COMPLEXES OF POLYADENYLIC ACID AND 7-METHYL-XANTHINE AND THEIR OPTICAL ROTATORY DISPERSION

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Summary: Polyadenylic acid forms a 2:1 complex with 7-methyl-xanthine at pH 7.0, 0.15 M Na^+, and a 1:1 complex at pH 6.0, 0.15M Na^+. The complexes have been characterized by equilibrium dialysis and by ORD measurements. Both complexes are laevorotatory at wavelengths greater than 286 nm, and the 1:1 complex is remarkably optically active: $[m] = -154{,}000^{\circ}$ at 292 nm. The properties and structures of the complexes are discussed.

Introduction

Complex formation between polyribonucleotides and monomeric purine derivatives (1-8) represents an important class of macromolecule-small molecule interactions which is of interest in the contexts of the structure and function of nucleic acids, and of their prebiotic synthesis and replication. At present, only the monomers 3-methyl-xanthine and formycin B have been reported (5,8) to complex with polyadenylic acid (poly(A)). Monomer complexes of poly(A) may have some biological significance in relation to the widespread occurrence of poly(A) tracts in informational RNA molecules. Although the function of these poly(A) sequences is not yet established, it is likely to be modified if complexing with monomer molecules occurs. This communication reports the formation, under appropriate conditions, of a 1:1 and a 2:1 complex between poly(A) and 7-methyl-xanthine (also known as heteroxanthine), and the optical rotatory dispersion (ORD) of these complexes.

Materials and Methods

7-methyl-xanthine was purchased from Fluka AG. Its elemental analysis was satisfactory and it was used without further purification. Poly(A), obtained from British Drug Houses, was specified as having a molecular weight in excess of 10^5; it was used without further purification. The concentration of poly(A) solutions was determined by hydrolysis to AMP and is given as moles of AMP/litre.

The preparation of the buffers, and the procedures used for equilibrium dialysis and ORD measurements have been described previously (8). Values for mean residue rotation apply to AMP residues in poly(A) and are accurate to ± 5%.

Results and Discussion

The solubility and pK of a particular purine derivative are crucial factors determining its capability for forming soluble complexes with complementary polynucleotides. The binding studies reported here were carried out in either pH 7.0, 0.15 M Na^+ or pH 6.0, 0.15 M Na^+ buffer at $4.0^\circ \pm$ 0.5 degree C. Under these conditions, 7-methyl-xanthine, which has pK = 8.4 at 20° (9), exists almost entirely as the neutral species. Although its solubility at 4° in pH 6 buffer is 8.7×10^{-4}M, slightly supersaturated solutions of 7-methyl-xanthine (up to 2.5×10^{-3}M) are stable for long periods in the absence of seed crystals. It was therefore possible to carry out equilibrium dialysis experiments with concentrations of 7-methyl-xanthine in excess of it solubility limit. The binding isotherms thus obtained (Figure 1) show that under neutral conditions, at pH 7.0, 0.15 M Na^+, poly(A) forms a 2:1 complex with 7-methyl-xanthine. In contrast, at pH 6.0, 0.15 M Na^+, where poly(A) becomes protonated and assumes the double-helical acid form (10) below 15°, a 1:1 complex is formed. The binding isotherms for the formation of both complexes are strongly co-operative. For the 1:1 complex the slope of the isotherm at the mid-point is directly related to the stacking free energy, ω, between adjacent monomer molecules in the complex (5); in this case $\omega \sim -6$ kcal.mol^{-1}.

ORD spectra were obtained using a thermostatted 1 mm pathlength cell.

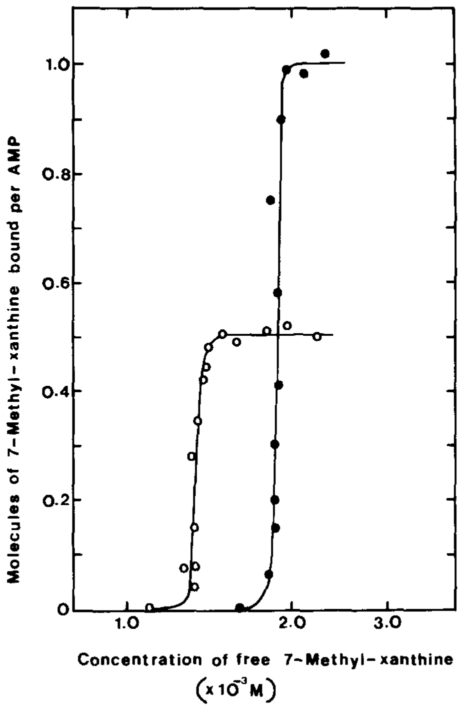

Figure 1. Binding isotherms for formation of complexes between poly(A) and 7-methyl-xanthine at 4°. ○, 2.00×10^{-3}M poly(A), pH 7.0, 0.15 M Na^+; ●, 1.53×10^{-3}M poly(A), pH 6.0, 0.15 M Na^+.

Solutions at dialysis equilibrium at 4°, shown by absorbance measurements to contain either the 2:1 or 1:1 complex, were transferred directly to this cell without an increase in temperature and the spectra shown in Figures 2 and 3 were recorded. At 25°, where the complexes were fully dissociated, the ORD spectra were those expected for poly(A) alone at this temperature. Due to the high absorbance of the solutions, reliable measurements could not be made at wavelengths shorter than approximately 280 nm. Unlike the vast majority of base-paired polynucleotide complexes, which exhibit positive rotation at long wavelengths (2,4,11), both the complexes of poly(A) with 7-methyl-xanthine are laevorotatory at long wavelengths (286 to 400 nm). This behaviour is also shown by complexes of poly(A) with formycin B (8), xanthine, 8-aza-xanthine and 3-methyl-xanthine

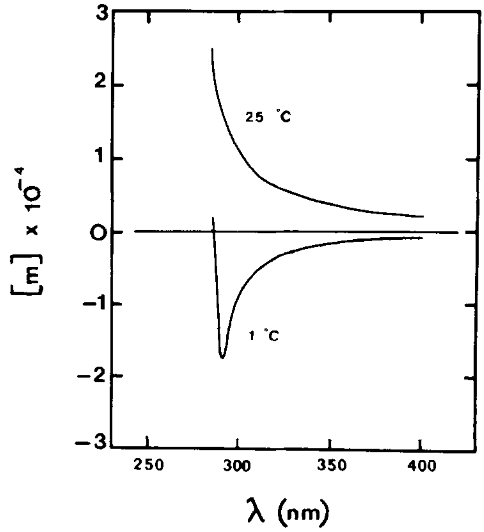

Figure 2. ORD spectrum of 2.00 x 10^{-3}M poly(A) + 3.00 x 10^{-3}M 7-methyl-xanthine, pH 7.0, 0.15M Na^+, at 1° (containing the 2:1 complex) and at 25°.

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(12), and appears to be a general feature of poly(A)-monomer complexes. For the 2:1 complex (Figure 2) there is a trough at 292 nm with mean residue rotation [m] = -17,500°, and crossover occurs at 286 nm. The optical activity of the 1:1 complex (Figure 3) is particularly striking: there is a trough at 292 nm with [m] = -154,000°; crossover occurs at 283 nm. While the ORD of polynucleotide complexes depends upon the overall pattern of base interactions and is not necessarily determined by the sense of the helix (13), the very high rotational strength of the 1:1 complex might be indicative of a left-handed helical structure in this case.

The thermal dissociation of the complexes was investigated by following the optical rotation at 295 nm as a function of temperature. Melting curves for the 2:1 and 1:1 complexes are shown in Figures 4 and 5; in both cases there is evidence for a co-operative helix-coil transition. In Figure 5, the melting curve for the double-helical acid form of poly(A), which is present at low temperatures at pH 6, is shown for comparison. Clearly, the 1:1 complex with 7-methyl-xanthine is formed in preference to

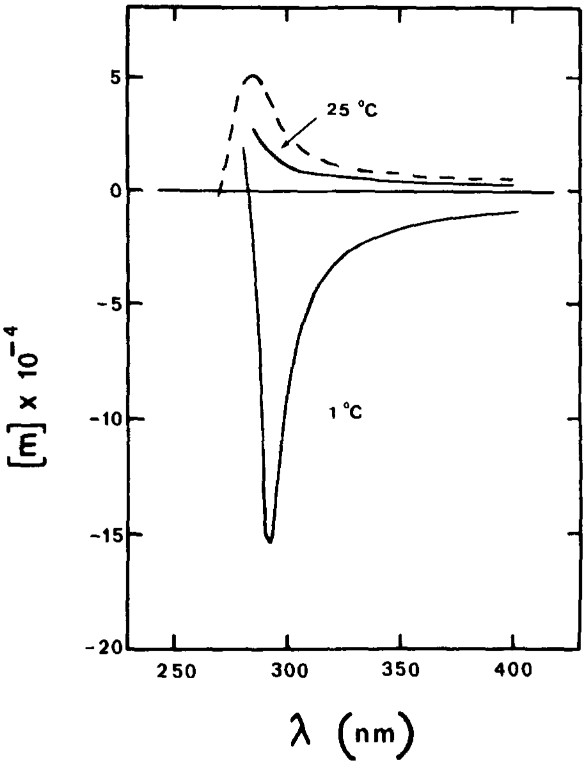

Figure 3. ORD spectra of solutions at pH 6.0, 0.15 M Na^+. - - -, poly(A) at 1°; ———, 1.53 x 10^{-3}M poly(A) + 3.55 x 10^{-3}M 7-methyl-xanthine at 1° (containing the 1:1 complex) and at 25°.

the acid form of poly(A) even though it dissociates at a lower temperature. This is also true of the poly(A)-formycin B complex at pH 6 (8). Annealing experiments proved that formation of the 1:1 complex is a very slow process requiring many hours for completion; the melting curve is therefore not rapidly reversible. In contrast, the formation and dissociation of the 2:1 complex at pH 7 are relatively rapid processes and the melting curves are readily reversible upon heating and cooling. From the dependence of the T_m (the temperature at the mid-point of the optical rotation v. temperature profile) upon the concentration of 7-methyl-xanthine free in solution at this temperature, it is possible to estimate the partial molar enthalpy (ΔH_t) and entropy (ΔS_t) of formation of the complex at the mid-point of

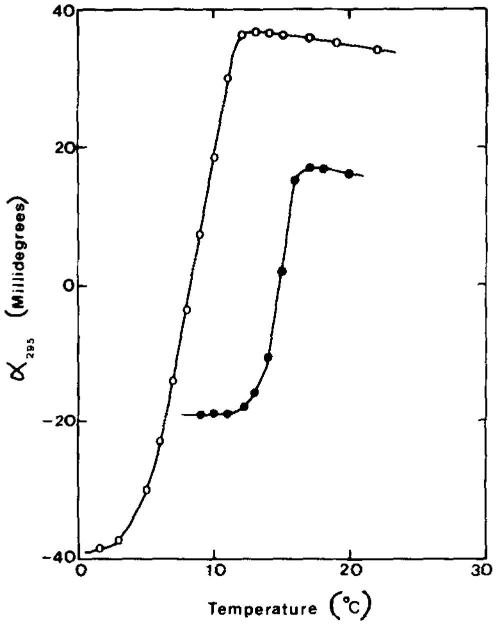

Figure 4. Observed optical rotation at 295 nm, as a function of temperature, for mixtures of poly(A) and 7-methyl-xanthine at pH 7.0, 0.15M Na^+. Cell pathlength is 1 mm. ○, 2.00 x 10^{-3}M poly(A) + 2.50 x 10^{-3}M 7-methyl-xanthine; ●, 1.00 x 10^{-3}M poly(A) + 3.30 x 10^{-3}M 7-methyl-xanthine.

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the helix-coil transition (6). From measurements over a limited temperature range (T_m varying from 4° to 15°), these quantities were estimated as ΔH_t = -12.2 ± 1.0 kcal.mol^{-1}, $\Delta S_t = -37$ cal.deg^{-1}mol^{-1}, for the 2:1 complex at pH 7.0, 0.15 M Na^+. The value of ΔH_t is the same, within experimental error, as that for the 2:1 complex of poly(A) with formycin B (8), and considerably smaller than the values for the 2:1 complexes of poly(U) and poly(C) with complementary monomers (6,7).

Possible hydrogen-bonding schemes for the 2:1 and 1:1 complexes are shown in Figure 6. It is assumed that at pH 6.0, at low temperature, the adenine bases of the poly(A) are protonated at N_1 as in the double-helical acid form (10). Other hydrogen-bonding schemes may be drawn which also satisfy the observed stoichiometries. The scheme suggested for the 2:1 complex, in Figure 6(b), is unusual in that all the hydrogen-bonding sites

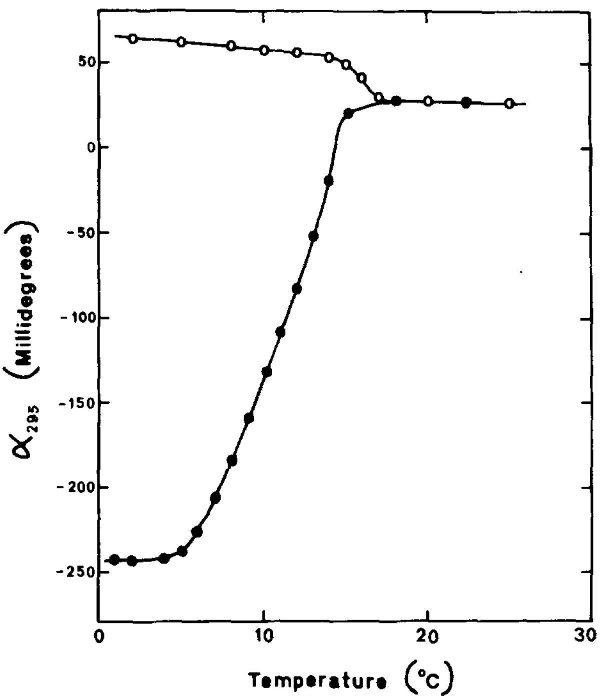

Figure 5. Observed optical rotation at 295 nm, as a function of temperature, for solutions at pH 6.0, 0.15 M Na^+ in 1 mm pathlength cell. ○, 1.53 x 10^{-3}M poly(A); ●, 1.53 x 10^{-3}M poly(A) + 3.59 x 10^{-3}M 7-methyl-xanthine.

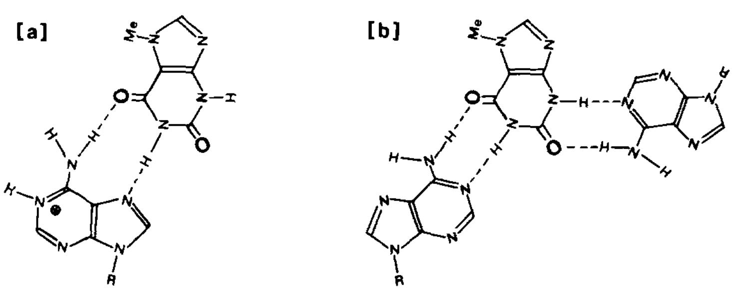

Figure 6. Possible hydrogen-bonding schemes for (a) the 1:1 complex, (b) the 2:1 complex.

of the purine monomer are confined to the six-membered pyrimidine ring. It is interesting that poly(A) also forms a 2:1 complex at pH 7, and a 1:1 complex at pH 6, with 3-methyl-xanthine (5). While the hydrogen-bonding pattern for the 1:1 complexes could involve the same ring positions in both 7-methyl-xanthine and 3-methyl-xanthine, this could not be so for the 2:1 complexes. Certainly, the rates of formation of these 2:1 complexes differ markedly. While formation of the 2:1 complex between poly(A) and 7-methyl-xanthine occurs rapidly, it is extremely slow in the case of 3-methyl-xanthine. The slow rate of formation of the 1:1 and 2:1 complexes of poly(A) with 3-methyl-xanthine upon cooling is responsible for the anomalous melting behaviour of these complexes which has been reported previously (5).

The reasons for the different stoichiometries of the complexes at pH 6 and pH 7 are not clear. Although protonation of poly(A) at the lower pH is evidently an important factor, it should not necessarily preclude hydrogen-bonding to give a 2:1 complex. The interaction of a number of xanthine derivatives with poly(A) is currently being studied. It is hoped that the results of this investigation will account for the different stoichiometries at pH 6 and pH 7, and also for the unusual optical rotatory properties of the poly(A)-monomer complexes.

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