FORMATION OF A NEW 5'-GUANYLIC ACID HELIX IN NEUTRAL SOLUTION

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Summary

5'-GMP forms in neutral solution a regular, ordered structure, presumably helical. The ordered structure is different from that previously reported at pH 5 and is not associated with gel formation. The transition is more cooperative than that of the pH 5 helix, but has a lower T_m.

Temperature-dependent gel formation by 5'-GMP occurs in moderately acid solution but not at neutral pH(1,2). The nucleotide molecules in the anisotropic gel are hydrogen bonded to each other in a helical array (2). Formation of this ordered structure has been studied by X-ray fiber diffraction (2), ultraviolet spectroscopy (2), infrared spectroscopy (3,4), optical rotatory dispersion (5), and viscometry (6).

We report here infrared spectroscopic evidence of formation by 5'-GMP of a new, regularly ordered structure in neutral or slightly alkaline solution.

The infrared spectrum of unassociated 5'-GMP has four double bond vibrations between 1500 cm^{-1} and 1700 cm^{-1} (Fig. 1; references 3,4,7). The band at 1665 cm^{-1} is assigned to a carbonyl stretching vibration (4) and that at ~1568 cm^{-1} to a normal mode which also involves, to a

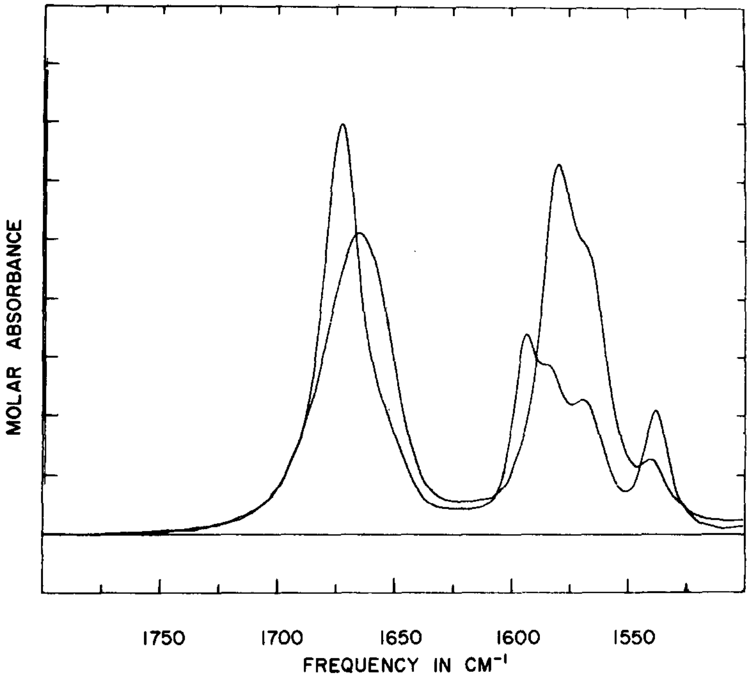

Figure 1. Infrared spectra in D_2O of 0.1 M 5'-GMP at pD8.0. $[Na^+]$, 1.0 M. The spectrum of the ordered form (-4.2°) has νmax (ϵmax) at 1672 cm^{-1} (1400), 1593 cm^{-1} (680), 1585 cm^{-1} (585) 1570 cm^{-1} (460), and 1538 cm^{-1} (420). The unassociated nucleotide (32°) has νmax (ϵmax) at 1665 cm^{-1} (1030), 1579 cm^{-1} (1260), and 1540 cm^{-1} (250). The spectroscopic methods employed have been described previously (3,4,7). The ordinate index marks are 200 units apart.

much smaller extent, some motion of the carbonyl oxygen (4). The intense band at 1580 cm^{-1} is a ring vibration and may have a major contribution from a C=C stretching motion. The band at 1540 cm^{-1} probably has a major contribution from a C_8=N_7 vibration (8).

The infrared spectrum of the low temperature form of 5'-GMP at pD8 shows marked changes from that of the unassociated nucleotide (Fig. 1) and is also quite different from that of the helix formed at pH 5-6(3,4). The carbonyl band becomes sharper ($\Delta\nu_{1/2}$ decreases from 35 cm^{-1} to 20 cm^{-1}), and νmax shifts to 1672 cm^{-1} (Fig. 1). The spectrum of the gel formed at pD5, on the other hand, has νmax = 1680 cm^{-1} (3,4). The ring vibrations between 1550 cm^{-1} and 1600 cm^{-1} undergo a large intensity decrease at low temperature and have maxima at 1593 cm^{-1}, 1585 cm^{-1}, and 1570 cm^{-1}. In the 5'-GMP gel (pD 5) a band also occurs at 1593 cm^{-1}, but no resolved peaks are observed between this frequency and 1550 cm^{-1}. The ring vibration at 1540 cm^{-1} in unassociated 5'-GMP is intensified in the ordered form and shifted slightly to 1538 cm^{-1} (Fig. 1). This band exhibits very similar changes in the gel formed at pD5 (3).

Temperature profiles of infrared absorbance are shown in Fig. 2 for 5'-GMP at pD8 and at pD5.5 (cf.3,4) under comparable conditions of nucleotide and salt concentration. Under these conditions (Fig. 2) the familiar 5'-GMP gel at pD5.5 has T_m = 22°, whereas the form observed at pD8 has T_m = 9°. The transition at pD8 is more cooperative than that at pD 5.5 (breadth ~13° for the former and ~34° for the latter, estimated as distance between intercepts of line of maximum slope with upper and lower plateaus). These results show the formation of a regular, ordered structure, presumably helical, by 5'-GMP in neutral or slightly alkaline solution. Gel formation, which led to initial observation of the ordered structures of 5'-GMP at pH 5, does not occur in neutral solution (1,2). Gelation, however, is not a necessary concomitant of helix formation. Flory (9,10, and references cited there) has shown that an isotropic dilute solution of helices may be in equilibrium with a tactoidal anisotropic solution phase, the properties of which depend on the alignment of the helices with respect to each other. A theoretical and experimental examination of the viscosity of 5'-GMP in acid solution has been made by Peticolas (5). In the neutral or slightly alkaline solutions reported here we are dealing with isotropic solutions of helices, even at relatively high concentrations

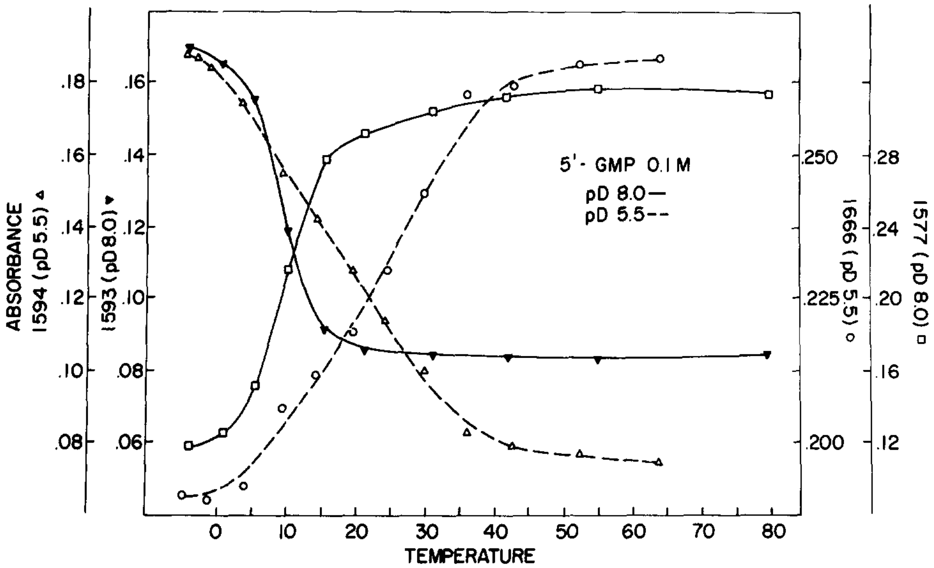

Figure 2. Infrared melting curves of 5'-GMP self structures formed at pD8.0 (solid lines: nucleotide, 0.1 M; phosphate buffer, 0.05 M, pD8.0, Na$^+$, 0.8 M) and at pD5.5 (dashed lines: nucleotide, 0.1 M; cacodylate buffer, 0.1 M, pD5.5; Na$^+$, 1.0 M). Path length for both solutions, 24.8 μ.

of nucleotide and salt. Greater coulombic repulsion between helices (resulting from two rather than one negative charge on each phosphate residue) probably plays a role in causing the properties of 5'-GMP to differ in neutral solution from those observed previously at pH 5.

Flory (9) has pointed out that formation of a tactoidal, anisotropic phase is a necessary consequence of the asymmetry of the particles rather than of attractive forces between them. If the degree of polymerization is very high, a disordered, isotropic solution of helices can exist only at sufficiently high dilutions (9). While we have no evidence on the extent of aggregation of the 5'-GMP helices in neutral solution, it appears likely that they are relatively small. The NMR signal of the H8 proton of 5'-GMP broadens progressively as the ordered structure

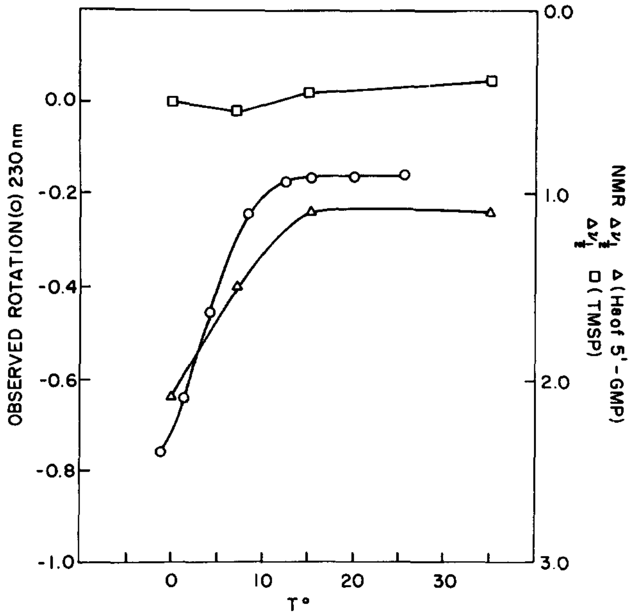

Figure 3. Band width ($\Delta\nu_{1/2}$ in Hz) of the H8 proton of 0.1 M 5'-GMP (Na^+, 0.7 M, pH 7.6) as a function of temperature (Δ). The band width of the internal standard, sodium trimethylsilylpropanesulfonate (TMSP, □) is independent of temperature over the same range. The optical rotation (O) and infrared absorbance (Fig. 2) show temperature dependances approximately parallel to that of the NMR band width.

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is formed (Fig. 3) but remains nevertheless much sharper at 0° than we would expect for a rigid rod of great length.

The infrared spectrum of the ordered form of 5'-GMP (Fig. 1) shows that the guanine residues exist in the keto-amino tautomeric form and that they are neither protonated nor ionized (4,7). Establishing these points of chemical fine structure limits the hydrogen bonding possibilities to those based upon the pairs listed by Donohue (11). Of these the only plausible bonding schemes appear to be the planar tetramer arrangement proposed by Gellert, Lipsett, and Davies (2) and a scheme consisting of a pair of G residues with two-fold rotation axis, involving mutual bonding of the carbonyl and 1H atoms of two guanine residues (Donohue, reference 11, scheme 9). We observe that the carbonyl band of the 5'-GMP ordered structure reported here and that of the 3'-GMP gel at pD5 (3) have the same frequency, 1672 cm^{-1}. We would not expect the difference between isotropic solution and gel to have an important effect on this spectroscopic property of the two helices. Though the spectroscopic similarity is suggestive, the extent to which it reflects a similarity in secondary structure is not clear at present.

We have also observed ordered structure formation with 0.2 M 5'-dGMP (2.0 M Na^+) at pD8. The infrared spectrum of a solution at -7° is qualitatively similar to that in Fig. 1 at -4°, though interaction is incomplete. The self-structure is fully melted by + 7°. The carbonyl band has ν_{max} at 1671 cm^{-1} at -7° but is broader than in Fig. 1.

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