Role of Alkali Metal and Ammonium Cations in the Self-Assembly of the 5′-Guanosine Monophosphate Dianion

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Abstract: When sodium and potassium ions are present in conjunction, an organized structure $[(G_4, K^+, G_4), 4Na^+]^{11-}$ forms. Potassium ions bind selectively to the inner site, where they are sandwiched in between two GMP tetramers. Sodium ions bind selectively to the outer sites, screening the electrostatic repulsion of the negatively charged phosphate groups. They do so by true site binding, whereas K^+ or Rb^+ ions undergo only atmospheric condensation, on the outer sites. Ammonium ions duplicate octamers into hexadecamers, with synergism of NH_4^+ and K^+ ions.

Introduction

In the accompanying article,[1] ^1H, ^{23}Na, and ^{31}P NMR are applied to elucidating the structure and dynamics of the self-ordered structures spontaneously formed by 5′-GMP, Na_2 in aqueous solution at neutral pH. These different spectroscopies provide complementary information and are mutually consistent in support of the aggregates consisting predominantly of octamers, with a contribution from hexadecamers. ^{23}Na NMR gives direct and crucial evidence for inclusion of Na^+ ions in the central cavity present in tetramers of 5′-GMP, which are important building blocks in the self-assembly process.

Such aggregates, which could form readily under prebiotic conditions, may have had an important role in prebiotic processes. It thus becomes important to examine the self-ordering of 5′-GMP^{2-} in the presence of *both* Na^+ and K^+ ions, or K^+ and NH_4^+ ions, since these cations coexisted in the primeval ocean. We have recently shown the crucial role of K^+ with respect to other alkali cations in the 5′-GMP^{2-}, $2Na^+$ self-assembly.[2] In this report, we examine such mixed aggregates, using jointly ^{23}Na, ^{39}K, ^{87}Rb, and ^{31}P NMR in order to elucidate their structure and microdynamics. Pinnavaia et al.[3] have already examined by ^1H NMR aggregates formed by 5′-GMP^{2-}, $2M^+$, when M^+ is an alkali metal cation. Our work complements and extends their earlier study, since they examined separately the effect of each of the alkali metal cations on the stability and structure of the 5′-GMP self-orderings. We find a remarkable duality of sites, each type of sites being selective toward different cations, in the interaction between octameric aggregates and alkali metal ions. We also find synergism between K^+ and NH_4^+ ions in the buildup of hexadecameric aggregates.

Experimental Section

Preparation of Solutions. See ref 1 for purity control of the commercial 5′-GMP^{2-}, $2Na^+$ and for the pH measurements. The alkali chlorides NaCl, KCl, and KBr (Merck Suprapure), KI and NH_4Cl (UCB Pour analyse), and RbCl (Pierce Inorganic Ultrapure) are dissolved in D_2O (Merck Uvasol 99.8%) or in twice-distilled water. Prior to preparation of solutions, salts are dried under vacuum at 70 °C for 6 h. No influence of the anion (Cl^-, Br^-, I^-) on the results could be detected (K^+ salts). Samples of 5′-GMP, Na_2 (0.1 M), upon addition of KCl (>0.5 M) at ambient temperatures display a light blue cloudiness that disappears in a few hours. Systematically, spectra are recorded 12–20 h after sample preparation. Samples containing RbCl, but not samples with KCl, show a time evolution in their spectrum, so that standardized conditions are important.

Measurements of NMR Chemical Shifts and Relaxation Times. Sodium-23. See ref 1.

Potassium-39. NMR spectra are obtained on a WH-90 Bruker spectrometer (Karlsruhe), at 4.20 MHz, with 9-μs 90° pulses, using 3-kHz spectral windows. The line widths are of the order of 50 Hz, corresponding to 2000 scans (8 K) for S/N ratios of 10:1. The other conditions are similar to those for ^{23}Na.

Rubidium-87 NMR spectra are obtained on a Bruker WP-80 spectrometer (Liège), at 26.16 MHz, with 8.2-μs 90° pulses, using 12-kHz spectral windows. The line widths are of the order of 10^2–10^3 Hz, requiring typically 5000 scans (8 K) for S/N ratios of 40:1.

Phosphorus-31. See ref 1.

Carbon-13 NMR spectra are obtained on a Bruker HFX-90 spectrometer at 22.6 MHz (Liège). See ref 4 for further details.

Results

The self-ordering process requires lower concentrations of 5′-GMP^{2-}, $2Na^+$ when potassium ions are present.[2] Comparison of K^+ with the other alkali metal cations shows a marked selectivity favoring potassium, with the sequence $K^+ > Rb^+, Na^+ \gg Li^+, Cs^+$ being observed.[2,3] The stability of the aggregates and their polymorphism also depend on the nature of the alkali metal cation.[3,4] Synergistic effects are also present. We discovered them upon investigating competition between Na^+ and K^+ for binding to the 5′-GMP aggregates: to our surprise, the ^{23}Na NMR line width *increases* upon admixture of KCl to the NaCl aqueous solution. The presence of potassium ions facilitates binding of sodium ions by the aggregates of 5′-GMP! This observation led us to a detailed study of such synergistic effects in multi-component systems consisting of 5′-GMP, $2Na^+$ and various salts in aqueous solution.

Self-Assembly in the Presence of Potassium. Let us focus first upon self-ordered structures formed by 5′-GMP^{2-}, $2Na^+$ in potassium chloride containing aqueous solutions. This self-ordering is conspicuous by several observational methods; for instance, using ^{32}Na NMR to look at the nucleotide counterions, aggregation leads to a considerable increase in the relaxation rate and therefore in the line width (Figure 1). In a complementary manner, the line acquires non-Lorentzian character (Figure 2), which is diagnostic of a correlation time greater than ca. 5 ns.[4]

^{31}P NMR shows spectra for the phosphate group consisting of a singlet coexisting with more complex spectral features (Figure 3A); their intensity ratio R, of the intensity of all the other lines in the spectrum to the intensity of the singlet, also measures the degree of self-assembly, which increases with the concentration of potassium ions at constant temperature (Figure 3B), or with decreasing temperature at constant K^+ concentration.

We have presented in detail elsewhere[4] the method for extracting from the non-Lorentzian line shape of a slowly reorienting spin $^3/_2$ nucleus (such as ^{23}Na, ^{39}K, or ^{87}Rb) both the value of the correlation time τ_c, and that of the product $p_B\chi^2$. In the cases to be analyzed in this section, p_B is the mole fraction of sodium ions bound to the aggregates, and χ is the

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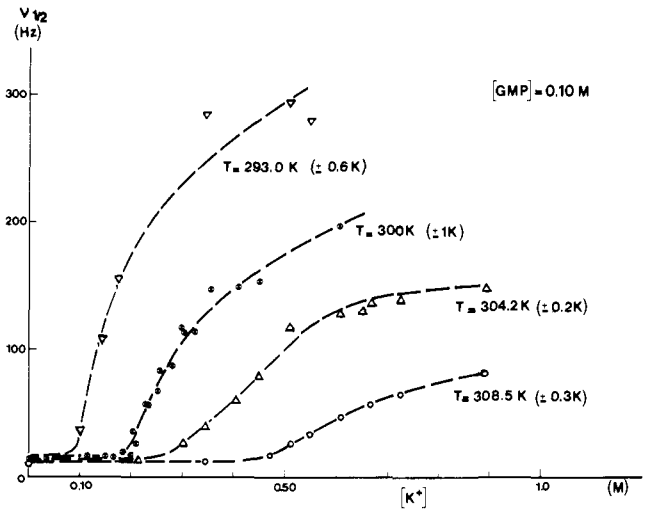


Figure 1. ^{23}Na line width $\nu_{1/2}$ as a function of KCl concentration for 5′-GMP, Na_2 (0.10 M) at various temperatures in D_2O solution.

quadrupolar coupling constant (e^2qQ/h) for these ions in the bound state. A very important clue is the finding of a *single* τ_c value of 9.4 ± 1 ns for a wide variety of experimental conditions (Figure 4); it strongly suggests formation of well-defined aggregates of a given size.

Going to the $p_B\chi^2$ values, they increase regularly with the concentration $[K^+]$ of potassium ions. At the lower concentrations in 5′-GMP (0.1 M), they even vary linearly with $[K^+]$ (Figure 5). It is comforting to note, as a further authentification of the procedure for obtaining τ_c and $p_B\chi^2$ separately from the experimental ^{23}Na line shape, that the $p_B\chi^2$ values thus obtained are correlated linearly with the fractional population of aggregates R obtained directly by a measurement of relative intensities, since these two values are linearly correlated with $[K^+]$ (Figures 3B and 5).

Besides the $p_B\chi^2$ and the R intensity ratios, a third line of evidence to be used below comes from the *observation of critical concentrations* when the sodium-23 NMR line width is plotted against reciprocal K^+ concentrations,[1] at each temperature (see Figure 1 for a plot of the data against K^+ concentrations). As potassium ion concentration reaches its critical value, the relaxation rate undergoes a cardinal change and increases very greatly, a feature suggestive of either an increase in size of the chemical species in which sodium ions attach themselves, or an increase in binding of the sodium ions, or both. As noted above, there is something of a paradox in this event, for one would expect that, upon increasing the concentration of potassium ions which compete with sodium ions for binding to the self-ordered structures, sodium ions would be displaced, leading conversely to a decrease in their relaxation rate and line width.

A Chemical Equilibrium Model. Our model accounts for all three types of observations. The contributing chemical equilibria are:

$$G_4 + M^+ \overset{K_1}{\rightleftharpoons} G_4, M^+ \quad (1)$$

$$G_4, M^+ + G_4 \overset{K_2}{\rightleftharpoons} G_8, M^+ \quad (2)$$

$$G_8, M^+ + nNa^+ \overset{K_3}{\rightleftharpoons} G_8, M^+, nNa^+ \quad (3)$$

In the first two steps, a dimer of tetramers forms in the presence of the nucleating cation M^+—preferentially K^+, even though Na^+ or Rb^+ can also serve this purpose. In the last step, metal ions bind, and sodium ions appear to bind preferentially, in order to neutralize further the two formal negative charges per phosphate group brought together in the self-assembled structure. This model yields from the experimental data the number n of metal ions thus bound. ^{31}P NMR measures, according to this simple treatment, the population ratio between the initial monomeric G or tetrameric G_4 states and the final aggregated state (G_8, M^+, nNa^+). The determination of the $p_B\chi^2$ product yields a parameter proportional to the mole fraction p_B of sodium ions bound to this final aggregated state (G_8, M^+, nNa^+). The critical concentration of potassium (Figure 1) is that necessary for efficient coupling between

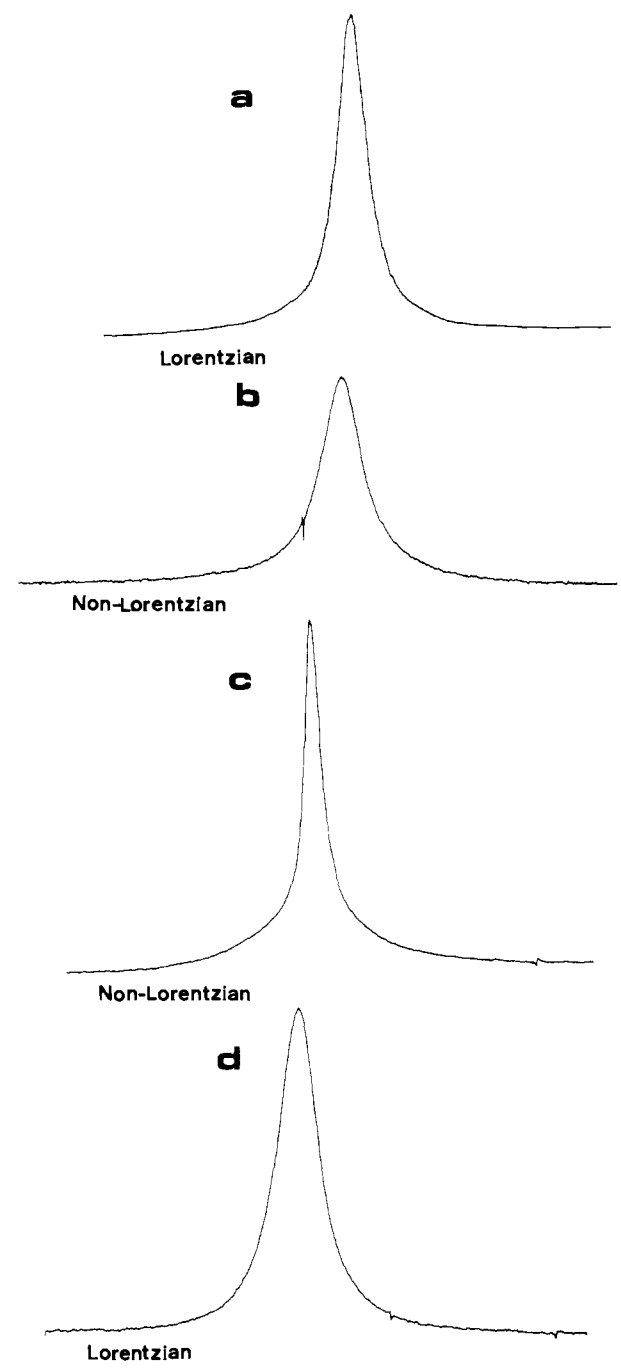


Figure 2. Comparison of the ^{23}Na and ^{87}Rb line shapes under various conditions: (a) ^{23}Na spectrum (21.15 MHz) of 0.10 M GMP, Na_2 with 0.15 M KCl at 300 ± 1 K. (b) ^{23}Na spectrum (21.15 MHz) of 0.70 M GMP, Na_2 at 285 ± 1 K. (c) ^{23}Na spectrum (21.15 MHz) of 0.10 M GMP, Na_2 with 0.63 M KCl and 0.32 M NH_4Cl.[18] (d) ^{87}Rb spectrum (26.16 MHz) of 0.20 M GMP, Na_2 with 0.40 M RbCl at 302 ± 1 K.

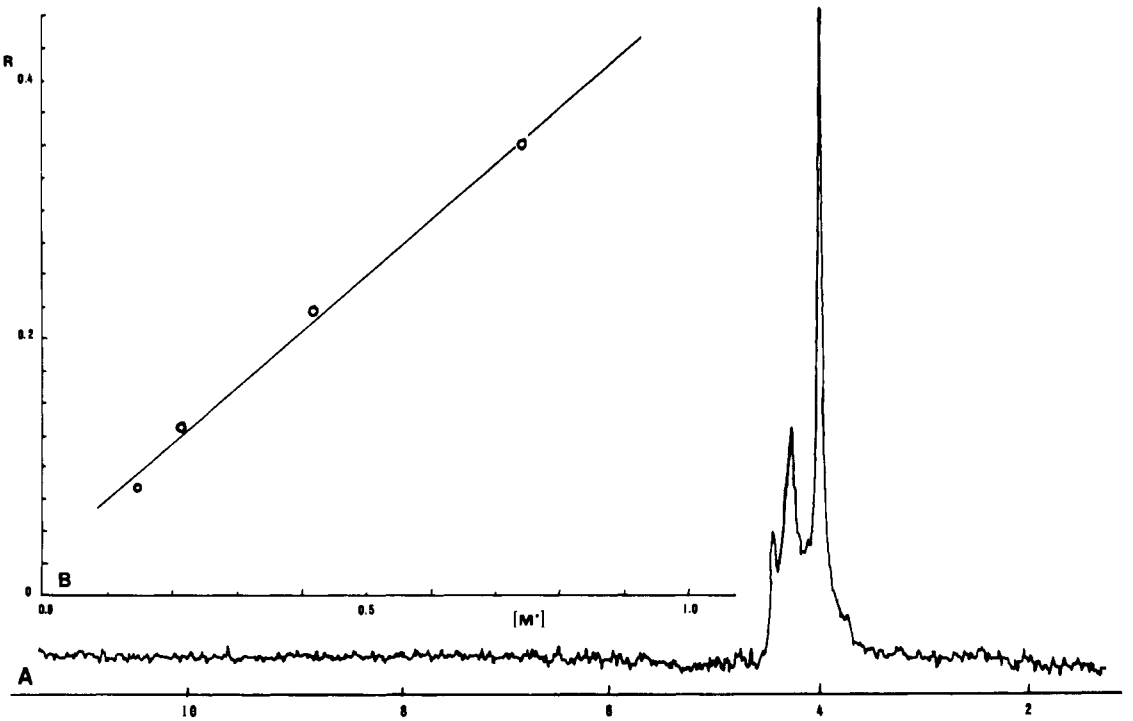


Figure 3. (A) ^{31}P NMR spectra for 5'-GMP, Na_2 (0.10 M) in D_2O solution, at 36.43 MHz (278 K), with 0.419 M KCl. (B) (Inset) Plot of the intensity ratio R against KCl concentration (M) for 5'-GMP, Na_2 (0.1 M) in D_2O solution.

equilibria 1-2, on one hand, and equilibrium 3. Then:

$$K_3 = \frac{[G_8, M^+, nNa^+]}{[G_8, M^+][Na^+]^n}$$

$$= \frac{p_B}{n(1-p_B)^n[Na^+]_t^{n-1}[G_8, M^+]}$$

$$\rightarrow K_3 \simeq \frac{p_B}{n(1-np_B)[Na^+]_t^{n-1}[G_8, M^+]}$$

for values of $p_B \ll 1$, limiting the series expansion of $(1-p_B)^n$ to the first term. Hence:

$$p_B = \frac{nK_3[Na^+]_t^{n-1}[G_8, M^+]}{1 + n^2K_3[Na^+]_t^{n-1}[G_8, M^+]} \quad (4)$$

which, if $n^2K_3[Na^+]_t^{n-1}[G_8, M^+] \ll 1$, reduces to:

$$p_B = nK_3[Na^+]_t^{n-1}[G_8, M^+] \quad (5)$$

Reduction of eq 4 to the simplified form (5) will hold if $p_B \ll 1$ and n is small. Since:

$$[G_8, M^+] = K_1K_2[G_4]^2[M^+]$$

$$p_B = nK_1K_2K_3[Na^+]_t^{n-1}[G_4]^2[M^+] \quad (6)$$

Now, by resorting to a phase-separation treatment[1] applicable to the coexistence of the (G_8, M^+) intermediate state and of the final (G_8, M^+, nNa^+) state:

$$RT \ln [G_8, M^+]_c = -RT \ln K_3 \quad (7)$$

or

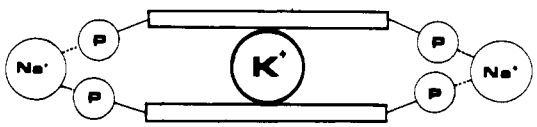
$$K_2[G_4][G_4, M^+]_c = K_3^{-1}$$
$$K_1K_2[G_4]^2[M^+]_c = K_3^{-1} \quad (8)$$

Combining eq 6 and 8:

$$p_B = n[Na^+]_t^{n-1}[M^+]/[M^+]_c \quad (9)$$

This key equation (9) predicts a linear dependence of p_B upon $[M^+]$, as indeed observed (Figure 4). Analyzing the data of Figure 4 ($p_B\chi^2$ as a function of $[M^+]$) with eq 9 yields a linear dependence of $p_B\chi^2$ upon the $[M^+]/[M^+]_c$ ratio, temperature independent to a very good approximation (correlation coefficient = 0.90 for 18 experimental points).

Scheme I



Letting the quadrupolar coupling constant χ span the whole range accessible to ^{23}Na NMR, i.e., 0.2-2 MHz,[7] eq 9 yields:

$$2.1 < n < 5.6 \quad (10)$$

The number n must be an integer, either 3, 4, or 5 from the above inequality (eq 10). Because of the fourfold symmetry of the tetramers, the value 4 is very likely. It corresponds to a highly reasonable value of the quadrupolar coupling constant $\chi = 0.68 \pm 0.08$ MHz. This number $n = 4$ of bound sodium ions corresponds to the sharing by two phosphate groups of one sodium ion; a plausible structural interpretation is for Na^+ ions to bridge the doubly charged phosphate groups, thus screening part of their electrostatic repulsion, as in Scheme I.

Such a geometry is indeed feasible. With the planes of the tetramers at the 3.3-Å spacing characteristic of base stacking,[8] together with a central cavity diameter distance between O_6 oxygens of approximately 2.5 Å,[2] the central potassium cation would be slightly beyond van der Waals contact with the eight O_6 oxygens to which it coordinates: 3.0 Å, a distance to be compared with 2.7-2.8 Å which is the $K^+ \cdots O$ distance reported for a number of antibiotic ionophores.[9] The sodium cations can now be placed upon this structure; it is possible to coordinate a sodium cation with two phosphate groups, one from the top tetramer, one from the bottom tetramer (as in Scheme I), at van der Waals contacts of 2.5-2.6 Å between sodium and the phosphate oxygens. If each phosphate ligates Na^+ in a bidentate manner, only two or three hydration mol-

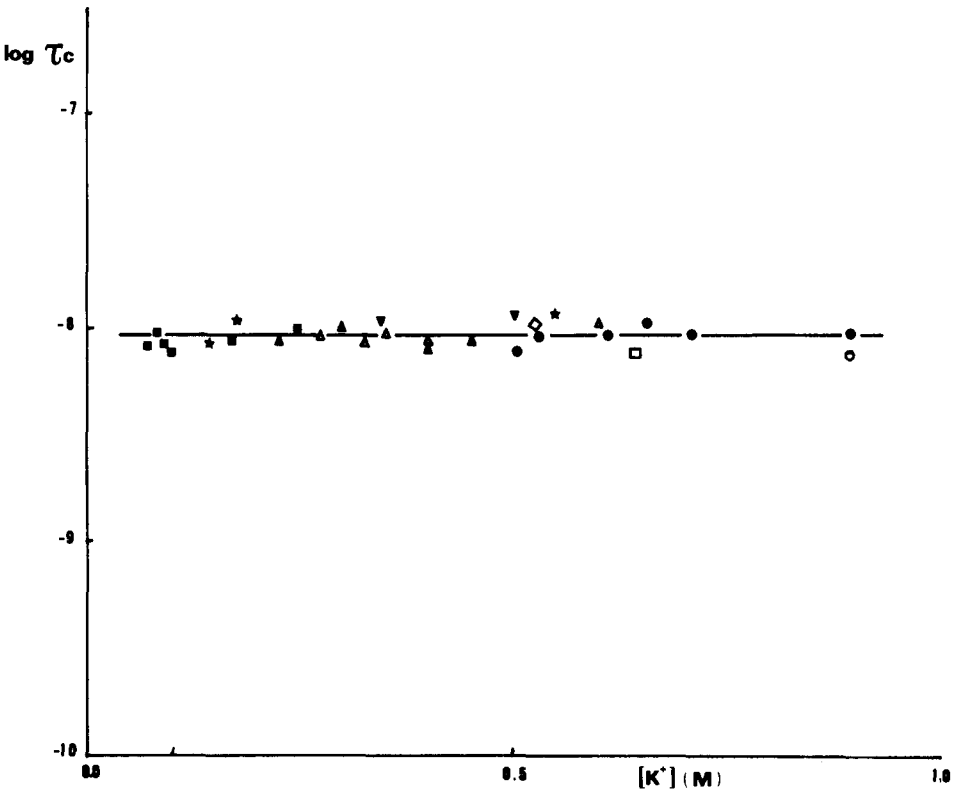


Figure 4. Correlation times τ_c (ns) for sodium bound to the aggregate against KCl concentration (M) for 5′-GMP, Na_2 at various temperatures and concentrations.

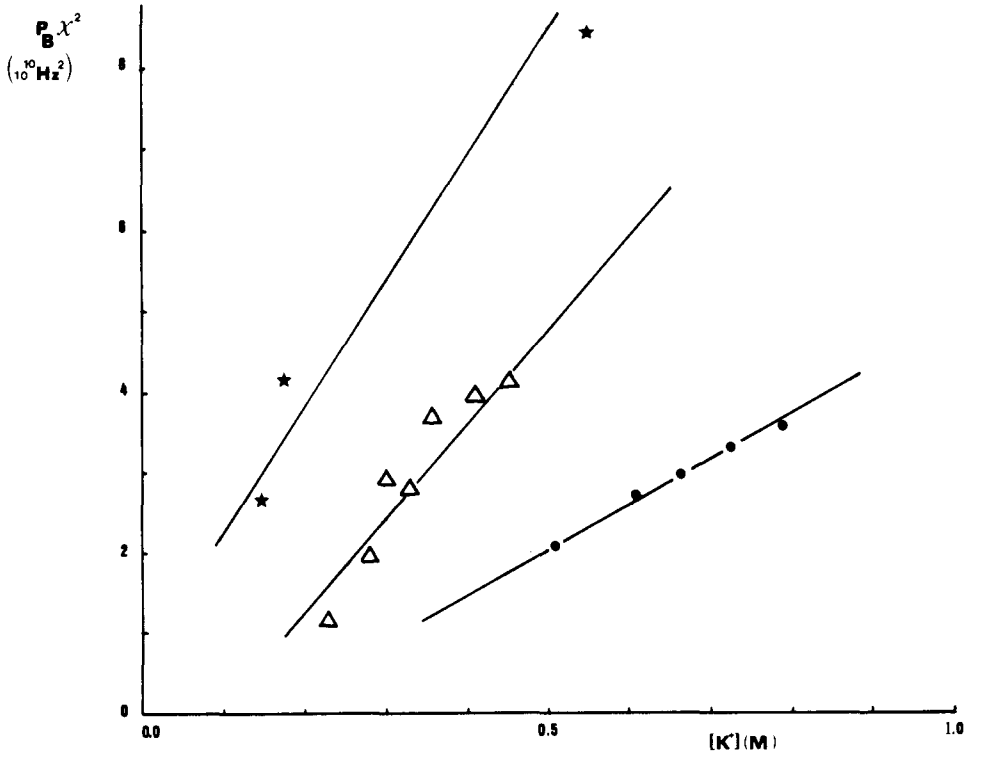


Figure 5. Plot of $p_B\chi^2$ (10^{10} Hz^2) for sodium bound to the aggregate against KCl concentration (M) at various temperatures: (★) 293 ± 0.6 K; (△) 300 ± 1 K; (●) 304.2 ± 0.2 K.

ecules would remain on the outside face of the Na^+ cation. This geometry assumes an anti conformation for 5′-GMP, of the guanine relative to the ribose. Indeed, the H-8 resonance shifts downfield by about 0.1 ppm when the pH changes from 4.5 to 8 for the monomeric nucleotide. This feature, which 5′-GMP shares with other 5′-purine nucleotides but not with the 2′- and 3′-nucleosides and nucleotides,[10] indicates an anti conformation bringing the phosphate group close to the H-8 proton.

Is the 9.4-ns correlation time τ_c consistent with the (G_8, K^+, nNa^+) species we postulate? Yes, assuredly. The corresponding Stokes radius of 13 Å is to be compared with a radius of 9–10 Å for the dimer of tetramers (G_8, K^+),[4] augmented

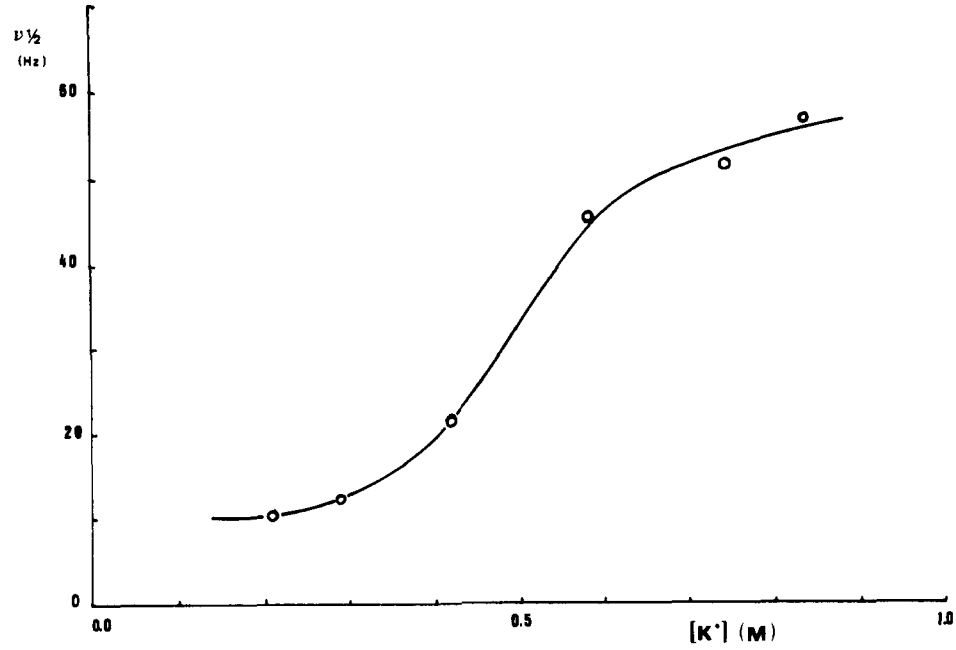


Figure 6. Variation of the ^{39}K line width $\nu_{1/2}$ (Hz) as a function of the concentration of KCl (M) added to a 5'-GMP, Na_2 (0.10 M) in D_2O solution at 304 ± 1 K.

by the dimension of the partly hydrated Na^+ cation bound to phosphate oxygens (Scheme I), i.e., a particle 4-5 Å in length.

We apply the model of eq 1-4 to ^{23}Na NMR spectra in the range 293-308.5 K, where ^{31}P spectra (Figure 3B) display a predominant singlet resonance ($R < 0.1$). The rest of the treatment hinges upon attribution of this signal either to free monomers G, or to hydrogen-bonded tetramers G_4. In the latter case, eq 7 with $n = 4$, for $[Na^+]_t = 0.2$ M and $[G_4] = (1/4)[G]_t$, gives:

$$K = K_1K_2K_3 = 5 \times 10^4 p_B/[M^+] \qquad (11)$$

The values of K as a function of temperature correspond to the following enthalpy and entropy changes: $\Delta H = -26 \pm 4$ kcal·mol^{-1} and $\Delta S = -68 \pm 10$ cal·mol^{-1}·K^{-1}. The intensity ratio from ^{31}P spectra is:

$$R = 2[G_8, M^+, nNa^+]/[G_4] \qquad (12)$$

(twice as many phosphate groups are present in an octameric species as in a tetrameric species). Since $[G_8, M^+, 4Na^+] = K_1K_2K_3[G_4][M^+][Na^+]^4$, $[Na^+] = 2[G]$ and $[G_4] = [G]_t/4$. Hence, $[G_8, M^+, 4Na^+] = K[G]^6[M^+]$ and:

$$R = 8K[G]^5[M^+] \qquad (13)$$

The intensity ratio, according to this model, ought to be proportional to the potassium ion concentration $[M^+]$, as is indeed the case ($\rho > 0.99$ for 4 points at each of the three temperatures 283, 293, and 298 K). From the temperature dependence of K, a second set of ΔH and ΔS parameters can be obtained:

$$\Delta H = -22 \pm 3 \text{ kcal·mol}^{-1}$$

$$\Delta S = -58 \pm 7 \text{ cal·mol}^{-1}\text{·K}^{-1}$$

A third way of determining these parameters is through a phase-separation formalism, in which:

$$\Delta G = \Delta H - T\Delta S = -RT\left(1 + \frac{n}{p}\right) \ln [G_8, M^+]_c \qquad (14)$$

where n/p is the fraction of sodium ions condensed (or bound) onto the aggregates. This reduces to:

$$\Delta G = -1.25RT[\ln K_1K_2[G_4]^2 + \ln [M^+]_c]$$

and

$$\ln [M^+]_c = \frac{\Delta H'}{1.25R}\left(\frac{1}{T}\right) - \frac{\Delta S'}{1.25R}$$

with $\Delta H' = -22.5 \pm 3$ kcal·mol^{-1} and $\Delta S' = -71 \pm 7$ cal·mol^{-1}·K^{-1}. Since $\Delta H' \simeq \Delta H$, the perturbation from the $K_1K_2[G_4]^2$ term is very small, as it should be. Comparing $\Delta S'$ and ΔS, the product K_1K_2 is relatively small and K_3 is relatively large, in determining the overall equilibrium constant $K = K_1K_2K_3$.

These are *apparent* equilibrium constants, which have not been corrected for activities instead of concentrations, due to the difficulty of making an accurate estimation of the activity coefficients in such a relatively complex system. The thermodynamic parameters ΔH and ΔS that have been quoted, however, are very little affected by the use of concentrations rather than activities. We have examined in depth the possibility that the initial state be the equilibrium $4G \rightleftarrows G_4$ rather than the equilibrium $G_4 + M^+ \rightleftarrows G_4, M^+$ (eq 1). Then the singlet resonance in the ^{31}P spectra (Figure 3A) would correspond to free monomers G, rather than tetramers G_4. However, and in contrast to the success and internal consistency of the model just presented, incorporation of monomeric species with nonnegligible concentrations leads to severe discrepancies between the ΔH and ΔS values obtained in three different manners as indicated above. We feel for this reason that, at concentrations of 5'-GMP superior or equal to 0.1 M, the $4G \rightleftarrows G_4$ equilibrium favors tetramers. Klump had also inferred from calorimetric data that for [5'-GMP] > 0.1 M, only tetramers G_4 or aggregates of tetramers are present.[11] We note the good agreement of these values of $\Delta H \simeq -22$ kcal and $\Delta S \simeq -65$ cal·K^{-1} per mol of octamer with those earlier determined by Klump,[11] $\Delta H \simeq -19$ kcal and $\Delta S \simeq -70$ cal·K^{-1} per mol of octamer, for [5'-GMP] = 0.1 M. Chantot[12] found an energy of interaction between two tetramers in the octameric arrangement of 16 kcal per mol of octamer.

Another comment is in order. In our model (eq 1-3), binding of sodium ions is (formally) subsequent to octamers' formation around a potassium core. However, it would be a completely equivalent process to start with partial neutralization of the charged phosphate groups by sodium binding, prior to the self-assembly proper. Whatever the description, self-ordered

Table I. ^{23}Na and ^{87}RB Line Widths, at Various Temperatures, for Samples of 0.4 M RbCl, in the Presence of GMP, $2Na^+$ [a]

[GMP, $2Na^+$], M	T, K (±1 K)	$\nu_{1/2}(^{23}Na)$, Hz	$\nu_{1/2}(^{87}Rb)$, Hz
0.00	313		126 ± 3
0.20	313	16 ± 1	243 ± 5
0.00	308		127 ± 3
0.20	308	16 ± 1	279 ± 5
0.00	303		138 ± 3
0.20	303	18 ± 1	300 ± 6
0.00	295		165 ± 4
0.20	295	80 ± 2[a]	950 ± 30

[a] Non-Lorentzian line shape: τ_c = 7.2 ns, $p_B\chi^2 = 1.62 \times 10^{10}$ Hz^2.

structures can only form in the presence of a nucleating cation in their core (which we shall refer to as the inner site) *and* if in addition cations occupy a number n of outer sites where they effect partial neutralization of the phosphate charges. To a very good first approximation, the inner site is potassium-ion selective, while the outer sites are sodium-ion selective.

Atmospheric Condensation of K^+ and Rb^+ on the Outer Sites. The analogy between the charged (G_8, M^+) species and a polyelectrolyte was very tempting. Two modes of interaction can occur between a polyelectrolyte and its counterions: site binding or atmospheric condensation.[13] Thus, one could expect sodium cations either to bind to the outer sites (equilibrium 3), or to undergo atmospheric condensation. The very success of our equilibrium model (eq 1-3) shows that sodium binding, rather than condensation, takes place. The evidence is the following: the value of $n < 16$; the equilibrium constant K_3 in the range 10^2-10^3 at 300 K, significantly greater than for ion pairing of Na^+ with the 5′-GMP monomer; the reorientation correlation time τ_R = 9.4 ns for the rigid binding of Na^+ to the potassium-centered octamers; and the quadrupolar coupling constant χ = 0.68 ± 0.08 MHz. All these results point unambiguously toward sodium cations undergoing site binding on the outer sites of the aggregates, rather than merely condensed in the surrounding region.

How do other alkali metal cations attach themselves to these outer sites at the periphery of the aggregates? We have measured ^{39}K NMR line widths on the same samples[2] (Figure 6). Comparison of the ^{23}Na and ^{39}K results, together with the known values[14] for the quadrupolar moments Q and the Sternheimer antishielding factors ($1 + \gamma_\infty$), yields a ratio $(p_Bq)_{Na^+}/(p_Bq)_{K^+}$ of 58, at 304 K. The only reasonable inference from this very high ratio is that *both* the fraction of bound ions p_B and the electrostatic field gradient at the nucleus q are significantly greater for Na^+ than for K^+. The unavoidable inference is for potassium ions to be capable of atmospheric condensation only, whereas sodium ions form true contact ion pairs with the charged phosphate groups.

Going to the Rb^+ cations, we were highly gratified to note that, despite a quadrupolar moment greater than that of Na^+ by almost 50%, and a Sternheimer antishielding factor greater by one order of magnitude (all things being equal, the combination of these two factors would lead to relaxation rates for ^{87}Rb *greater* than for ^{23}Na by a factor of 140), the ^{87}Rb spectra (5′-GMP = 0.2 M + 0.4 M RbCl, at 295-320 K) remain nicely Lorentzian (Figure 2) and display line widths (Table I) greater than the ^{23}Na line widths by a mere factor of 10-15. Again, the conclusion is that the Rb^+ cations undergo only atmospheric condensation about the outer sites, while the Na^+ cations are capable of true site binding at the interface between the aqueous solution and the 5′-GMP self-assemblies.

Self-Assembly in the Presence of Ammonium. We were led by the similarity between the ionic radii for K^+ (1.33 Å) and for NH_4^+ (1.45 Å) to investigate the possible role of ammonium ions in the 5′-GMP self-assembly. Also, an ammonia-rich primitive atmosphere[15-17] ties in with the same idea. We indeed found facilitation of 5′-GMP aggregation in the presence of ammonium ions, together with the interesting feature of synergism between K^+ and NH_4^+ action.[18] Furthermore, if one starts with already-formed 9.4-ns aggregates, addition of NH_4Cl leads to gradual shift from a correlation time below 10 ns to a correlation time of approximately 20 ns.[18]

These observations show that, perhaps unexpectedly, ammonium ions compete neither with the potassium cations for occupation of the inner sites, nor with the sodium cations for occupation of the outer sites, in the postulated (G_8, K^+, $4Na^+$) species. Rather, they appear to effect the duplication to a hexadecameric ammonium-ion bridged (G_8, K^+, $4Na^+$/NH_4^+/G_8, K^+, $4Na^+$) species. Assuming that these τ_c values, of ca. 10 and 20 ns, respectively, are indeed reorientational correlation times, to which the Debye-Stokes-Einstein relation applies, such a doubling in value implies formation of a particle with twice its earlier volume. (Whereas the octameric species can be approximated validly as a sphere, the hexadecameric entity might deviate significantly from a spherical shape; however, τ_R is a good first approximation for ellipsoids with an axial ratio of up to 5.[19]) This finding of a dimerization from G_8 to G_{16}, brought about by ammonium ions, is thus in harmony with the postulated co-existence between G_8 and G_{16} species for 5′-GMP, Na_2, especially at the higher concentrations.[1] It would occur by the bridging, via an NH_4^+ ion, of two phosphate groups belonging to separate (G_8, K^+, nNa^+) species. If biological molecules, such as enzymes, can discriminate between NH_4^+ and the isosteric K^+ ions, it is truly remarkable that aggregates formed spontaneously by 5′-GMP, a relatively small molecule, are also capable of making the K^+/NH_4^+ distinction.

Since the aggregates interact strongly with ammonium ions, we have investigated their interaction with amino acids. Starting again with preformed 9.4-ns aggregates obtained by the admixture in aqueous solution of 5′-GMP, Na_2 and KCl, we have shown that the system discriminates between glycine and alanine, two amino acids formed in abundance under prebiotic conditions from the primitive atmosphere constituents.[20,21] Glycine, which in proteins is a strong helix breaker,[21] destroys the 5′-GMP clusters, perhaps because the amino acid competes with potassium ions for occupation of the core positions in the tetramers.[13] No measurable effects, by contrast, are found with L-alanine.

Discussion

A single correlation time of 9.4 ns characterizes the aggregates formed by 5′-GMP, Na_2 in the presence of potassium ions, to which we attribute the composition (G_8, K^+, $4Na^+$). The reorientational correlation time obtained by ^{23}Na NMR for this species comes very close to that derived from measurement of the ^{13}C-$\{^1H\}$ nuclear Overhauser effect:[4] the complex formed between the ionic partners has the structuring and rigidity normally associated with covalently bound compounds. It is a species to which the Na^+ ions are tightly and durably bound. This self-ordered structure formed in the presence of potassium ions is either a single one or, if several self-ordered states coexist, as implied by the ^1H NMR results of Pinnavaia et al.,[3,6] they have identical or nearly identical sizes (i.e., degrees of aggregation) so that their microdynamics can be described with a correlation time of approximately 10 ns.

The buildup of the (G_8, K^+, $4Na^+$) occurs with a cooperativity that shows up in the difference between the equilibrium constants for the two main steps in our model: (1) (G_4, K^+) +

$G_4 \rightleftarrows (G_8, K^+)$; (2) formation of $(G_8, K^+, 4Na^+)$. In the preceding article,[1] we apply the Hill formalism[22] to analyze the cooperativity of the self-assembly of 5′-GMP, Na_2. Unfortunately, this treatment cannot be extended to aggregates formed in the presence of potassium ions. The data of Figure 6 can be combined with the model of an octamer (G_8, K^+, nNa^+). Taking $(p_B)_{lim} = [G_8, K^+, 4Na^+]_{max}/[K^+]_t = [G]_t/8[K^+]_t$, it is straightforward to plot $p_B\chi^2/(p_B)_{lim}$ as a function of $[K^+]$. However, one obtains in this manner only the rising portion of a Hill plot. All the points remain too far from saturation. Therefore, it is not feasible to translate these results into expression of a saturation percentage as a function of $[K^+]$. Even if a quantitative analysis fails, a qualitative description can be provided. Self-assembly is a strongly cooperative process. Polarization of the guanine base by ligation of an alkali metal cation, at the O_6 oxygen, reinforces its hydrogen-bond donor and acceptor abilities. Potassium ion encapsulation in the spheroidal cavity lined by the eight O_6 carbonyl oxygens of two tetramers stacked in parallel planes provides additional stability. Sodium binding and potassium or rubidium condensation on the outer sites of the octamers are additional stabilizing influences, necessary for the shielding of the closely packed phosphate charges. Ammonium ions display an astonishing amount of synergism with potassium ions: they appear to effect duplication of the $\tau_c \simeq 10$ ns aggregates into $\tau_c \simeq 20$ ns aggregates ($G_8 \rightarrow G_{16}$ possibly).[18]

Our model is based on coexistence of two kinds of sites, inner sites selective toward potassium ions and outer sites that display a strong apparent preference for sodium ions over potassium or rubidium ions. Likewise, poly(I) helix formation occurs with nucleation by alkali metal ions at two kinds of sites: inner sites which are Li^+/Na^+-specific, together with another type of sites binding K^+, Rb^+, and Cs^+ ions.[23] Only by considering sodium binding to outer sites of the 5′-GMP self-assembly is it possible to account for the enhancement in the ^{23} Na relaxation rate upon introduction of K^+ ions. Admittedly, this is an indirect inference, but the most likely positions for Na^+ attachment, after a K^+ ion has occupied the core position between the planes of two tetramers (Scheme I), are the phosphate groups on the periphery. Both the number $n = 4$ of bound Na^+ ions on these outer sites and the magnitude of the quadrupolar coupling constant χ are consistent with bridging of two phosphate groups by an Na^+ cation, as in Scheme I.

Sodium- and potassium-cation binding are essential to aggregate formation. Sodium and potassium ions are not only the most effective agents for aggregation, they are also necessary building blocks (or, perhaps more appropriately, cornerstones). Cation binding is indeed a prerequisite for the aggregation of 5′-GMP at neutral and slightly basic pH, a heretofore enigmatic process: potassium entry, for instance, nucleates the self-assembly. Self-assembly of 5′-GMP, unlike a normal stacking interaction,[24] is not determined predominantly by hydrophobic forces, but by cation binding. The self-assembly is *enthalpy driven*, since the entropy change upon self-association is strongly negative.

The self-assembly of 5′-guanosine monophosphate provides a handsome prototype for the self-assembly of bigger and more complex biomolecules; with each entity appears a new set of integral properties that the constituent parts lacked.[25] Hydrogen bonding of the guanines transforms normal 5′-GMP monomers, which bind Na^+ preferentially to K^+, into tetramers that are capable of binding potassium ions selectively with respect to lithium, sodium, rubidium, and cesium ions. Stacking of two tetrameric units and the attendant coming together of phosphate groups lead to aggregates that bind sodium ions on the outer sites selectively with respect to lithium, potassium, and cesium ions.

Self-ordering of a biomolecule, especially one that could form readily under prebiotic conditions, invites comment: "biological activity demands organization. Aggregation provides one level of organization of molecules and it is reversible".[26] One cannot but speculate at this time about possible prebiotic functions of such a highly ordered noncovalent structure. Potassium enrichment of the inside of proto-cells; a primitive ionophore with respect to both K^+ and Na^+ ions; joint stacks of 5′-GMP and porphyrin[27] aggregates; perhaps even a primitive enzyme: these are questions for which we are now seeking experimental answers. The 5′-GMP self-assembly could also have been important for the appearance of the first polynucleotides, as a template. We note in this respect that the homocodon GGG is related to glycine, unique among amino acids for its abundance in the prebiotic soup and for its archaic character. Association between a structured noncovalent assembly of G, Na^+, K^+, and Gly could have been a primitive step in the emergence of the genetic code. It is important to note in this respect the discrimination that we have observed[18] between the interactions of the aggregates with glycine and with alanine.

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References and Notes

(1) M. Borzo, C. Detellier, P. Laszlo, and A. Paris, *J. Am. Chem. Soc.*, preceding paper in this issue.
(2) C. Detellier, A. Paris, and P. Laszlo, *C. R. Hebd. Seances Acad. Sci., Ser. D*, **286**, 781–783 (1978).
(3) T. J. Pinnavaia, C. L. Marshall, C. M. Mettler, C. L. Fisk, H. T. Miles, and E. D. Becker, *J. Am. Chem. Soc.*, **100**, 3625–3627 (1978).
(4) A. Delville, C. Detellier, and P. Laszlo, *J. Magn. Reson.*, **34**, 301–315 (1979).
(5) A. Paris and P. Laszlo, *C. R. Hebd. Seances Acad. Sci., Ser. D.*, **286**, 717–719 (1978).
(6) T. J. Pinnavaia, H. T. Miles, and E. D. Becker, *J. Am. Chem. Soc.*, **97**, 7198–7200 (1975).
(7) P. Laszlo, *Angew. Chem.*, **90**, 271–283 (1978); *Angew. Chem., Int. Ed. Engl.*, **17**, 254–266 (1978).
(8) A. W. Hanson, *Acta Crystallogr.*, **17**, 559–568 (1964).
(9) B. T. Kilbourn, J. D. Dunitz, L. A. R. Pioda, and W. Simon, *J. Mol. Biol.*, **30**, 559–563 (1967); M. Pinkerton, L. K. Steinrauf, and P. Dawkins, *Biochem. Biophys. Res. Commun.*, **35**, 512–518 (1969); M. Dobler, J. D. Dunitz, and J. Krajewski, *J. Mol. Biol.*, **42**, 603–606 (1969).
(10) S. S. Danyluk and F. E. Hruska, *Biochemistry*, **7**, 1038–1043 (1968).
(11) H. Klump, *Ber. Bunsenges. Phys. Chem.*, **80**, 121–124 (1976).
(12) J. F. Chantot, *Arch. Biochem. Biophys.*, **153**, 347–356 (1972).
(13) G. S. Manning, *J. Chem. Phys.*, **51**, 924–933; 3249–3252 (1969); *Biopolymers*, **11**, 937–949 (1972); *Annu. Rev. Phys. Chem.*, **23**, 117–140 (1972); *J. Chem. Phys.*, **62**, 748–749 (1975).
(14) H. Weingärtner and H. G. Hertz, *Ber. Bunsenges. Phys. Chem.*, **81**, 1204–1221 (1977).
(15) M. Calvin, *Angew. Chem., Int. Ed. Engl.*, **12**, 121–131 (1975).
(16) M. Calvin, "Chemical Evolution. Molecular Evolution towards the Origin of Living Systems on the Earth and Elsewhere", Clarendon Press, England, 1969.
(17) C. C. Price, Ed., "Synthesis of Life", Dowden, Hutchinson and Ross, Stroudsburg, Pa., 1974.
(18) C. Detellier and P. Laszlo, *Helv. Chim. Acta*, **62**, 1559–1565 (1979).
(19) T. Tao, *Biopolymers*, **8**, 609–632 (1969).
(20) R. H. Lemmon, *Chem. Rev.*, **70**, 95–109 (1970).
(21) A. Brack and L. E. Orgel, *Nature (London)*, **256**, 383–387 (1975).
(22) A. V. Hill, *J. Physiol.*, **40**, 4–7 (1910).
(23) H. T. Miles and J. Frazier, *J. Am. Chem. Soc.*, **100**, 8037–8038 (1978).
(24) E. Plesiewicz, E. Stepien, K. Bolewska, and K. L. Wierzchowski, *Biophys. Chem.*, **4**, 131–141 (1976).
(25) E. Antonini and E. Chiancone, *Annu. Rev. Biophys. Bioeng.*, **6**, 239–271 (1977).
(26) A. T. Florence, in "Micellization, Solubilization, and Microemulsions", Vol. 1, K. L. Mittal, Ed., Plenum Press, New York, 1977, pp 55–74.
(27) W. I. White, in "The Porphyrins", Vol. V, Academic Press, New York, 1978, pp 303–339.