Copolymers of Adenylic Acid with Inosinic and Cytidylic Acids

R. F. Steiner*

From the Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland

(Received for publication, September 6, 1960)

In earlier publications the molecular properties of copolymers of adenylic and uridylic acids have been described (1, 2). In brief, evidence was obtained for the occurrence of ordered regions, probably of a helical character, which persisted for copolymers containing quite high uridylic contents. Although collectively accounting for a large portion of the molecule, these regions of order were apparently too limited in extent to confer a high degree of rigidity. Thus, at ionic strengths of the order of 0.1 (or higher) the over-all shape of the molecular domain approximated that of a random coil. The molecular properties of the AU copolymers thus showed many points of analogy to those of natural ribonucleic acid.

It was furthermore found that the AU copolymers with uridylic contents up to about 50% retained the ability to interact with polyuridylic acid, although the stability of the resultant complexes, as measured by the midpoint of the thermal transition, showed a progressive decrease (1, 2). Fresco and Alberts have likewise observed an interaction between poly AU and polyuridylic acid (4). These authors have suggested that the uridylic groups of poly AU are folded into loops lying outside the helical duplex, thereby permitting 1:1 pairing between the adenes of poly AU and the uracils of polyuridylic acid. The available spectral evidence is consistent with this model, as the observed mixing curves show minima when the ratio of adenine to uracil (in polyuridylic acid) is 1:1 or 1:2, depending upon whether the doubly stranded A + U or the triply stranded A + 2U complex species is formed.

It is of interest to extend these studies to other biosynthetic copolymers. The use of the currently available nucleotide-polymerizing enzymes permits the systematic variation of composition and hence affords one useful approach to the problem of RNA structure.

The present investigation is primarily concerned with copolymers of adenylic and inosinic acids with a few observations upon copolymers of adenylic and cytidylic acids.

The hypoxanthine base differs from adenine in that the C-6 amino group of the latter is replaced by a carbonyl. It thus is structurally analogous to guanine, except for the presence of an external amino group at the C-2 position of the latter base. As the amino group is not involved in several of the hydrogen-bonded base pairs which have been shown to be sterically feasible for guanine, it would be expected that hypoxanthine could replace guanine in these (5). The individual homopolymers differ considerably in their molecular properties. Polyadenylic acid at neutral and alkaline pH appears to consist of randomly coiled single strands containing helical regions of limited extent, rather like RNA and poly AU (6). Polyinosinic acid, on the other hand, is believed to exist as a triply stranded helix at neutral pH and ionic strengths of the order of 1.0 (7). However, this helical structure does not appear to persist at ionic strengths of 0.1 or lower (7), at least by the criterion of ultraviolet hypochromism.

Polyadenylic and polyinosinic acids have furthermore been shown to interact in solution at neutral pH to form, depending upon the mole ratio, a doubly stranded A + I complex or a triply stranded A + 2I species (8). Although stoichiometrically analogous to the interaction of polyadenylic + polyuridylic acids, (9) the stability of the A + I complex species is much lower, as is indicated by its considerably lower “melting point,” or midpoint of its thermal denaturation (3).

Polyctydylie is much less well characterized than either polyadenylic or polyinosinic acid. The available evidence appears to suggest that its configuration at neutral pH is similar to that of polyadenylic acid. No interaction between polyadenylic and polyctydylie acids in solution has been reported, although the theoretical considerations of Donohue indicate that adenylic-ctydylie hydrogen-bonded base pairs are sterically feasible (5).

It is the purpose of the present paper to examine the physical properties of the AI and AC copolymers with particular regard to their apparent helical content and its dependence upon composition. A secondary purpose is to determine the degree of substitution of the polyadenylic acid chain which permits reaction with polyuridylic acid.

It should be recognized that the available criteria for the detection of helical regions in polynucleotides in solution, principally ultraviolet hypochromism and optical rotation (3, 6), are by no means wholly satisfactory. The basis for their use in this case is really semi-empirical, and definite reservations should be retained with regard to conclusions derived solely from these methods. This is especially true in the case of ultraviolet hypochromism, for which an adequate theory is still unavailable. Also, relatively little theoretical effort has been devoted to the optical rotation of helical polynucleotides, as compared with the polypeptides.

**EXPERIMENTAL PROCEDURE**

*Preparation of Copolymers—Copolymers of adenylic and inosinic acids (poly AI) and of adenylic and cytidylic acids (poly AC) were prepared via the action of the polynucleotide phosphorylase of *Micrococcus lysodeikticus* upon mixtures of the corresponding nucleoside diphosphates. The enzyme preparations were ob-

---

* The opinions or assertions contained herein are those of the author and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

1 The abbreviations used are: poly AU, biosynthetic copolymers of adenylic and uridylic acids; poly AI, copolymers of adenylic and inosinic acids; poly AC, copolymers of adenylic and cytidylic acids; CMP; cytidine monophosphate.
tained through the courtesy of Dr. R. F. Deers and were prepared by the method described in detail elsewhere (10). They were supplied in solution and were stored in the frozen state at -5°C until use.

A typical reaction mixture was as follows: 100 mg of nucleoside diphasphate; 15 ml of H2O; 0.5 ml of 0.5 M Tris buffer, pH 9.5; 0.5 ml of 0.01 M dithiothreitol; 0.1 to 1.0 ml of enzyme solution. The amount of enzyme added was adjusted so as to permit completion of the reaction within 3 hours at 37°C. The appropriate amount was determined by a trial run on a reduced scale. The mixtures were incubated at 37°C, and the reaction was followed by the rate of liberation of inorganic phosphate, which was determined by the Fiske-SubbaRow procedure (11).

When the concentration of inorganic phosphate attained a plateau, the reaction was stopped by chilling the mixture to 0°C. The pH was then adjusted to about 7 by the dropwise addition of 1 M acetate pH 4.5, and the solution was made 0.5 M in KCl. Deproteinization was carried out at 3°C by repeated emulsification with CHCl3 until no denatured protein collected at the H2O-CHCl3 interface after centrifugal separation of the two phases (12). Finally the polymer was precipitated by the addition of 2 volumes of ethanol. The precipitate was collected centrifugally, washed successively with 80% ethanol, absolute ethanol, and absolute ether, and then dried in a vacuum.

**Composition of Copolymers**—The nucleotide composition of the AI and AC copolymers was determined by paper chromatographic analysis of 2% solutions of complete alkaline hydrolysates (1 M NaOH for 24 hours at 25°C). Whatman No. 1 paper and a descending system were employed. Two kinds of solvent were used, of the following composition. Solvent A: 600 g of (NH4)2SO4, 20 ml of n-propanol, 1 liter of H2O. Solvent B: 79% saturated (NH4)2SO4, 19% 0.1 M phosphate (pH 6.0), and 2% isopropanol (volume per volume). The spots were located by their quenching of the ultraviolet-excited fluorescence of the filter paper. They were then cut out and eluted with 0.01 M HCl. The absorbancies of the eluates at 260 mp were measured. When corrected for the differing molar absorbancies of the nucleotides, the ratio of absorbancies yielded the mole ratio (Table I).

**Physical Measurements**—Light scattering measurements were made with a Phoenix light scattering photometer. Measurements over a range of angles were made with a cylindrical cell, with planar entrance and exit windows, furnished by the same company. The general technique of light scattering and the details of performing and interpreting measurements have been amply described elsewhere (13). Before measurement, each solution was clarified by repeated centrifugation at 20,000 x g centrifugal force in a Serva centrifuge until constant scattering properties were attained.

Viscosities were measured with Ostwald viscosimeters having efflux times of the order of 220 seconds. Sedimentation coefficients were measured with a Spinco analytical ultracentrifuge, with schlieren optics.

Measurements of ultraviolet absorbancy were made with a Beckman DU spectrophotometer, equipped with a thermostatted cell holder through which water from a constant temperature bath could be circulated. In this manner, temperature could be controlled within ±0.3°C from 7°C to 70°C.

Optical rotation was measured with a polarimeter attachment of the Keston type which was furnished by the Standard Polarimeter Company. It was used in conjunction with a Beckman spectrophotometer.

Concentration was determined from the absorbancy at 280 mp of a suitably diluted alkaline hydrolysate (1 M NaOH, 24 hours at 25°C).

**Titrations Curves**—Hydrogen ion titration data were obtained with a thermostatted, magnetically stirred titration cell, equipped with a nitrogen bubbler. Standard acid (0.1 M HCl) or base (0.1 M NaOH) was introduced with a calibrated microsyringe.

Solvent, 7 ml, was introduced into the cell, titrated to pH 3.8, and flushed with nitrogen for 15 minutes. The solvent was then back-titrated to pH 7, 3 to 5 mg of solid polymer were added, and the titration cycle was repeated. The corresponding blank value was subtracted for each pH.

**RESULTS**

**General Remarks**—A fair degree of success was obtained in preparing AI copolymers of reasonably high degrees of polymerization. The sedimentation coefficients of the preparations examined varied from 5 to 12 S, corresponding to molecular weights in the range 104 to 106 (Table III). In what follows, physical data upon these preparations will be combined and discussed in a unified manner, despite the differences in molecular weight. The justification for this procedure is admittedly incomplete. However, measurements with polyadenylic acid preparations ranging in molecular weight from 1.1 x 106 to 2.0 x 106 have indicated little if any variation in specific rotation, hypochromism, or thermal profile with molecular weight in this molecular weight range, the extreme deviation in the first two of these parameters being only 3% and 4%, respectively.2

However, the AC copolymers prepared were of relatively low molecular weight, with sedimentation coefficients of 3.4 S or less. The reason for the smaller size of these preparations remains obscure. It is possible that this arises from their greater susceptibility to the action of a nuclease contaminant in the enzyme preparation. Clearly molecular weight cannot be ignored as a variable in this case. However, as will be seen later, their lower order of molecular weight does not entirely vitiate the comparison with poly AI.

2 Steiner, R. F., unpublished observations.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Mole fraction:inosinic (or cytidylic) (±5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AI II</td>
<td>0.59</td>
</tr>
<tr>
<td>AI III</td>
<td>0.17</td>
</tr>
<tr>
<td>AI IV</td>
<td>0.51</td>
</tr>
<tr>
<td>AI VI</td>
<td>0.46</td>
</tr>
<tr>
<td>AI VII</td>
<td>0.70</td>
</tr>
<tr>
<td>AI VIII</td>
<td>0.46</td>
</tr>
<tr>
<td>AI IX</td>
<td>0.54</td>
</tr>
<tr>
<td>AI X</td>
<td>0.23</td>
</tr>
<tr>
<td>AI XI</td>
<td>0.27</td>
</tr>
<tr>
<td>AI XII</td>
<td>0.56</td>
</tr>
<tr>
<td>AI XIII</td>
<td>0.39</td>
</tr>
<tr>
<td>AI XIV</td>
<td>0.22</td>
</tr>
<tr>
<td>AI XV</td>
<td>0.48</td>
</tr>
<tr>
<td>AC II</td>
<td>0.39</td>
</tr>
<tr>
<td>AC III</td>
<td>0.40</td>
</tr>
<tr>
<td>AC IV</td>
<td>0.22</td>
</tr>
</tbody>
</table>


### Table II

Comparison of exhaustive alkaline and ribonuclease digests of poly AC IV

<table>
<thead>
<tr>
<th></th>
<th>Alkaline digest</th>
<th>RNase digest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio of absorbancy of eluted CMP spot to absorbancy of remainder</td>
<td>0.118 ± 0.005</td>
<td>0.041 ± 0.01</td>
</tr>
<tr>
<td>Ratio corrected for different absorbancies of AMP and CMP and for hypochromism of AC oligonucleotides</td>
<td>0.28 ± 0.01</td>
<td>0.067 ± 0.015</td>
</tr>
<tr>
<td>Fraction of CMP occurring as free CMP</td>
<td>1.0</td>
<td>0.28 ± 0.04</td>
</tr>
<tr>
<td>Fraction predicted for a random sequence of nucleotides</td>
<td>0.22 ± 0.01</td>
<td></td>
</tr>
</tbody>
</table>

* Composed of 0.1 M NaOH; 1 hour, 100°.
† Composed of 1 mg. per ml of RNase, 0.1 M KCl, 0.01 M Tris pH 8.1; 48 hours at 37°.
‡ Average of four determinations.

Heppel et al. early obtained evidence that the AU copolymers produced by the action of the phosphorylase from Azotobacter vinelandii did not deviate markedly from randomness of nucleotide sequence (15). A similar conclusion, based on considerably less complete data, was reached in the case of poly AU prepared with the enzyme from M. lysodeikticus (2). However, Simha and Zimmerman have recently shown by a more sophisticated analysis that the nucleotide sequence of poly AU is not truly random, sequences of the alternating A and U type being definitely favored (10). It is doubtful whether the analytical procedures described in reference (2) and subsequently in the present paper are precise enough to detect this minor degree of deviation from true randomness. In all probability, the most which can be claimed is that gross deviations from randomness are absent and that, in particular, extended sequences of like nucleotides do not occur. This is, however, sufficient for the purposes of the present paper.

Evidence that the above holds in the case of the AC copolymers prepared by the M. lysodeikticus enzyme was sought via a paper chromatographic analysis of the products of exhaustive alkaline and ribonuclease digestion.

In the former case, the hydrolysis goes to completion yielding a mixture of the 2'- and 3'-nucleotide monophosphates. However, the specificity of ribonuclease is such that phosphodiester linkages of the Crp and Cpa types are attacked, but not linkages of the ApC type. Thus, the products of its exhaustive action upon an AC copolymer should consist of CMP and a mixture of oligonucleotides terminating in 3'-CMP of the type ApCp, ApApCp, ApApApCp, etc. This every CMP unit preceded by an AMP unit in the copolymer will occur as a terminal group of oligonucleotide in the ribonuclease digest. Only the CMP residues preceded by other CMP units will occur as free CMP in this digest. For a random distribution the fraction of CMP units preceded by other CMP units will be equal to the mole fraction of CMP in the original copolymer.

Thus if \( y \) = fraction of total nucleotide occurring as free 3'-CMP in exhaustive ribonuclease digest and \( x \) = mole fraction of CMP in the AC copolymer, then \( y/x \) = fraction of CMP units preceded by CMP units in the original copolymer. If the sequence is random, then \( y/x = x \).

Table II compares the paper chromatographic analyses of complete alkaline and ribonuclease digests of poly AC preparation IV. Relative concentrations of the material corresponding to the various spots were obtained from the absorbancies at 260 ma of eluates in 0.01 M HCl, as described earlier.

As Table II shows, the fraction of CMP units preceded by (linked in the 5' position to) other CMP units is in fair accord with what would be expected if the nucleotide sequence were truly random.

Unfortunately, there is no known enzyme which displays any analogous selectivity with regard to adenylic and inosinic residues. Hence, it is not at present possible to demonstrate the randomness of sequence of the AI copolymers directly. However, since the other copolymers examined do appear to be essentially random, it is probably a good working hypothesis to assume randomness of sequence in this case as well. Certainly the physical properties of the AI copolymers are completely at variance with those expected if protracted sequences of adenylic and inosinic residues occurred.

### Molecular Properties

Table III summarizes the molecular kinetic and light scattering properties of the various AI and AC copolymers studied. At an ionic strength of 0.1, none of the copolymers showed any streaming birefringence, despite quite high molecular weights obtained in many cases. This virtually eliminates the possibility of a rodlike configuration under these conditions, and hence of a highly helical conformation.

The available light scattering data upon the AI copolymers likewise indicate that, at an ionic strength of 0.1, the end to end separation is very much too small to be consistent with a completely extended rod-like shape. The ratio of end to end separation to contour length is well below the figure of 0.3 which roughly marks the upper limit of extension of the Gaussian configuration, and the intrinsic viscosity of an AI copolymer containing 46% inosinic residues is profoundly dependent on ionic strength, as Table III shows. The intrinsic viscosity increases with decreasing ionic strength, indicating that these copolymers undergo the characteristic polyelectrolyte inflation at low ionic strengths.

On the whole, the available data are consistent with, and suggest the presumption that the over all shape of the AI copolymers at ionic strengths of the order of 0.1 is that of Gaussian coils. The coils are flexible and expand under electrostatic stress at low ionic strengths. Clearly they cannot contain helical regions persisting over a major fraction of the contour length.

### Ultraviolet Spectra

Fig. 1 summarizes the effect of progressive increase in the fraction of inosinic residues upon the ultraviolet hypochromism of the AI copolymers and upon its thermal dependence. In general, these effects present some degree of contrast to those observed in the case of the AU copolymers described in an earlier publication (1).

Ultraviolet hypochromism at pH 6.5 decreases continuously with increasing inosinic substitution. This progressive drop in hypochromism is paralleled by a corresponding decrease in the thermal dependence of the absorbancy (Fig. 1). However, neither the hypochromism nor the thermal variation disappears, even for fractional inosinic contents as high as 70%. The former, in particular, remains quite significant.
March 1961

R. F. Steiner

TABLE III

Molecular properties of copolymers

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Solvent</th>
<th>pH</th>
<th>$s_{20,w}$</th>
<th>Mol. wt.</th>
<th>$R_f$ (A)</th>
<th>$R_f$ L</th>
<th>$\beta$</th>
<th>[a] int.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AI II</td>
<td>0.1 M KCl, 0.01 M NaOAc¢</td>
<td>6.5</td>
<td>9.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AI III</td>
<td>0.1 M KCl, 0.01 M NaOAc¢</td>
<td>6.5</td>
<td>5.0</td>
<td>2.0</td>
<td>&lt;200</td>
<td>&lt;0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AI IV</td>
<td>0.1 M KCl, 0.01 M NaOAc¢</td>
<td>6.5</td>
<td>9.1</td>
<td>4.2</td>
<td>300</td>
<td>.065</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AI VI</td>
<td>0.1 M KCl, 0.01 M NaOAc¢</td>
<td>6.5</td>
<td>11.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AI V1</td>
<td>0.0001 M NaOAc, 0.1 M KCl</td>
<td>6.5</td>
<td>5.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AI VII</td>
<td>0.1 M KCl, 0.01 M NaOAc¢</td>
<td>6.5</td>
<td>5.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AI VIII</td>
<td>0.1 M KCl, 0.01 M NaOAc¢</td>
<td>6.5</td>
<td>6.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AI IX</td>
<td>0.1 M KCl, 0.01 M NaOAc¢</td>
<td>6.5</td>
<td>6.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AI X</td>
<td>0.1 M KCl, 0.01 M NaOAc¢</td>
<td>6.5</td>
<td>6.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AI XI</td>
<td>0.1 M KCl, 0.01 M NaOAc¢</td>
<td>6.5</td>
<td>6.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AI XII</td>
<td>0.1 M KCl, 0.01 M NaOAc¢</td>
<td>6.5</td>
<td>7.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AI XIII</td>
<td>0.1 M KCl, 0.01 M NaOAc¢</td>
<td>6.5</td>
<td>7.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AI XIV</td>
<td>0.1 M KCl, 0.01 M NaOAc¢</td>
<td>6.5</td>
<td>8.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AI XV</td>
<td>0.1 M KCl, 0.01 M NaOAc¢</td>
<td>6.5</td>
<td>8.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC II</td>
<td>0.1 M KCl, 0.01 M NaOAc¢</td>
<td>6.5</td>
<td>2.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC III</td>
<td>0.1 M KCl, 0.01 M NaOAc¢</td>
<td>6.5</td>
<td>2.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC IV</td>
<td>0.1 M KCl, 0.01 M NaOAc¢</td>
<td>6.5</td>
<td>3.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Weight average molecular weight, as determined by light scattering.
† Root-mean-square end to end separation, computed upon the assumption of a randomly coiled configuration.
‡ Ratio of end to end separation to contour length (computed assuming a nucleotide separation of 3.4 Å).
§ Sodium acetate.

The data of Fig. 1 display some scatter. This may well be due to the fact that data from preparations differing widely in degree of polymerization are combined. However, the over-all trend is unmistakable.

The behavior of the AC copolymers appear to be somewhat different, as Fig. 1 shows. There appears to be less decrease in either hypochromism or thermal dependence with increasing cytidylic content. However, the relatively low molecular weights of these preparations render a direct comparison with the AI copolymers hazardous.

The pH dependence of absorbancy in the acid range likewise decreases continuously with increasing inosinic content (Figs. 2 and 3). In particular, the discontinuity in the vicinity of pH 6, which is characteristic of polyadenylic acid itself (17-19) is lost at relatively low degrees of substitution. Copolymers with 50% inosinic groups show little or no pH dependence.

Hydrogen Ion Titrations Curves—The binding of protons by the AI copolymers at pH values acid to 7 is entirely a consequence of the presence of adenine, as the hypoxanthine base has no pK in this region and the secondary phosphates are too few in number to account for an appreciable fraction of the binding sites. The structural transition which polyadenylic acid itself undergoes in the vicinity of pH 6 is reflected by a pronounced abnormality in its hydrogen ion titration curve in this region. There is an abrupt and almost vertical rise at a critical pH, which depends upon the ionic strength and temperature, with a subsequent more gradual increase after about 50% of the adenines have bound protons (17, 18). The region of the titration discontinuity coincides with the transition to the ultraviolet spectrum characteristic of the acid form (or forms).

Fig. 2 illustrates the effect of a progressive replacement of adenylic by inosinic residues. The midpoint of the curve, corresponding to the effective pK of the adenine group, is displaced progressively to lower pH values. Furthermore, the sharpness of the titration curve is lost, and the binding becomes much more gradual. For AI copolymers with inosinic contents of 50% or more, the midpoint of the titration curve is displaced to below pH 5. The titration curves in 0.1 M KCl were completely reversible and showed no time dependence.

These results are in harmony with the ultraviolet spectral data discussed earlier and are reminiscent of the behavior of the AU copolymers which were the subject of an earlier publication (1).

Thus the "dilution" of the polyadenylic acid A chain by sufficient inosinic residues appears to eliminate the cooperative features of its titration curve, and by implication, the structural transition responsible for these features.

Optical Rotation—Polyadenylic acid of reasonably high molecular weight (>10²) has been shown to have a specific rotation which is positive and fairly large, lying about midway between the values for the unorganized polynucleotide polyuridylic acid and the almost wholly helical complex of polyuridylic and polyadenylic acids (6). The thermal profile of the specific rotation parallels that of the ultraviolet absorbancy. The positive rotation of polyadenylic acid has been attributed to the presence of numerous short helical regions, analogous to those postulated for the AU copolymers and for natural RNA, and indeed provides the principal evidence for such regions (6).

The magnitude of the dextrorotation of the AI copolymers declines rapidly with increasing inosinic content (Fig. 3), and becomes small for copolymers with over 50% inosinic residues. This result is in definite contrast to the behavior of the AU copolymers (1). It is of interest that the specific rotation appears...
The stoichiometry of the process is consistent with the mechanism proposed by Fresco and Alberts (4), provided that the triply stranded (A + 2U) complex species is formed at high U:A mole ratios. Some reservations as to the correctness of this model must, however, be retained, pending its confirmation by some other means, in view of the lack at present of an adequate theory of the hypochromatic effect.

The thermal profiles of the 1:1 mixtures of poly AI and poly U are definitely dependent upon the composition of the former (Fig. 5). With increasing inosinic content, the midpoint of the thermal transition falls dramatically. The transition also becomes more gradual. This result is again in harmony with that obtained in the case of AU (1, 2, 4). The thermal data of Fig. 5 are uncorrected for the variation of the absorbancy of the AI copolymers themselves with temperature. Inasmuch as any correction would have to be based upon the doubtful assumption that the splitting off of each AI strand was "all or nothing" (since a retention of the linkage to polyuridylic acid would certainly interfere with the reformation of any internal hydrogen bonding of the AI copolymer), it was thought better to omit the correction.

**Discussion**

The over-all molecular properties of the AI and AC copolymers appear to have much in common with other members of the numerous class of incompletely organized polynucleotides, in-
cluding the alkaline form of polyadenylic acid, poly AU, polyuridylic acid, and RNA. Thus, the shape of the molecular domain at ionic strengths of the order of 0.1 or higher is best described as that of a Gaussian coil. Again in analogy to the examples cited above, the special extension of the molecular domain is ionic strength-dependent and displays the familiar inflation at low ionic strengths characteristic of unorganized polyelectrolytes. Thus these copolymers appear to retain a definite molecular flexibility.

Clearly the helical content of the AI and AC copolymers must be of a fractional character and insufficient in extent to confer any appreciable rigidity upon the molecule. The most plausible form for this helical content is that of numerous short helical regions separated by amorphous regions. This picture of the molecule is not necessarily in conflict with an over-all highly coiled shape. As in the case of the AU copolymers, the question of whether the helical regions involve the interaction of two or more chains or the intramolecular folding of a single chain to form hairpin-like helical zones must remain open for the present.

Quantitatively, the estimate of helical content must be based upon the rather crude and empirical yardsticks of optical rotation and ultraviolet hypochromism. Both appear to indicate that the progressive replacement of adenylic by inosinic residues results in a decrease in helical content. The two criteria are not completely in harmony, as the optical rotation results indicate a somewhat more drastic change with inosinic substitution than do the spectral data. It is, of course, possible that interactions

![Figure 1](http://www.jbc.org/)

**Fig. 1.** *Upper:* Fall in relative absorbancy at 260 mμ for mixtures of AI copolymers and polyuridylic acid such that the adenylic concentration is equal to the uridylic concentration (0.1 M KCl, 0.01 M NaOAc, 20°). *Lower:* Mixing curve with polyuridylic acid of poly AI XIV (0.22 inosinic) (0.5 M KCl, 0.01 M NaOAc, pH 6.5). The ordinate is the absorbancy at 260 mμ relative to that expected for a non-interacting mixture of the two components.

![Figure 3](http://www.jbc.org/)

**Fig. 3.** *Upper:* Specific rotation at 589 mμ as a function of inosinic (or cytidylic) content (0.1 M KCl, 0.01 M NaOAc, pH 6.5, 26°). *poly AI; poly AC. *Lower:* The pH dependence of the absorbancy at 260 mμ in the acid range as a function of inosinic content for the AI copolymers (0.1 M KCl, 26°).

![Figure 4](http://www.jbc.org/)

**Fig. 4.** *Upper:* Variation of the midpoint (uncorrected) of the thermal transition of equimolar mixtures of polyuridylic acid (tₚ₂₀ = 5.1 S) with AI copolymers (0.1 M KCl, 0.01 M NaOAc, pH 6.5) with the fraction of inosinic residues in the copolymer. *Lower:* Thermal variation of relative absorbancy at 260 mμ for equimolar mixtures of poly AI X (0.22 inosinic) and polyadenylic with polyuridylic acids (0.1 M KCl, 0.01 M NaOAc, pH 6.5).
of a nonhelical type may contribute to the ultraviolet hypochromism, although no other known examples are available.

The implication of the above is that adenine-hypoxanthine and hypoxanthine-hypoxanthine pairings are relatively ineffective in stabilizing very short helical regions of the type discussed earlier. This is consistent with the relatively weak character of these interactions, as revealed by thermal data upon the A + I and I + I complexes (3).

The AC copolymers appear to show considerably less decline in helical content with increasing cytidylic content. Thus, although the data are less complete, they seem to resemble the AU copolymers more closely than does poly AI.

As regards interaction with polyuridylic acid, the behavior of the AI copolymers is very reminiscent of poly AU. The interaction with polyuridylic acid appears to persist to surprisingly high extents of substitution in both cases. However, the resultant complex species become progressively less stable, as judged by the position of the midpoint of the thermal transition, as the inosinic content increases. Again, in analogy to the case of AU, the minima in the mixing curves with polyuridylic acid appear to correspond to equivalence of adenine to uracil, as is predicted by the Fresco-Alberts model (4).

**SUMMARY**

The progressive introduction of inosinic residues into the polyadenylic acid chain results in:

1. The disappearance of the characteristic structural transition of polyadenylic acid at acid pH values;
2. A fall in helical content, as measured by ultraviolet hypochromism and optical rotation;
3. A lowering of the "melting point" of the complex with polyuridylic acid.

**REFERENCES**

12. **Seyaq, M., Biochem. Z.*, 273, 419 (1934).
18. **Steiner, R. F., and Beers, R. F., Biochim. et Biophys. Acta, 32, 196 (1959).**
Copolymers of Adenylic Acid with Inosinic and Cytidylic Acids
R. F. Steiner