Optical Rotatory Dispersion and Conformation of Polyadenylic and Polyuridylic Acids*

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The application of optical rotary dispersion to nucleic acids and polynucleotides is fairly recent (1–6). Doty et al. (2) made a comprehensive study of the optical rotatory dispersion of deoxyribonucleic acid, ribonucleic acid, and complexes of some homopolynucleotides; their results strongly suggested a close relationship between the optical rotations and the secondary structure of nucleic acids. A systematic study by Ts'o and his co-workers (4–7) for the optical rotatory dispersion of poly- and mononucleotides in both aqueous and organic solvents also revealed the important contributions to optical rotatory dispersion of the secondary structures and base interactions of polynucleotides. Almost all these investigations, however, were made in the visible region of the spectra because of the limitation of the instrument. Since the rotatory properties in the visible region are directly influenced by the Cotton effects in the absorption bands, the extension of the optical rotatory dispersion measurements toward the ultraviolet region is therefore expected to provide more information than those in the visible region alone. Some such results on DNA, RNA, and mononucleotides have already been reported elsewhere (8, 9). In this report we present results not only corroborate data in the corresponding absorption bands, we checked the base-line below 300 mp, which occasionally shifted and had to be corrected, by comparing the specific rotation position for the complex formation appears to favor the addition of the second poly U strand to the poly (A + U) complex under conditions similar to those used here (13, 14).

EXPERIMENTAL PROCEDURE

Materials—Poly A and poly U were purchased from the Miles Chemical Company, Clifton, New Jersey. The protein impurities were removed by shaking a concentrated aqueous solution with an equal volume of amyl alcohol-chloroform (1:3) in the presence of 1% sodium lauryl sulfate. The supernatant (after centrifugation) was treated similarly several times until no protein precipitate occurred at the interfacial layer. Finally, 3 volumes of 95% ethanol were added to the solution to precipitate the polymer, which was then washed with ethanol and ether and dried. The purified polymer was then dissolved in a minimum volume of water and dialyzed against 0.01 M EDTA to remove any heavy metal ions and finally against the appropriate solvent for experiments. The concentrations of the polynucleotides were determined by phosphorus analysis (10).

Poly (A + U) and poly (A + 2U) were prepared by mixing equimolar solutions of poly A and poly U in appropriate proportions. The formation of the complexes was detected by ultraviolet spectrophotometry at 259 mp (11–13). A mixture of poly A and poly U in 0.01 M sodium cacodylate and 0.1 M NaCl had an absorbance minimum at an A:U molar ratio of 1:1, whereas in 0.15 M KF without Mg++ ions the ratio was 1:2. The latter minimum was reached within 15 minutes after mixing. This is in line with recent observations that the equilibrium position for the complex formation appears to favor the addition of the second poly U strand to the poly (A + U) complex under conditions similar to those used here (13, 14).

Methods—Optical rotatory dispersion was measured with a Cary 60 recording spectropolarimeter (190 to 600 mp) (8). The water-jacketed cell of 1- or 10-cm path length was connected to a Haake circulating constant temperature bath, and the actual temperature of the solution was read with a metallic probe of a Yellow Spring telethermometer. Because of the small magnitude of rotations for very dilute solutions in the absorption bands, we checked the base-line below 300 mp, which occasionally shifted and had to be corrected, by comparing the specific rotation with that obtained with a more concentrated solution (usually 100 times that used in the ultraviolet) in a 10-cm cell for the visible region which overlapped portions of the ultraviolet region. The blank for the 1-cm cell was then slightly adjusted, if necessary, so that the overlapping portions gave identical specific rotations. The absorbance of the samples was always kept below 2 to avoid possible artifacts (15). All optical rotatory dispersion data were expressed in terms of mean residue rotation, [ml, which equals (10α/d·m)] where m is the molar concentration of phosphorus, d the path length in decimeters, and α the rotation in degrees.

The absorption spectra of the samples were measured in a Cary 14 spectrophotometer equipped with a thermostated cell compartment. The cells were covered with alkathene stoppers to prevent evaporation at elevated temperatures.

RESULTS

Polyadenylic Acid—The optical rotatory dispersion of poly A was measured in three solvents (acetate buffer, pH 4.88; Tris...
buffer, pH 7.8; and KF, pH 7.5) at different temperatures (1–80°C). Some of the representative data are shown in Figs. 1 and 2 and the numerical values are summarized in Table I. In the ultraviolet region poly A exhibits multiple Cotton effects; in all cases a positive peak (p1) appeared at 282 to 283 μm, a negative trough (t1) around 252 to 257 μm, and a second small peak (p2) near 240 μm. The shape of the optical rotatory dispersion curves in Figs. 1 and 2 is actually very similar, but the magnitude of the Cotton effects and also the temperature profiles (see insets) strongly depend on the pH used. In acetate and Tris buffer, measurements were limited to about 220 μm because of strong solvent absorption; in KF, however, we were able to detect another trough (t2) near 212 μm. The data and their mixtures...

Table I

<table>
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<th>Polymer and solvent*</th>
<th>Temperature</th>
<th>λ_{p1}</th>
<th>[λ_{p1} - 10^4]</th>
<th>λ_{t1}</th>
<th>[λ_{t1} - 10^4]</th>
<th>λ_{max}</th>
<th>λ_{p2}</th>
<th>λ_{t2}</th>
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<tr>
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<td>88</td>
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<td>290</td>
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*Acetate: 0.1 M sodium acetate, 0.1 M NaCl, pH 4.85; Tris: 0.05 M Tris, 0.1 M NaCl, pH 4.85; KF: 0.15 M KF, pH 4.75; cacodylate: 0.01 M cacodylate, 0.1 M NaCl, pH 1.0.
†The symbol p denotes a peak, and t a trough, in the dispersion curve (see text). The symbols k and λ_α are constants which were found to fit the dispersion data in the visible region by means of a simple Drude equation (Equation 1 of the text).

Fig. 1. Ultraviolet rotatory dispersion of polyadenylic acid in 0.15 M KF (pH 7.5). Inset: variation of the mean residue rotation at the 283 μm peak with temperature.

Fig. 2. Ultraviolet rotatory dispersion of polyadenylic acid in 0.1 M sodium acetate plus 0.1 M NaCl (pH 4.85). Inset: variation of the mean residue rotation at the 282 μm peak with temperature.
shows a sharp transition in acetate buffer with $T_m$ of about 60°, whereas at neutral or slightly alkaline pH the transition is quite broad. These again agree well with the evidence obtained from other physical methods; for example, the change in $[\alpha]$ with temperature parallels the hyperchromicity of the polymer on melting. Decreasing the temperature of the solution in acetate buffer to 1-2° did not result in any significant increase in the rotations of $p_t$, indicating that the polymer had reached the maximum ordered structure even at room temperature. This is not true for poly A at neutral pH, the rotations ($p_t$) of which continued to increase and did not reach a plateau even at 1-2°.

**Polyuridylic Acid**—The optical rotatory dispersion of poly U in the ultraviolet region is shown in Fig. 3 and that in the visible region in Fig. 4. The results in 0.15 M KF (Fig. 3) and in Tris buffer (not shown here) were essentially identical; the low absorption of KF made it possible to extend the measurements to about 200 mμ. Like poly A, poly U has a $p_1$ at 284 mμ, a $t_1$ near 260 mμ, and a $p_2$ around 230 mμ. The magnitude of $p_1$ and $t_1$ of poly U (except at 1-2°) was, however, much less than that for poly A. At temperatures above 20° there was no significant change in rotations with temperature; below 20° poly U underwent a sharp increase in the magnitude of the Cotton effects (Fig. 3, inset). These findings are consistent with the conclusions drawn from optical, hydrodynamic, and titration studies of poly U, which seems to lack any ordered structure at room temperature, but shows a sharp reversible transition at lower temperatures with a $T_m$ of about 10° (18). The data in the visible region

![Fig. 3. Ultraviolet rotatory dispersion of polyuridylic acid in 0.15 M KF (pH 7.5). Inset: variation of the mean residue rotation at the 284 mμ peak with temperature.](image)

![Fig. 4. Visible rotatory dispersion of polyuridylic acid. Symbols: solid lines, in 0.05 M Tris buffer plus 0.1 M NaCl (pH 7.8); broken lines, in 0.15 M KF (pH 7.5).](image)

![Fig. 5. Ultraviolet rotatory dispersion of 1:1 mixture of polyadenylic and polyuridylic acids in 0.15 M KF (pH 7.5). Inset: variation of the mean residue rotation at the 284 mμ peak and the optical density with temperature.](image)

![Fig. 6. Ultraviolet rotatory dispersion of 1:2 mixture of polyadenylic and polyuridylic acids in 0.15 M KF (pH 7.5). Inset: variation of the mean residue rotation at the 284 mμ peak and the optical density with temperature.](image)
Poly (A + U)—The optical rotatory dispersions of poly (A + U) in 0.15 M KF (Fig. 5) and in 0.1 M NaCl-0.01 M sodium cacodylate (not shown here) were virtually identical at 27°, but at elevated temperatures the complex in cacodylate showed a much sharper reversible transition (between 55 and 60°) than that shown in the inset of Fig. 5 (between 40 and 70°). In both solvents, however, the peak \( p_1 \) occurred at 286 m\( \mu \) and the trough \( t_1 \) at 250 m\( \mu \). The latter underwent a red shift with increasing temperature and eventually reached 257 m\( \mu \) at 80°, as did the \( t_1 \) of poly A alone (pH 4.85) (Fig. 1). The variation of \( p_1 \) (see the inset) parallels the hyperchromicity of the complex at elevated temperatures.

Earlier we mentioned that the 259 m\( \mu \) absorbance of mixtures of poly A and poly U in 0.15 M KF reached its minimum at a molar ratio of 1:2 rather than 1:1. It is therefore highly probable that in the molar ratio of 1:1 in 0.15 M KF, portions of poly A chains are not associated with those of poly U but may interact among themselves to form some secondary structure. This perhaps explains why the mixture of poly A and poly U had a broader transition in 0.15 M KF than in the cacodylate, where a sharp transition \( (T_m = 58°) \) has been observed.

Poly (A + 2U)—The optical rotatory dispersion pattern of poly (A + 2U) complex (Fig. 6) is similar in many respects to that of poly (A + U) (Fig. 5). The trough \( t_1 \) also underwent a red shift at elevated temperature. On the other hand, the change in the magnitude of \( p_1 \), with temperature was much sharper than that for poly (A + U) in the same solvent (0.15 M KF, pH 7.5), indicating perhaps a more ordered structure for the triple stranded complex. Here again the effect of temperature on \( p_1 \) parallels the hyperchromicity of the complex, and the transition is essentially reversible as in the cases of other polynucleotides and their mixtures.

**DISCUSSION**

**Optical Rotatory Dispersion in Ultraviolet Region**—Poly A, poly U, and their complexes all show multiple Cotton effects, the magnitude of which is dependent on temperature and solvent. In all cases the first strong peak, \( p_1 \), and trough, \( t_1 \), occurred at 282 to 286 m\( \mu \) and 252 to 260 m\( \mu \), respectively, followed by a comparably weak peak, \( p_2 \), near 230 to 240 m\( \mu \). In a recent theory, Tinoco (17) pointed out that helical conformations of nucleic acids and proteins could initiate a new type of circular dichroism and optical rotatory dispersion curves; the shape of the former resembles that for a Cotton effect and the optical rotatory dispersion curves have two peaks and one trough (or two troughs and one peak). Qualitatively, our results in this study, and those of Brahms and Mommaerts and their co-workers on circular dichroism of nucleic acids and polynucleotides (18-22), agree well with this new theory; in particular, our \( t_1 \) was close to the absorption maximum and the circular dichroism was near zero at the same wave length (18), just as Tinoco predicted. These findings are certainly consistent with the idea that the secondary structures of the polynucleotides contribute to the experimentally observed multiple Cotton effects. In KF solutions, where measurements were extended to about 200 m\( \mu \), we detected a second trough, \( t_2 \), near 210 to 212 m\( \mu \), which is very likely the beginning of another Cotton effect. Indeed, Samejima and Yang (8, 23) show that both RNA and DNA do have a third peak, \( p_3 \), around 195 to 200 m\( \mu \). Note that Voet et al. (24) reported a second absorption band near this wave length in addition to the well known 260 m\( \mu \) band. This second absorption band also exhibited hyperchromism upon disruption of the secondary structure of nucleic acids. This absorption band, however, was not discussed in the theoretical calculations of Tinoco.

Optical rotatory dispersion alone cannot prove any secondary structure of a macromolecule, but it does lend support to the conclusions drawn from other physicochemical studies. The large magnitudes of the multiple Cotton effects of polynucleotides imply strong interactions among the bases, and a helical conformation certainly provides an optimal condition for the stacking of the bases. The close parallel between the reduction in the magnitude of the Cotton effects and the hyperchromicity of the polynucleotides at elevated temperatures, especially when the transition is very sharp, corroborates the idea of the melting of the helical conformation. On the other hand, our optical rotatory dispersion profiles seem to be unable to distinguish a double stranded helix from a single stranded or triple stranded one. On the basis of x-ray diffraction study of oriented fibers (25), poly A is known to exist as double stranded helices at acidic pH. From small angle x-ray scattering study, Luzzati et al. (26) now confirm the rod-like structure of poly A in acid solution (pH 5.0 to 5.9), and the calculated dimensions are consistent with the structure observed in fibers. That poly A at low pH is double stranded is very likely due to the presence of certain positively charged adenine groups which attract the phosphate groups of the opposite chains. At neutral pH, however, the small angle x-ray scattering curves show an intermediate form for poly A, which also appears to be rodlike but, unlike that in acid solution, with a linear dimension of one nucleotide per 3.4 A (26). Luzzati et al. then propose two structures for this intermediate form; one is a double stranded helix with the phosphodiester bonds fully elongated and no hydrogen bonds linking the bases, and the other is a single stranded helix in which one unit of the double stranded one has become separated from its complementary strand. These models differ from the hairpin model (with paired bases formed through loops) proposed several years ago (27). Note that the latter model was based on the concept that hydrogen bonding among the paired bases plays an important role for the stability of the helices. Now the hydrophobic interactions among the stacking bases are believed to stabilize the helical conformation even in the absence of hydrogen bonds. The hairpin model thus loses some of its former attractiveness. We are, however, unable to favor one structure over the other merely on the basis of our optical rotatory dispersion curves, which are similar for poly A in both acid and neutral solutions. In this respect the recent work by Faerman, Lindblow, and Grossman (28) shows that the Cotton effects of poly C at neutral pH remain essentially unchanged after the polymer reacts with formaldehyde. Thus, the influence of amino group hydrogen bonds on the stability of secondary structure of poly C at neutral pH is negligible. (On the other hand, from kinetic study of the reaction of RNA with formaldehyde, Doty et al. (2) presented evidence that the hydrogen bonding plays an important role in
the secondary structure of RNA.) On the basis of the broad temperature profile of poly A at pH 7.5, a single stranded helix as proposed by Luzzati et al. (26) appears more favorable than their double stranded helix without hydrogen bonds.

In comparison with poly A, poly U (above 10°) has smaller Cotton effects and smaller changes in their magnitude with temperature. These results are in accord with the idea that poly U has little secondary structure unless the solution is cooled below 10° (16). This sharp transition at low temperature indicates the melting of a regular cooperative structure, probably a double stranded helix. On the other hand, such helical conformation is rather unstable, as witnessed by its low melting temperature. The magnitude of the Cotton effects of poly (A + U) and poly (A + 2U) are intermediate between poly A and poly U. The sharp melting curves of these complexes again are in accord with their double and triple stranded helical conformations. In Fig. 7 we show the experimental curve of poly (A + U), which differs from that calculated (in a very elementary manner) from the mixture of poly A and poly U. Similar results are also obtained for the poly (A + 2U) complex. This again supports the idea of interaction of poly A and poly U, which in turn alters the spectral properties and consequently the interaction of the various electronic transitions. It is a complex theoretical problem and at present it is difficult to predict to what extent these newly formed interactions will change the Cotton effects.

Our data show that the Cotton effects of some of the homopolyribonucleotides and their mixtures are much larger than those of RNA (8, 23). For example, the magnitude of $p_1$ for poly A (pH 4.85) is $5.3 \times 10^4$, about 5 or 6 times that of rat liver RNA (8). Even poly U has an $[\alpha]$ comparable with that of RNA.

At elevated temperatures where any secondary structure of the polynucleotides is disrupted, the $p_1$ of poly A, poly U, and their complexes still retains $[\alpha]p_1 = 5 \times 7 \times 10^4$, about twice that of RNA under the same conditions. A satisfactory explanation for this difference between synthetic polynucleotides and RNA is still lacking. If one accepts the hairpin model, the fraction of the bases that can be matched in an RNA molecule is expected to be smaller than that of a double stranded poly A, for example, thus resulting in a smaller $p_1$. Likewise, imperfect helical segments in an RNA molecule due to elbows when the polynucleotide chain loops back on itself could also account for the weakening of the Cotton effects of RNA. According to the single stranded helix model, the base stackings in the RNA molecule must be much less than those in the homopolyribonucleotides. But the fact that poly U, which is known to have little ordered structure above $10°$, has a larger $p_1$ than RNA seems to rule out the above explanations as the major cause of the difference in $p_1$. Neither do we believe that difference in base composition can account for this difference in $p_1$. Another attractive suggestion (Drahms and Mommaerts (22), Drahms*) is that the two intertwined helical chains of poly A in acid solution may be parallel as contrasted with the antiparallel arrangement in DNA and RNA molecules. As a consequence, the rotational strengths of the base pairs are assumed to be additive in the former case and subtractive in the latter case, thus resulting in the large difference in $p_1$. The only difference between a parallel and an antiparallel helix seems to be that the orientation of the ribose residues in the two strands is in the same direction in one case and opposite in the other. It is difficult to see how such arrangement of the ribose residues could affect drastically the magnitude of the Cotton effects of polynucleotides. Furthermore, the large magnitude of the Cotton effects of poly U in its disordered form does not seem to support such a hypothesis. We suspect that the large magnitude of the Cotton effects of homopolyribonucleotides arises from the regularities in base sequences. It is quite possible that the interactions among an array of identical bases differ significantly from those of mixed bases. Samejima and Yang (23) show that poly dAT also has larger Cotton effects than those expected for the deoxyribonucleic acids; this seems also to support the contention that the regularity of the base sequences, as contrasted with their random distribution in nucleic acids, would enhance the magnitude of the Cotton effects, although too much speculation at this stage is unwarranted.

Optical Rotatory Dispersion in Visible Region—All optical rotatory dispersion data in the visible region in this study obeyed a one-term Drude equation, which is an approximation of many Drude terms

$$[\alpha] = \sum \frac{a_i \lambda^i}{(\lambda^2 - \lambda_c^2)} \cong \frac{k}{\lambda^2 - \lambda_c^2}$$

The one-term Drude equation is of course entirely empirical, since the theoretical treatment predicts that the summation of $a_i$, in Equation 1 must be zero. Here, $a_i$ values are proportional to the rotational strengths of the dichroic bands which are responsible for the Cotton effects in the ultraviolet region. Furthermore, since a single Cotton effect would reduce to one Drude term in regions away from the absorption bands (29), several Drude terms are needed to account for the multiple Cotton effects observed for the polynucleotides. In principle, we can estimate the rotational strengths from Figs. 1 to 3, and 5 to 7 and calculate the rotations in the visible region that are attributable to these Cotton effects. In the absence of direct precise determination of the rotational strengths by circular dichroism, such calculations are usually risky. At present the instrumental difficulties limit the measurement of circular dichroism to wave lengths greater than about 220 m$\mu$ (22), thus excluding the measurement of the dichroic band below 200 m$\mu$, which from our optical rotatory dispersion results seems to be very important. Therefore we will not attempt to fit the experimental data in the visible region with several Drude terms.

* J. Drahms, private communication.
We believe that any curve fitting with several parameters, even with the aid of a computer program, might be fortuitous, until we have precise circular dichroism data down to below 200 µm. In the meantime, the rotations at any wave length in the visible region can of course be used to follow the “melting” process of the polynucleotides, provided we have sufficient sample to warrant the accuracy of the measurements.

SUMMARY

Optical rotatory dispersion of polyadenylic acid (poly A), polyuridylic acid (poly U), and their 1:1 (poly(A + U)) and 1:2 (poly(A + 2U)) mixtures was measured between 190 and 600 µm. All exhibit multiple Cotton effects below 300 µm similar to those observed for ribonucleic acid. A large peak and trough occur at 282 to 286 µm and 250 to 260 µm and another, considerably smaller, peak is near 230 to 240 µm. For poly A in acetate buffer (pH 4.85), raising the temperature of the solution results in a sharp drop in the magnitude of the Cotton effects near 65°. In Tris buffer or KF solution at neutral pH, however, the same polymer undergoes a broad transition with increasing temperature. These findings are consistent with the contention that poly A exists in a double stranded helical form in neutral poly A exists in a double stranded helical form in acid solution, temperature, on the other hand, is very small above 10°, but a solution. The variation of the Cotton effects of poly U with temperature of the solution results in a sharp drop in the magnitude of the Cotton effects near 65°. In Tris buffer or KF solution at neutral pH, however, the same polymer undergoes a broad transition with increasing temperature. These findings are consistent with the contention that poly A exists in a double stranded helical form in acid solution, but probably in a single stranded, less ordered form in neutral solution. The variation of the Cotton effects of poly U with temperature, on the other hand, is very small above 10°, but a sharp increase in the magnitude of the peak and trough occurs below 10°. Poly (A + U) and poly (A + 2U) complexes also show transitions at elevated temperatures. All these changes are essentially reversible. The optical rotatory dispersion data of all polynucleotides studied obey a one-term Drude equation in the visible region.

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REFERENCES

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