1 °C. The chemical shift of H_γ for the disordered nucleotide, which is described as monomer in rapid equilibrium with an irregularly stacked monomer,[4] is temperature and concentration dependent, but it usually appears between H_β and H_δ.

The concentration dependence of the H(8) resonances of 5′-GMP in 0.85 M NaCl at 15 °C has been shown to be consistent with the formation of ordered octamers:[11]

$$8(5'\text{-GMP}) \rightleftarrows (5'\text{-GMP})_8 \qquad (1)$$

$$K = [(5'\text{-GMP})_8]/(5'\text{-GMP})^8$$

$$K = (4.0 \pm 0.6) \times 10^4$$

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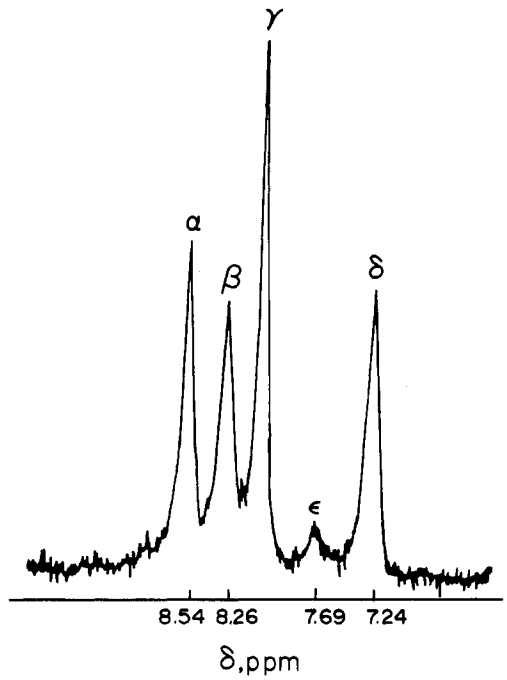

Figure 1. A typical ^1H NMR spectrum for the H(8) protons of Na_2-(5′-GMP) in D_2O solution under conditions of concentration (0.46 M) and temperature (0 °C) where both ordered (H_α, H_β, H_ϵ, H_δ) and disordered nucleotides (H_γ) are present (ref 10).

where K is an apparent equilibrium constant with concentrations expressed in mol/L. An average of 4.1 ± 0.1 Na^+ are complexed per mole of octamer. Also, the octamers are capable of binding 1 and 2 mol of the drug ethidium in a stepwise fashion.

On the basis of the above results, along with symmetry considerations and model-building studies,[11] estimates of the size of the ordered structure,[10] and the results of IR studies,[3] we previously assigned H_α and H_δ to rapidly interconverting C_4 octamers with normal (head to tail) tetramer stacking.[11] These octamers are represented by structures II and III in Figure 2. Also, we attributed H_β and H_ϵ to rapidly interconverting D_4 isomer pairs IV ⇄ V and VI ⇄ VII with inverted (head to head and tail to tail) tetramer stacking (cf. Figure 2). The interchange of isomer pairs was explained by rapid twisting of the tetramer units about the C_4 axis to give intermediates in which the twist angle between tetramers is 45° and the ribose groups on adjacent tetramers are staggered. Alternatively, in the absence of rapid twisting, the H(8) resonances could be attributed to one isomer in each of the three isomer pairs.

The results of the present work will be presented in light of the tetramer model and the above structural assignments. Consideration will be given later to the stacked asymmetric dimer model of Peterson et al.[9]

^1H NMR Spectra of H_2O. Figure 3 illustrates a typical spectrum in the 5.8–11.5-ppm range for the sodium salt of 5′-GMP (Na^+/5′-GMP = 2.0) in H_2O solution under conditions where both the ordered and disordered forms of the nucleotide are present. The chemical shifts and relative intensities of H_α, H_β, and H_δ for the major ordered octamers are the same as those observed in D_2O solution. H_ϵ for the minor ordered octamer is obscured by line H_F. The H_F line along with lines H_A through H_E are not observed in D_2O solution. Accordingly, we assign the H_A–H_F lines to exchangeable protons of the ordered nucleotide involved in H bonding.

The $(NH_2)_d$ line in Figure 3 is assigned to the exchangeable amino protons of disordered 5′-GMP. This assignment is made on the basis of the following observations: (i) the tetramethylammonium (TMA^+) salt of 5′-GMP, wherein TMA^+ is structure inert,[11] exhibits the $(NH_2)_d$ line in H_2O but not in D_2O; (ii) the TMA^+ salt in H_iO exhibits no additional resonances below 6.0 ppm, other than an H(8) resonance corresponding to H_γ; (iii) as the extent of nucleotide ordering is increased with increasing concentration of Na_2(5′-GMP), the relative intensity of $(NH_2)_d$ decreases while the relative intensities of H_A–H_F increase. Thus it seem clear that $(NH_2)_d$ is due to disordered nucleotide and is not related to the H-bonded protons of the ordered nucleotide.

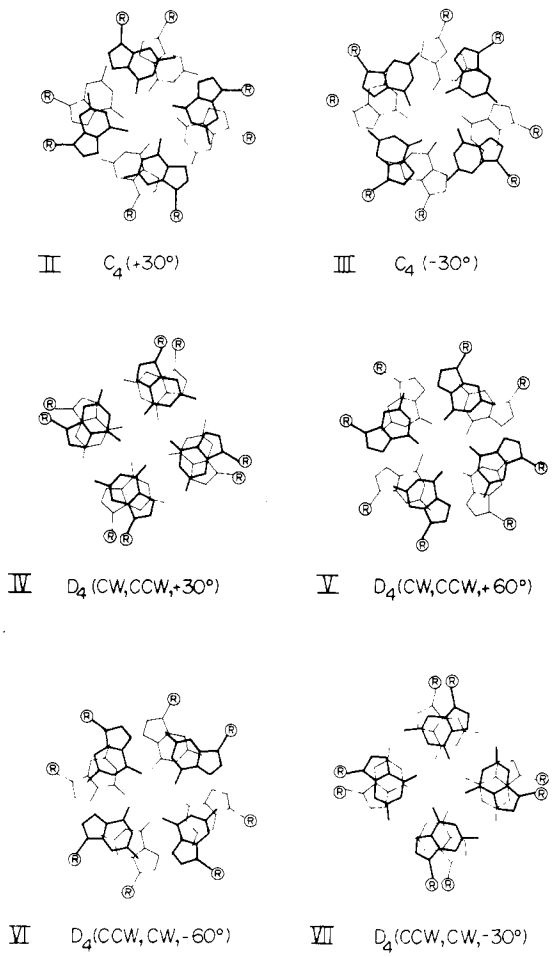

Figure 2. The six possible diastereomers resulting from normal stacking (II and III) and inverted stacking (IV–VII) of planar 5′-GMP tetramer units. The isomers with "normal" stacking have the tetramer units stacked head to tail and the H-bonding directionality is the same for the two tetramers. In the "inverted" isomers the tetramer units are stacked head to head or tail to tail and the H-bonding directionality is opposite for the two tetramer units. R represents the chiral ribophosphate moiety. The numbers in parentheses specifiy the twist angle between tetramer plates (±30° or, equivalent, ±60°). The CW and CCW notation for the D_4 isomers indicates the clockwise sense of the intratetramer H bonding for the upper and lower tetramer units.

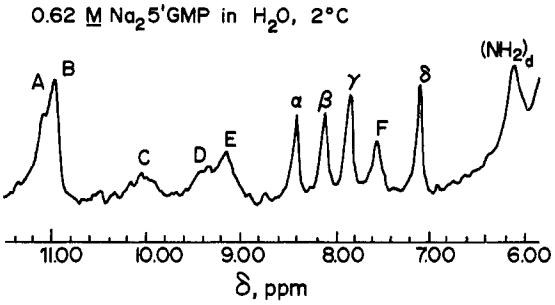

Figure 3. ^1H NMR spectrum (5.7–11.5-ppm region) of 0.62 M Na_2-(5′-GMP) in H_2O at 2 °C. The lines labeled α through δ are H(8) resonances of ordered nucleotide, and the line γ is the H(8) resonance of disordered nuclide. Lines A through F arise from exchangeable protons of the ordered nucleotide involved in H bonding. The weak ε resonance for the ordered species presented in minor amount (<5% abundance) occurs under resonance F. The line labeled $(NH_2)_d$ is the amino proton resonance of disordered 5′-GMP. The ribose proton resonances which occur at higher fields (not shown) are obscured by the intense H_2O line.

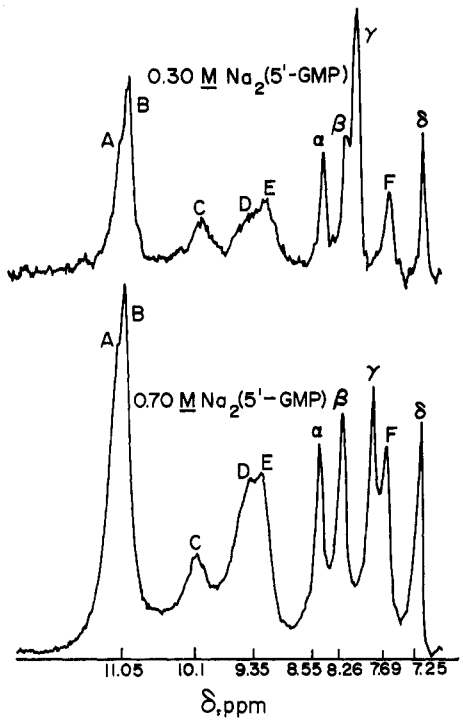

Figure 4. ^{1}H NMR spectra of the H-bonded protons (lines A–F) and H(8) protons (lines α–δ) of Na_2(5′-GMP) in H_2O at two different concentrations at 2 °C.

TABLE I: Intensity Relationships for the H-Bonded Protons of Ordered Na_2(5′-GMP) in H_2O at 1 °C

H-bonded proton resonances	protons per ordered 5′-GMP unit	assignment
$H_A + H_B$ (11.1–11.3 ppm)	0.96 ± 0.12[a]	N(1)H
$H_C + H_D + H_F$ (8.8–10.4 ppm)	1.1 ± 0.1[b]	N(2)H
H_F (7.69 ppm)	0.33 ± 0.06[b]	2′- or 3′-OH

[a] Mean of values obtained for eight independent solutions in the concentration range 0.27–0.75 M. [b] Mean obtained for six independent solutions.

There are four types of exchangeable protons in 5′-GMP which could be involved in H bonding and account for the H_A–H_F resonances of the ordered octamers: N(1)H, N(2)H, 2′-OH, and 3′-OH. In arriving at assignments for H_A–H_F, we have normalized their intensities with regrad to H_α, H_β, H_δ of the ordered octamers. The contribution of H_ϵ was disregarded, justifiably, because the normalized intensity of this line (<5%) is less than the random errors encountered in determining the normalized intensities of H_A–H_F. Within experiment uncertainty, the normalized intensities are invariant over the concentration range 0.27–0.75 M. The invariance in the relative intensities of the H-bonded and H(8) proton resonances can be qualitatively seen from the spectra presented in Figure 4 at different concentrations. Table I provides the normalized intensities of lines $H_A + H_B$, $H_C + H_D + H_E$, and H_F for six to eight independent solutions in the 0.27–0.75 M range.

The H_A and H_B resonances may be assigned on the basis of their normalized intensity (0.96 ± 0.12) to the H-bonded N(1)H protons of structural unit I.[19] This assignment is also in agreement with the chemical shift position at 11.1–11.3 ppm. For instance, the N(1)H guanine resonance of a hexanucleotide containing a central G–C pair is observed at 12.7 ppm for the double helical form and at 10.2 ppm for the coil form.[20,21] In a pentanucleotide

TABLE II: Percent Decrease in Signal Intensity, $100(I_0 - I_d)/I_0$,[a] for the H-Bonded and H(8) Proton Resonances of Na_2(5′-GMP) upon Saturation of Selected Nuclei

resonance saturated	H-bonded protons $H_A + H_B$	H_C	$H_D + H_E$	H_F	H(8) protons H_α	H_β	H_γ	H_δ
$(NH_2)_d$[b]	0	−5	10	0	−10	−15	−30	0
H_γ	10	5	−10	−15	−10	60		−10
H_F	−10	30	5		20	10	0	+5
H_2O	85	90	90	70	80	30	30	85

[a] I_0 is the integral intensity with no applied radio frequency; I_d is the intensity in the presence of the applied radio-frequency field. Absolute values of 15 or less are experimentally insignificant. Data were obtained for 0.62 M Na_2(5′-GMP) at 2 °C in H_2O. [b] Amino proton resonance in disordered nucleotide.

the central G–C pair exhibits a guanine N(1)H resonance at 12.4 ppm.[22] Also, the N(1)H proton of metastable poly-G, which is believed to have a four-stranded structure containing tetramer units, occurs at 11.1 ppm.[23]

Resonances H_C, H_D, and H_E exhibit a combined normalized intensity (1.1 ± 0.1) and chemical shifts (9–10 ppm) consistent with the values expected for the H-bonded N(2)H protons of I. Though the amino protons of nucleotides often are observed in the 6.3–7.5-ppm range,[24,25] H bonding of amino protns to an acceptor position in an aromatic ring causes the resonance to shift downfield. For instance, in certain oligonucleotides and in tRNA, the resonances of amino protons H bonded to a ring nitrogen occur in the 9–10-ppm region.[26–29]

It should be noted at this point that since the ordered octamers give rise to three magnetically nonequivalent H(8) environments, we should expect three nonequivalent N(1)H and N(2)H environments for the ordered forms. Indeed, three N(2)H environments are observed (H_C, H_D, H_E) but there are only two distinguishable N(1)H environments (H_A, H_B). Apparently, two of the structurally nonequivalent N(1)H protons have very similar magnetic environments and essentially coincident chemical shifts.

The normalized intensity of resonance H_F (0.33 ± 0.06) is too low to assign this line to amino protons in I which are not involved in H bonding.[30] However, the line could arise from an amino proton or a ribose 2′- or 3′-OH in an ordered octamer which is involved in extratetramer H bonding. We tend to favor the ribose OH assignment, in part, because the line is comparable in width ($\nu_{1/2}$ = 30 Hz) to the H(8) resonances and significantly narrower than the H-bonded amino protons ($\nu_{1/2}$ = 85 Hz) attached to the quadrupolar nitrogen nucleus (cf. Figure 4). Also, the chemical shift of H_F (7.69 ppm) is near the region found for a H-bonded 2′-OH proton in RNA and in some cyclic nucleotides (6.2–7.3 ppm).[24,31]

Spin Saturation Transfer. In an attempt to further verify some of the above H-bonded proton assignments, we performed a series of spin saturation transfer experiments wherein one resonance is continuously irradiated to saturate the resonance while other

(19) In a previous brief investigation of ordered Na_2(5′-GMP) in water (ref 4), H_A and H_B along with H_C were assigned to N(1)H of the ordered nucleotide. However, the present work shows that all of the expected intensity for N(1)H is contained in resonances H_A and H_B alone.

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(30) Though the disordered nucleotide exhibits the $(NH_2)_d$ resonance for amino protons which are not involved in ordered H bonding, the non-H-bonding amino protons in ordered 5′-GMP tetramer units are not observed by NMR due to rapid exchange with bulk water. Future studies will examine non-h-bonded amino proton exchange in ordered guanine nucleotides.
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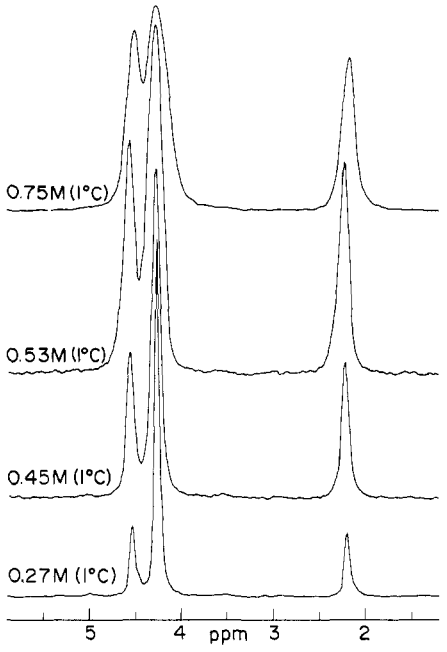

Figure 5. Concentration dependent ^{31}P NMR spectra of Na_2(5′-GMP) in D_2O at 1 °C.

resonances are being observed. If dipolar interactions or rapid chemical exchange reactions occur between the irradiated and observed nulei, spin saturation will be transferred to the observed nuclei, resulting in an intensity change in the observed resonances.[32]

Because the disordered nucleotide is in equilibrium with the ordered octamers, it was hoped that chemical exchange would be sufficiently fast to observe transfer of amino proton spin saturation in the disordered nuclotide to the amino protons of the ordered nucleotide. However, as can be seen from the data in Table II, saturation of $(NH_2)_d$ does not result in statistically significant changes in the intensities of any of the H-bonded protons of the ordered nucleotide. Apparently, chemical exchange between the ordered octamers and the disordered nucleotide is too slow to allow spin saturation transfer between amino proton environments. A further indication of relatively slow exchange is provided by results for saturation of H_γ for disordered 5′-GMP. Under these conditions essentially no change in intensity is observed for the C_4 octamers (H_α and H_δ), but a decrease in intensity does occur for the H(8) proton of the D_4 octamers giving rise to H_β. This latter result suggests that the disordered nucleotide exchanges more rapidly with the D_4 octamers than with the C_4 octamers.

Although exchange between disordered and ordered nucleotide is too slow to provide additional support for the assignment of H_C, H_D, and H_E to H-bonded amino protons of ordered octamers, the data in Table II do support the assignment of H_F to a H-bonded ribose OH and not a N(2)H proton. The saturation of H_F has little or no effect on the intensities of the remaining exchangeable protons, H_A–H_E. In fact, the intensity changes for the exchangeable protons are comparable to those experienced by the nonexchangeable H(8) protons, H_α–H_δ. This result is not compatible with H_F being a N(2)H proton involved in extratetramer H bonding. If this were the case we would expect dipolar interactions to lead to a change in the intensity of one of the intratetramer H-bonded amino proton resonances (H_C, H_D, or H_E) when the extratetramer H-bonded proton line is irradiated.

That dipolar effects can be important is evident from the results obtained when the H_2O protons are magnetically saturated (cf. Table II). As expected, all of the exchangeable protons H_A–H_F undergo dramatic reductions in intensity. However, the resonances for the nonexchanging H(8) proton of the C_4 octamer (H_α and H_δ) also undergo dramatic reductions in intensity. Thus, dipolar interactions are almost certainly responsible for the loss in H_α and H_δ intensity. For dipolar interactions ot be important, the water molecules must be held very near the N(8) protons of the C_4 isomers, more so than in the D_4 isomers (H_β) or in the disordered nucleotide (H_γ). Such differences in the proximity and residence time of water in the vicinity of H(8) suggests marked variability in the position or conformations of strongly solvated dipolar groups (e.g., phosphate) for the ordered and disordered forms of the nucleotide.

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TABLE III: Extratetramer H-Bonding Schemes of Stacked 5′-GMP Tetramer Units Suggested by Model Building Studies

isomer[a]	type of stacking	type of H bond	no. of H bonds[b]
II	normal	OH(3′) → OP	4
III	normal	OH(2′) → OP	4
IV	inverted	OH(2′) → O(3′)	4
V	inverted	OH(2′) → OP	4
VI	inverted	N(2)H → OP	8
VII	inverted	N(2)H → OP	8

[a] Isomers are defined in Figure 1. [b] Maximum number of H bonds between tetramer units, disregarding the effects of Na^+–phosphate binding and solvation.

^{31}P NMR Spectra. Figure 5 illustrates the ^{31}P NMR spectra of Na_2(5′-GMP) solutions at 1 °C and at various concentrations. In addition to the spectra shown, the spectrum of 0.094 M Na_2(5′-GMP), where essentially all of the nucleotide is disordered, exhibits a single sharp resonance near 4.3 ppm with a half-width of 3.4 Hz. As the concentration is increased, two new lines appear, one slightly downfield near 4.5 ppm and another markedly upfield near 2.2 ppm. These latter two lines appear concomitantly with the proton lines for ordered nucleotide. At a concentration of 0.75 M, the 2.2- and 4.5-ppm lines account for ~50% of the total ^{31}P NMR intensity, whereas the relative H_α, H_β, and H_δ intensities in the ^1H NMR spectrum of the same solution indicates 75% nucleotide ordering. Thus, the ordered nucleotide also gives rise to a third resonance with the same chemical shift value as the disordered nucleotide (4.3 ppm). The presence of three phosphate environments for the ordered nucleotide is consistent with the three major H(8) environments observed in the ^1H NMR spectra.

The usual ^{31}P chemical shift for the disodium salts of nucleotide 5′-monophosphates is ~4 ppm.[33] For instance, disordered $(TMA)_2$(5′-GMP) and disordered alkali metal salts of 5′-GMP in dilute solution exhibit a single sharp resonance in the chemical shift range 3.8–4.5 ppm. Thus, the question arises as to the origin of the high-field ^{31}P line at 2.2 ppm for ordered 5′-GMP.

Although ^{31}P chemical shifts of phosphates are relatively insensitive to the nature of the groups bonded to the phosphate oxygen, they are highly dependent on phosphate ionization state,[34] O–P–O bond angles,[35] and, in phosphate diesters, to the phosphate–ester torsional angle.[35-37] Therefore, the high-field ^{31}P NMR line for ordered Na_2(5′-GMP) may represent changes in the conformation or bond angles of a phosphate involved in complexation of Na^+ or formation of an extratetramer H bond. Indeed, the normalized intensity of the 2.2-ppm line (0.30 ± 0.06) is equal to the normalized intensity of the H-bonded ribose OH resonance H_F (0.33 ± 0.06). This is compatible with the 2.2-ppm phosphate acting as the acceptor for the extratetramer H bond.

Extratetramer H-Bonding Schemes. Summarizing our results thus far, we find that the normalized intensities and chemical shift positions of exchangeable protons H_A through H_E are consistent with I acting as the structural unit for ordered 5′-GMP octamers. However, resonance H_F, which can be assigned to a ribose OH on the basis of chemical shift position, line width, and spin sat-

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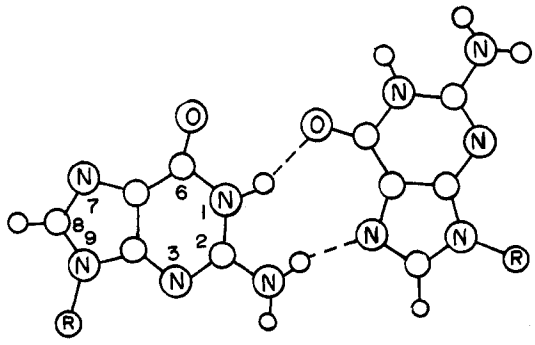
uration transfer results, represents an additional exchangeable proton involved in extratetramer H bonding. Because of the uncertainties introduced by the variability of nucleotide conformations and the absence of structural details for Na^+ binding and solvation, we are unable to unequivocally assign the extratetramer H bond to a specific isomer. Nevertheless, it may be useful to consider some plausible possibilities.

Earlier model-building studies recognized the possibility of H bonding between stacked tetramer units. A summary of the types and number of possible extratetramer H bonds for the six diastereomers resulting from normal and inverted tetramer stacking are provided in Table III. 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 ribose dihedral angles (ψ = 53 ± 7°, ϕ = 187 ± 24°, ω = 69 ± 4°) were maintained within the range of their commonly observed values.[38,39] Also, the effect of Na^+ binding to phosphate was disregarded. Within these structural limitations, we could immediately exclude D_4 isomer VI and VII as candidates for the observed extramer bond, because they utilize an N(2)H proton as the donor. The remaining isomers, however, utilized a ribose OH as the donor.

Before giving further consideration to the remaining possibilities in Table III, we note that the apparent equilibrium constant for conversion of C_4 octamers to D_4 octamers at 1 °C is 0.53 ± 0.03, as judged from the relative intensities of H_α, H_β, and H_δ over the concentration range 0.27–0.75 M. Thus, the normalized intensity of the ribose OH resonance (0.33 ± 0.06) corresponds to 4.0 ± 0.8 H bonds per C_4 octamer or to 7.9 ± 1.5 H bonds per D_4 octamer, depending on which type of isomer is assigned the extratetramer H bond. By comparison, the model-building studies predict four extratetramer H bonds for both C_4 isomers II and III and D_4 isomers IV and V.

The agreement between the number of H bonds allowed by experiment and the predicted number of H bond for the C_4 isomers, together with the 1:1 correlation between the normalized intensities of the H_F and 2.2-ppm ^{31}P resonances noted earlier, point to the plausibility of a ribose OH → OP bond between stacked tetramers with structures II and III. We emphasize, however, that this assignment must remain speculative, because other possibilities exist which cannot be readily tested. In D_4 isomers VI and VII, where the models predict a N(2)H → OP bond, the binding of Na^+ to phosphate may lead to conformational changes which promote a ribose OH → OP bond. Thus the assignment of the observed extratetramer H bond to a specific isomer must await future structural studies.

Stacked Tetramer vs. Stacked Dimer Models. Petersen et al.[9] have recently proposed that the H_α and H_δ lines of ordered Na_2(5'-GMP) are due to stacking of the asymmetric dimer unit VIII, which is equivalent to half of a tetramer unit I. The H_β



VIII

resonance was assigned to a second ordered form yet to be characterized. The complexation of Na^+, K^+, and Rb^+ (but not Li^+, Cs^+, or Mn^{2+}) was attributed to metal ion binding in a flexible "cation trap" defined by the four phosphate groups on every other

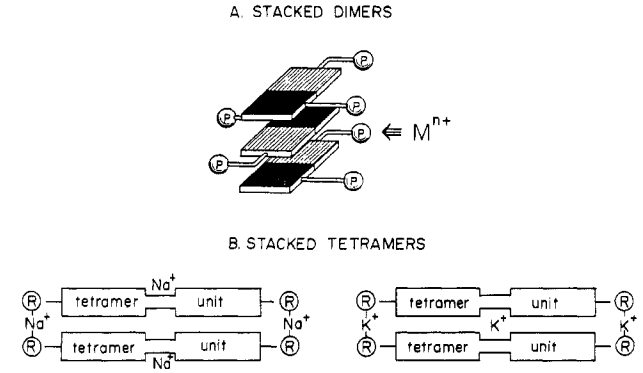


Figure 6. Schematic illustrations of the proposed metal ion binding sites for (A) the stacked asymmetric dimer model according to Petersen et al. (ref 9) and (B) the stacked tetramer model (ref 5 and 11). In the dimer model, a "cation trap" defined by the phosphate oxygens on every other dimer unit in the stack is proposed for the selective complexation of Na^+, K^+, and Rb^+, but not Li^+, Cs^+, or Mn^{2+}. In the tetramer model there are two possible binding sites. One is a structure-*directing* site at the central cavity of the tetramer units (Na^+) or at the eight-coordinate cavity between tetramer units (K^+, Rb^+). The other binding site is a structure-*stabilizing* site at chelating phosphate positions (Na^+, K^+, or Rb^+). The extent of tetramer stacking may differ for different structure-directing cations.

dimer unit in the stack, as illustrated in Figure 6A. In contrast, the stacked tetramer model explains both the H_α and H_δ resonances and the H_β resonance in terms of octamers with different symmetry. Also, the tetramer model provides two distinct metal ion binding sites. In the case of Na_2(5'-GMP), the structure-*directing* binding site is highly specific toward Na^+, as evidenced by the formation of a different set of ordered structures when Na^+ is completely replaced by K^+ or Rb^+.[5,11] The second binding site functions as a structure-*stabilizing* site, because Na^+ can be favorably replaced by K^+ at these secondary sites without changing the structure of the ordered aggregates.[11] As illustrated in Figure 6B, the structure-directing site is attributed to metal ion binding at the central O(6) cavity of the tetramer (Na^+) or to the eight-coordinate cavity between tetramer units (K^+, Rb^+). Chelation of metal ions by phosphate has been proposed as the structure-stabilizing site (Na^+, K^+, and probably other metal ions).

Unlike the metal binding site proposed for the stacked dimer model, the structure-directing binding sites in the stacked tetramer model are regular cavities, similar to those found in other classes of complexing agents which selectively bind alkali metal ions on the basis of size (for instance, cyclic polyether,[40] cryptands,[41] and certain polypeptides[42]). This regularity in the structure-directing binding sites allows the stacked tetramer model to be applied successfully to related ordering phenomena which are metal cation dependent, such as the gelation of guanine nucleosides[2] (no phosphate present) and the ordering of poly-I[43,44] and poly-X[45] with four-stranded structures. The stacked dimer model, however, is incapable of explaining the structure-directing influence of cations in these latter processes.

Regardless of the above inadequacies of the stacked dimer model, there are other deficiencies in the model, even when it is applied exclusively to Na_2(5'-GMP) as a special case. For the H(8) resonances of stacked asymmetric dimers to be independent of their position in the stack (cf. Figure 6), the extent of stacking must be so extensive that the magnetic anisotropy is the same for essentially all dimers in the stack. However, extensive dimer stacking is precluded by previous estimates of the number of 5'-GMP units per ordered aggregate (6.5 ± 1.6).[11] Also, there are two modes of binding of ethidium to the ordered nucleotide

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with stoichiometries corresponding to ethidium to ordered octamer ratios of 2:1 and 1:1.[11] One should expect only one mode of ethidium binding (intercalation) for stacked dimer units in which the extent of stacking is large. Moreover, the normalized intensities of the N(1)H and N(2)H lines found in the present work are incompatible with the stacking of dimers. The dimer model predicts 0.5 N(1)H and 0.5 N(2)H per ordered 5′-GMP, whereas the tetramer model predicts 1.0 N(1)H and 1.0 N(2)H, as observed. Finally, the estimated size of the ordered aggregate giving rise to H_α and H_δ is consistent with the stacking of two tetramers, provided anisotropic rotations and hydrated dimensions are taken into account.[10]

In considering the ^{13}C NMR spectra of ordered Na_2(5′-GMP), Petersen et al.[9] say that the downfield shifts observed for certain carbon centers (C(2), C(4), C(8)) are inconsistent with a model that exclusively involves tetramer stacking since this mode of association is expected to produce only upfield shifts on the basis of ring current effects. We have previously pointed out[10] that other factors also are likely to contribute to the observed chemical shifts, including (i) H bonding and Na^+ complexation, (ii) electric field gradients arising from the proximity to phosphate or Na^+, and (iii) solvation differences resulting form removal of the 5′-GMP base from a primarily aqueous to a more hydrophobic environment. Since these effects cannot be accurately assessed, it is impossible to draw meaningful structural conclusions on the basis of chemical shift data alone.

Despite our disagreement with the interpretation offered by Petersen et al.,[9] these workers have made important observatons which bear significantly on the dynamics of ordered Na_2(5′-GMP) structures. On the basis of spin saturation transfer measurements, they estimated the rate constant for interchange of H_α and H_δ environments in the ordered nucleotide (C_4 isomers) to be $k_{\alpha\delta} = 0.3\ s^{-1}$. By comparison, the rate constant for exchange of H_α or H_δ with H_γ of the disordered nucleotide was significantly lower than $k_{\alpha\delta}$ ($k_{\delta\gamma} = k_{\delta\gamma} = 0.1\ s^{-1}$). Also, the rate constant for exchange of H_β (D_4 isomers) with H_γ was larger than $k_{\alpha\delta}$ ($k_{\beta\gamma} = 1.2\ s^{-1}$). Thus it was concluded that $H_\alpha \rightleftarrows H_\delta$ interchange was *direct* (most likely, intramolecular). They also concluded that for octamers consisting of stacking tetramers there was no apparent mechanism which would permit direct exchange of H_α and H_δ to be faster than the exchange of these proton environments with H_γ of the disordered nucleotide. This latter conclusions, however, is incorrect.

The direct interchange of H_α and H_δ can be explained in terms of the tetramer model by ±60° rotations of one tetramer unit with respect to the other about the C_4 symmetry axis. However, we must revise our previous proposal[11] that the twisting rate is sufficiently rapid to time average the isomer pairs. Though isomers II and III are formally distinguishable by the presence of a D-ribophosphate attached to the left- and right-handed forms of a chiral core of stacked guanine tetramers, it is now apparent that the H(8) protons are magnetically equivalent, or nearly so, in the two isomers. Thus both isomers give rise to resonances at H_α and H_δ. Under these conditions isomers II and III would not be distinguishable by NMR, but the environments of the upper and lower tetramer units would be interchanged by twisting about the C_4 axis. An interchange mechanism of this type does not preclude extratetramer H bonding for a C_4 isomer. If the lifetime of the H bond is comparable to the lifetime before tetramer twisting (~3 s), the ribose OH resonance should remain sharp, as observed.

In summary, the stacked tetramer model for ordered Na_2(5′-GMP) is consistent with (i) the stoichiometry of nucleotide aggregation, (ii) the dependence of structural ordering on the nature of the alkali metal counterion, (iii) the bimodal binding of ethidium to the ordered forms, (iv) the estimates of aggregate size, (v) the number of exchangeable H-bonded protons per ordered nucleotide unit, (vi) the kinetics of H(8) environmental interchange, and (vii) the alkali metal ion dependence of ordering phenomena in related systems such as guanosine, poly-I, and Poly-X. The dimer model, however, is incompatible with these collective observations.

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Dielectric Properties and Molecular Structure of Amide Solutions. 2. 2-Azacyclotridecanone in Carbon Tetrachloride

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The thermodynamic theory of the nonlinear dielectric effect proposed by Rivail and Thiebaut for systems in which one chemical reaction takes place has been generalized for systems with many simultaneous reactions. Experimental results of the nonlinear dielectric effect and static dielectric polarization for 2-azacyclotridecanone (lauryl lactam) in CCl_4 solutions are presented. Conformations of hydrogen-bonded multimers of this lactam are discussed.

Introduction

In the first part of this paper[1] dielectric and spectroscopic studies of *N*-methylacetamide (NMA) solutions in CCl_4 were presented. Numerous studies devoted to this amide result from the fact that the compound can be regarded as a simple model of biological systems containing peptide groups. The trans configuration of this group leads to such a strong chain association of NMA molecules via NH···O=C hydrogen bonds that this amide in a pure state is one of the moss polar liquids ($\epsilon \approx 180$).

Our studies showed that, when highly diluted, the chain NMA multimers take conformations intermediate between linear (as in the crystalline state) and statistically random (as in the case of free rotation around hydrogen bonds). This conclusion agrees with the results of Bass, Nathan, Meighan, and Cole[2] obtained for pure NMA. Thus, the concentration of amide is not a main factor, implying the conformation of chains. It may be, to some extent, a surprise since it seemed that the distances between chains, determining the possibility of their appropriate packing (partic-

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