162. Influence of Amino Acids, Ammonium and Potassium Cations on the Self-Assembly of 5′-GMP

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Summary

The self-ordered supramolecular structures formed by 5′-guanosine monophosphate (disodium salt) in aqueous solution at pH 7.8 show pronounced interaction with ammonium ions. Rather than competing with potassium ions for the central cavity in hydrogen-bonded guanine tetramers, ammonium ions bring about – in synergism with potassium ions – further aggregation. Glycine appears to destroy the aggregates, by competing with potassium ions for the core positions within the tetramers. Conversely, alanine does not interact significantly with the system. These conclusions follow from analysis, at various concentrations, of the microdynamics and of the mole fractions of sodium ions bound to self-assembled 5′-GMP^{--}, obtained from relaxation rate measurements for the ^{23}Na nucleus, as the 5′-GMP^{--} counter-ion.

1. Introduction. – Guanosine monophosphate (GMP) is the only nucleotide capable of self-assembly in aqueous solution [1]. The guanine base and the ribose sugar can be formed under prebiotic conditions; likewise, condensation into a nucleoside and a nucleotide are feasible abiotic processes [2] [3]. The 5′-isomer (5′-GMP) forms highly ordered structures: not only at acidic pH's where gels occur, but also at the slightly basic pH's (7.8–8.4) characteristic of the present and perhaps also of the primeval ocean [4–6]. Another unique feature of the 5′-GMP aggregates is their rigidity: whereas other self-associated nucleosides and nucleotides show only time-averaged NMR. signals, large energy barriers ($\Delta G^{\neq} > 15$ kcal · mol^{-1}) prevent fast chemical exchange between sites [7] [8]. Aggregation of 5′-GMP occurs through hydrogen bonding of the guanines into planar centrosymmetric tetramers [9] which proceed to form higher order structures [10]. Cations are involved in the self-assembly; the central cavity circumscribed in the tetramers by the O–C(6) atoms selects cations according to their size: whereas Li^+ and Cs^+ are either too small or too large, Na^+, K^+ and Rb^+ manifest significant interaction, the maximum effect being that of K^+ [8] [11] [12]. Given the similarity of the ionic radii for K^+ (1.33 Å) and NH_4^+ (1.45 Å), together with the likelihood of an ammonia-containing primitive atmosphere [2] [3] [13] [14], we have investigated the influence of ammonium and

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of amino acids on the self-ordering of 5'-GMP. We report here facilitation of 5'-GMP aggregation by NH_4^+, with the novel feature of synergism between K^+ and NH_4^+ action. Our measurements also show that interaction of amino acids with the aggregates differs from that of NH_4^+, and discriminates between the two archaic [4] [15] [16] amino acids Gly and Ala.

2. Materials and Methods. – To attempt to mimic prebiotic conditions in or near the primeval ocean, we examine multi-component systems consisting of 5'-GMP^{--}, 2 Na^+ (0.1 M, a concentration at which no self-assembly occurs in the absence of K^+ ions [12]), KCl and NH_4Cl (or KCl, 0.633 M and variable amounts of the neutral amino acids), in a mixture of D_2O/H_2O 1:4 in order to provide a lock signal for the *Bruker* HFX-90 and WP-80 spectrometers operating in the ^{23}Na configuration at 23.81 and 21.16 MHz respectively.

Solutions of 5'-GMP^{--}, 2 Na^+ ½ H_2O *(Aldrich)* in H_2O/D_2O 0.1 M (*Merck*, Uvasol), are co-dissolved with variable concentrations of KCl (*Merck* Suprapure): the measured pH is 7.8 ± 0.2. KCl and NH_4Cl *(U.C.B. p.a.)* are dried under vacuum, at 60–70°, for 4–6 hours. Glycine *(U.C.B. p.a.)* and L-alanine *(Serva, p.a.)* are used without further purification.

^{23}Na-NMR. spectra are recorded 12 hours after sample preparation at 23.81 or 21.16 MHz. The usual spectral windows are 6 or 12 kHz. 2,000 to 10,000 scans are required, depending upon the sample. The *S/N* ratio for all of the absorption curves analyzed is better than 70:1. Temperature in the probe of the *Bruker* HFX-90 and

Table. *Fast* (prime) *and slow* (double prime) *relaxation components, correlation times, and mole fractions of bound Na^+ ions*, at 299 ± 1 K with [5'-GMP] = 0.107 M and [KCl] = 0.633 M

Added substance (concentration, M)	$\nu_{1/2}$ (± 5 Hz)	$\nu_{1/8}$ (± 15 Hz)	$1/\pi T'_{2B}$ (Hz)[a]	$1/\pi T''_{2B}$ (Hz)[a]	τ_c(ns)[b]	$p_B \cdot \chi^2$ (10^{-2} MHz^2)[b]	p_B[b][c]
	188	565	309	137	7.5	4.36	0.17[d]
NH_4^+ (0.101)	178	590	369	127	9.7	4.43	0.18
NH_4^+ (0.151)	165	583	394	119	11	4.35	0.17
NH_4^+ (0.220)	128	575	548	100	16	4.64	0.19
NH_4^+ (0.323)[e]	100	480	541	81	19	3.99	0.16
Gly (0.089)	152	455	246	111	7.5	3.44	0.14
Gly (0.129)	161	498	282	116	8.2	3.75	0.15
Gly (0.196)	143	440	249	103	8.3	3.27	0.13
Gly (0.266)	133	408	230	96	8.2	3.02	0.12
Gly (0.463)	108	345	205	77.5	9.2	2.46	0.10
Ala (0.085)	175	523	282	128	7.4	4.01	0.16
Ala (0.146)	170	535	311	122	8.6	4.02	0.16
Ala (0.216)	166	528	311	119	8.8	3.96	0.16

a) Error limit ± 10%.

b) Error limit ± 15%.

c) For $\chi = 0.50$ MHz, appropriate for Na^+ binding to the phosphate group of a nucleotide [24], assuming that the Na^+ ion is bound to a single site of the aggregate, and, consequently, is related to a single value of the quadrupolar coupling constant.

d) This value is quasi invariant upon the existence of 5'-GMP as the monomer or in the aggregated state.

e) At this high NH_4^+ concentration, a precipitate appears.

Bruker WP-80 spectrometers is measured with a thermocouple and a micro-voltmeter. Duration of the 90° pulses is 9 μs (HFX-90) or 19 μs (WP-80).

The method of study is lineshape analysis of the ^{23}Na-NMR. [17] for the counter-ion of the 5′-GMP dianion. The sodium cation exchanges fast between the free state in the solution, and the phosphate-bound ion pair. ^{23}Na with a spin quantum number of $\frac{3}{2}$ has a quadrupole moment whose interaction with the local fluctuating electric field gradients causes relaxation. Slow modulation occurs if a fraction of the total sodium is attached to a slowly reorienting entity having a correlation time $>$ *ca.* 10^{-9} s, and a non-lorentzian absorption is observed. It corresponds to coexistence, under assumptions which are valid here, of two relaxation times [18] [19]. Correspondingly, the experimental absorption is the superposition of two lorentzian components, corresponding to a fast relaxation and a slow relaxation, with relative intensities of 3:2 [18] [19]. Separation of these two components can be effected [20] and yields the values of τ_c, the reorientational correlation time [20] and of the product $p_B \cdot \chi^2$, where p_B is the mole fraction and χ is the quadrupolar coupling constant ($\chi = e^2qQ/h$), for Na^+ in the slowly reorienting bound state *(Table)*.

^{23}Na-NMR. [17] is uniquely well adapted for studying the 5′-GMP self-assembly: (a) the intrinsic receptivity of this nucleus, 525 times that of the widely used ^{13}C, is outstanding, and allows for the study of the interaction of other cations as well, from competition experiments; (b) the range of chemical shifts is large; for instance, it provides direct evidence for inclusion of Na^+ in the central square cavity of each tetramer where the cation ıtacts the O–C(6) atoms [11]; (c) because of the spin quantum number of $\frac{3}{2}$, observation of a non-lorentzian absorption uniquely characterizes the appearance of aggregates: for an 0.1 M concentration of 5′-GMP, as used here, we can detect reliably as little as 5–20% of aggregates relative to the total monomer concentration; (d) from the magnitude of the quadrupolar coupling constant, and that of the residence time τ_B for Na^+ in the bound state on the aggregates, it is possible to ascertain reliably whether binding occurs with or without interposition of a water solvent molecule in the case described herein when K^+ occupies the core positions within the tetramers, and Na^+ is thus constrained to ion pair with the peripheral phosphate groups.

3. Results. – This ^{23}Na probe into the microdynamics of the 5′-GMP aggregates is revealing. Addition of NH_4^+, while leaving virtually unchanged the proportion of phosphate-bound Na^+ ions on the aggregates, markedly increases the apparent correlation time for the aggregates *(Fig. 1)*.

Remarkably, the interaction with glycine is of an entirely different type. Glycine appears not to influence substantially the size of the aggregates, as measured by their τ_c which remains equal to 8.5 ± 1 ns. The main effect of glycine is a decrease of the $p_B \cdot \chi^2$ and therefore of the p_B terms *(Fig. 2)*.

Alanine is another very simple α-amino acid, which like glycine forms readily under prebiotic conditions [21] [22]. They differ however in their interaction with self-assembled 5′-GMP: while Gly binds strongly *(Fig. 2)*, with $K \sim 10$ M, L-Ala does not appear to interact appreciably, and gives values for τ_c and $p_B \cdot \chi^2$ equal to those for the reference sample within the combined experimental uncertainties.

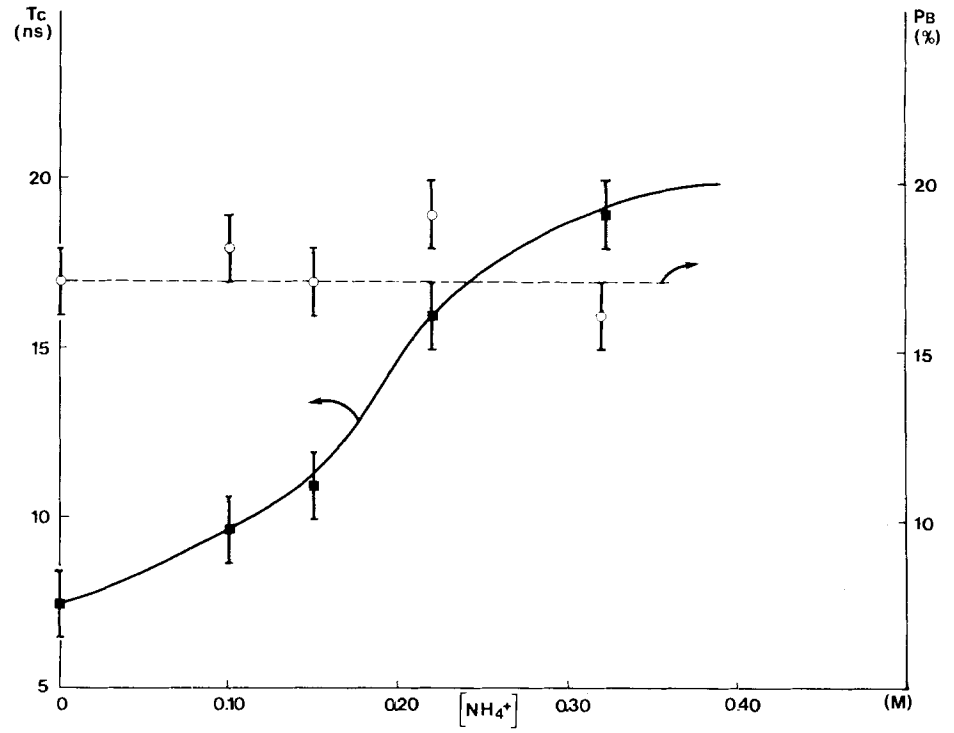

Fig. 1. *Variation of* τ_c (solid line) *and invariance of* p_B (dashed line) *as a function of* NH_4^+ *ion concentration, for* Na^+ *ions bound to self-aggregates of 5'-GMP* (monomer concentration = 0.107M) *formed in the presence of KCl* (0.633M), *at 299 K.* The mole fraction of Na^+ bound to the aggregates, p_B, is calculated with a quadrupolar coupling constant χ for phosphate-bound Na^+ of 0.5 MHz [17].

We have also investigated the joint influence on the self-ordering process of K^+ and NH_4^+ ions *(Fig. 3).*

Five distinct regions enclose the experimental points: in region 1, at low K^+ and NH_4^+ concentrations, no aggregation occurs; the borderline is drawn from the critical concentration at which sharp breaks occur in plots of the ^{23}Na linewidth against reciprocal K^+ or NH_4^+ concentration [11]. In region 5, conditions are intermediate between the extreme narrowing limit applicable to region 1 and the slow modulation regime characteristic of regions 2, 3 and 4; it is impossible to ascertain the extent of aggregation, if any, in this region. Region 2, along the K^+ ordinate, contains experimental points for which $\tau_c = 9.4 \pm 1.4$ ns, suggestive of existence of a single species (A) of aggregates, tentatively identified with dimers of tetramers [20]. Region 3 corresponds to experimental points characterized also by a single value of the correlation time $\tau_c = 21 \pm 2$ ns; this species B could be a K^+ (or NH_4^+) bridged dimer of A, *viz.* a K^+ (or NH_4^+)-containing 5'-GMP hexadecamer. In region 4, intermediate values of the correlation time τ_c point to coexistence of species A and B.

Obviously, K^+ and NH_4^+ ions play a different role and do not compete for identical sites. Furthermore, appearance of the highest aggregates at this

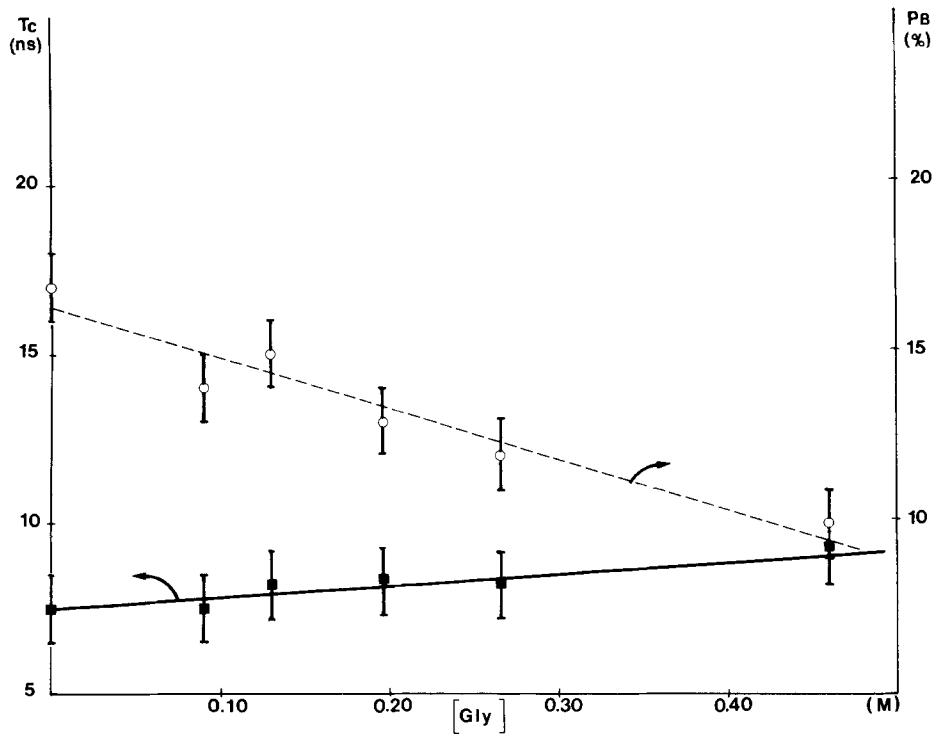

Fig. 2. *Variation of* τ_c (solid line) *and of* p_B (dashed line) *as a function of glycine concentration, for* Na^+ *ions bound to self-aggregates of 5′-GMP.* The other conditions are those of *Figure 1.*

temperature of 304 K (species B, in region 3) demands that both K^+ and NH_4^+ ions are present.

4. Discussion. – The effect of glycine could be due to removal by Gly of Na^+ ions from their phosphate binding sites. Such displacement does not reflect competition of the Na^+ ions between binding to the 5′-GMP phosphates or to the amino acid; in terms of binding constants, the former is approximately ten-fold greater than the latter [23] [24]. Decrease of $p_B \cdot \chi^2$ with increasing Gly concentration *(Fig. 2)* may be diagnostic of Gly binding more strongly than Na^+ to the phosphate groups in the 5′-GMP aggregates, a feature suggestive of chelation of Gly, as the zwitterion, by a phosphate group and the OH groups on a ribose ring. Least one should overemphasize this interaction between glycine and aggregates of its single base codon (G), the selectivity coefficients between nucleotides and L-amino acids on a chromatographic support [25] do not bear out the notion that specific interactions between amino acids and their codons, or anticodons, contributed significantly to emergence of the genetic code [26].

An alternate and we feel more likely explanation of the observations *(Fig. 2)* is for Gly to decrease p_B by lowering the mole fraction of aggregates rather than by chasing Na^+ from its phosphate binding sites. Displacement of K^+ ions from

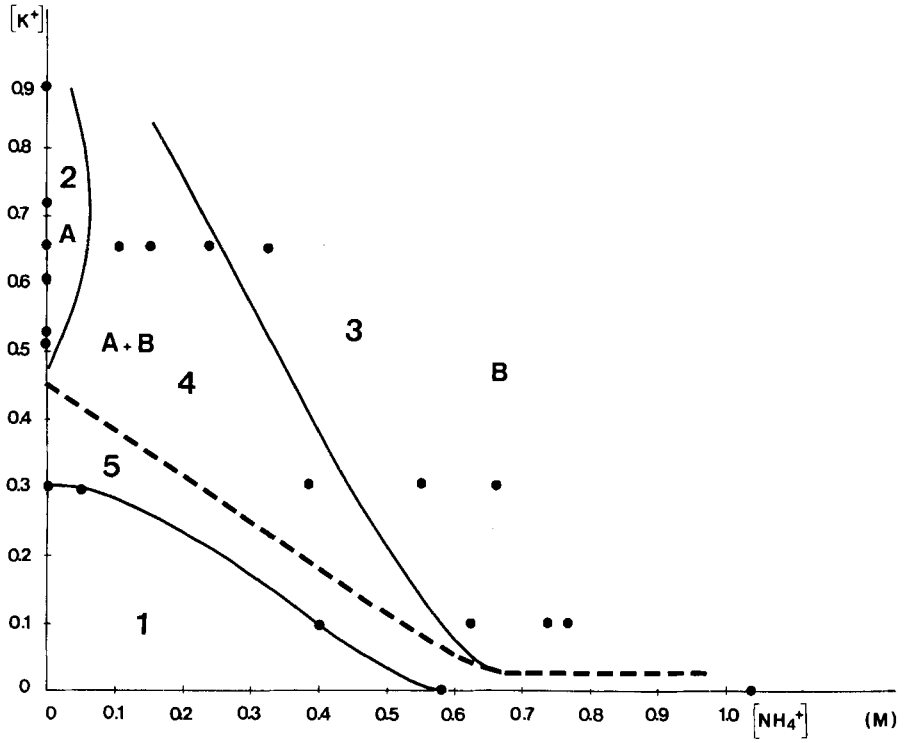

Fig. 3. *Representation of the apparent correlation times τ_c for Na^+ ions bound to the aggregates against K^+ ion concentration* (ordinate) *and NH_4^+ ion concentration* (abscissa), *for 0.10M 5'-GMP at 304 K* (see section 3 for other indications).

their core position in the tetramers [12] by the NH_3^+ polar head of the amino acid would sterically prevent further aggregation of the tetramers.

As for the lack of a measurable effect with Ala, under these conditions, it is consistent with binding either to the phosphate, or to the tetrameric core sites being blocked by the additional methyl group in L-Ala, as compared to Gly.

Besides the K^+/NH_4^+ synergism displayed by the results in *Figure 3*, we wish to draw attention to another effect, which appears to go beyond a mere coincidence and to be real; it is striking that correlation times of *ca.* 9.4 and 21 ns are consistently observed, with the normal exception of the transition range in *Figure 1* and of the intermediate region 4 in *Figure 3*. This suggests that well-defined structures form, whose possible prebiotic function(s) remains to be established.

5. Conclusions. – Pronounced interactions occur between the aggregates of 5'-GMP, formed in the presence of K^+ ions, and either the neutral (zwitterionic) Gly or the charged NH_4^+ ions. No measurable effects are found with L-Ala. With the amino acid glycine, the main effect appears to be partial destruction of the aggregates, perhaps because the amino acid competes with K^+ ions for occupation of the core positions in the tetramers. NH_4^+ ions give rise to an entirely different

phenomenon: they appear to effect the duplication of the $\tau_c \simeq 10$ ns aggregates into $\tau_c \simeq 20$ ns aggregates; we are inclined to identify tentatively the former with dimers of tetramers [20] and the latter therefore as NH_4^+ ion bridged-hexadecamers.

Because of the possibility of such interactions occurring in or near the ocean, under pre-biotic conditions, we believe these findings to be *pregnant* with implications which are now being actively explored.

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REFERENCES

[1] *W. Guschlbauer & J. F. Chantot,* in: 'Synthesis, Structure and Chemistry of Transfer Ribonucleic Acid and their Components', Proceedings of the International Conference held in Dymaczewo near Poznan, 13.–17.9.1976, Poznan, p. 96.
[2] *M. Calvin,* Angew. Chem. Int. Ed. *12,* 121 (1975); *M. Calvin,* 'Chemical Evolution. Molecular Evolution towards the Origin of Living Systems on the Earth and Elsewhere', Clarendon Press, Oxford 1969.
[3] *C. C. Price,* Ed., 'Synthesis of Life', Dowden, Hutchinson and Ross, Strondburg, Pa., 1974.
[4] *T. H. Jukes, R. Holmquist & H. Moise,* Science *189,* 50 (1975).
[5] *F. T. Mackenzie,* in: 'Chemical Oceanography', J.P. Riley & G. Skirrow, Eds., 2nd Ed., Academic Press London (1975), vol. 1, p. 309.
[6] *J. D. Burton,* Chemistry & Ind. *1977,* 550.
[7] *T. J. Pinnavaia, H. T. Miles & E. D. Becker,* J. Amer. chem. Soc. *97,* 7198 (1975).
[8] *T. J. Pinnavaia, C. L. Marshall, C. M. Mettler, C. L. Fisk, H. T. Miles & E. D. Becker,* J. Amer. chem. Soc. *100,* 3625 (1978).
[9] *M. F. Gellert, M. N. Lipsett & D. R. Davies,* Proc. Natl. Acad. Sci., USA *48,* 2013 (1962).
[10] *H. Klump,* Ber. Bunsenges. physik. Chem. *80,* 121 (1976).
[11] *A. Paris & P. Laszlo,* C.r. hebd. Séances Acad. Sci. *286D,* 717 (1978).
[12] *C. Detellier, A. Paris & P. Laszlo,* C.r. hebd. Séances Acad. Sci. *286D,* 781 (1978).
[13] *C. Sagan & G. Mullen,* Science *177,* 52 (1972).
[14] *C. Sagan,* Origins of Life *5,* 497 (1974).
[15] *T. H. Jukes,* Nature *246,* 22 (1973).
[16] *J. T. Wong,* Proc. Natl. Acad. Sci. USA *72,* 1909 (1975).
[17] *P. Laszlo,* Angew. Chem. Int. Ed. *17,* 254 (1978).
[18] *P. S. Hubbard,* J. chem. Physics *53,* 985 (1970).
[19] *T. E. Bull,* J. magn. Res. *8,* 344 (1972).
[20] *A. Delville, C. Detellier & P. Laszlo,* J. magn. Res. *34,* 301 (1979).
[21] *L. E. Orgel,* Israel J. Chemistry *14,* 11 (1975).
[22] *A. Brack & L. E. Orgel,* Nature *256,* 383 (1975).
[23] *R. M. Smith & R. A. Alberty,* J. phys. Chemistry *60,* 180 (1956).
[24] *G. A. Rechnitz & S. B. Zamochnick,* J. Amer. chem. Soc. *86,* 2953 (1964).
[25] *C. Saxinger & C. Ponnamperuma,* J. mol. Evol. *1,* 63 (1971).
[26] *G. W. Hoffmann,* Ann. Rev. phys. Chemistry *26,* 123 (1975).