Arch. Biochem. Biophys. 54, 162.
Kauzmann, W. (1959), *Advan. Protein Chem. 14*, 1.
Lipkin, M. R., Davison, J. A., Harvey, W. T., and Kurtz, S. S. (1944), *Ind. Eng. Chem., Anal. Ed.* (now *Anal. Chem.*) *16*, 55.
Ó hEocha, C. (1958), *Arch. Biochem. Biophys. 73*, 207.
Ó hEocha, C. (1962), Physiology and Biochemistry of Algae, Lewin, R. A., ed., New York, Academic, p. 421.
Ó hEocha, C. (1963), *Biochemistry 2*, 375.
Reithel, F. J. (1963), *Advan. Protein Chem. 18*, 123.
Ropp, G. A. (1960), *J. Am. Chem. Soc. 82*, 4252.
Schachman, H. K. (1959), Ultracentrifugation in Biochemistry, New York, Academic.
Scheraga, H. A. (1961), *J. Phys. Chem. 65*, 1071.
Singer, S. J., and Campbell, D. H. (1952), *J. Am. Chem. Soc. 74*, 1794.
Steinberg, I. Z., and Scheraga, H. A. (1963), *J. Biol. Chem. 238*, 172.
Strain, H. H., Thomas, M. R., Crespi, H. L., and Katz, J. J. (1961), *Biochim. Biophys. Acta 52*, 517.
Strain, H. H., Thomas, M. R., and Katz, J. J. (1963), *Biochim. Biophys. Acta 75*, 306.
Streitwieser, A., Jr., and Klein, H. S. (1963), *J. Am. Chem. Soc. 85*, 2759.
Svedberg, T. (1937), *Nature 139*, 1051.
Svedberg, T., and Eriksson, I.-B. (1932), *J. Am. Chem. Soc. 54*, 3998.
Svedberg, T., and Katsurai, T. (1929), *J. Am. Chem. Soc. 51*, 3573.
Svedberg, T., and Lewis, N. B. J. (1928), *J. Am. Chem. Soc. 50*, 525.
Tanford, C. (1955), *J. Phys. Chem. 59*, 798.
Tiselius, A., and Gross, D. (1934), *Kolloid Z. 66*, 11.

Optical Activity and the Conformation of Polyinosinic Acid and Several Other Polynucleotide Complexes*

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ABSTRACT: The optical rotatory dispersion (ORD) and absorption spectra of poly-I, poly-C, poly-(I + C), poly-(A + 2I), and poly-(G + C) were measured at various temperatures. All the polymers exhibited multiple Cotton effects below 300 mμ, but poly-I is the first polynucleotide studied that shows two troughs and one peak between 240 and 300 mμ instead of two peaks and one trough in the same wavelength range, as observed for nucleic acids and other polynucleotides. The complex formations of poly-I with poly-C or with poly-A immediately inverted the ORD profile to one characteristic of other polynucleotides.

This must be attributed to the difference in base interactions, since an inverse ORD profile for polynucleotides does not necessarily indicate a change in the handedness of the helices. In all cases the temperature curves of ORD paralleled those of the hyperchromic effect of the polymers. Poly-I showed a broad melting temperature in 0.01 M NaCl but a sharp transition in 1 M NaCl. This supports the contention that poly-I in low salt solution has very little, although not negligible, secondary structure. The three complexes, poly-(I + C), poly-(A + 2I), and poly-(G + C), all showed sharp helix-coil transitions, some of which were irreversible and some partially reversible.

ORD[1] of nucleic acids and polynucleotides in the visible region shows that their secondary structure generally contributes a positive rotation to that of their constituent mononucleotides (Doty *et al.*, 1959; Ts'o *et al.*, 1962; Samejima and Yang, 1964, 1965; Sarkar and Yang, 1965a). All these polynucleotides exhibit multiple Cotton effects with two peaks and one trough in the wavelength range of 230 and 300 mμ, which are drastically diminished at elevated temperature (Samejima and Yang, 1964, 1965; Sarkar and Yang, 1965a). In this communication we report the ORD of poly-I, since inosine is an analog of guanosine while poly-G is difficult to prepare and not available to us (Fresco and Su, 1962). We will show that poly-I is the first polynucleotide studied that distinguishes itself from the others by having two troughs and one peak between 240 and 300 mμ. Once poly-I is complexed with poly-C or poly-A, however, the ORD profiles immediately reverse to those resembling nucleic acids

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[1] Abbreviations used in this work: ORD, optical rotatory dispersion; IMP, inosine 5′-phosphate; AMP, adenosine 5′-phosphate; CMP, cytidine 5′-phosphate; T_m, melting temperature.

and other polynucleotides. Likewise, poly-(G + C) shows two peaks and one trough in the same wavelength range. The changes in ORD and absorption spectra of polynucleotides and their complexes with temperature and/or solvent will be described in terms of helix-coil transition and compared with other physicochemical properties pertinent to the understanding of their conformations.

Experimental Procedures

Materials. Poly-I and poly-C were purchased from Miles Chemical Co., Clifton, N.J. The polymers were deproteinized by dissolving them in 0.1 M NaCl containing 0.01 M EDTA and stirring them vigorously with phenol saturated with water (for poly-I) or with 3:1 chloroform–amyl alcohol (for poly-C). The process was repeated until no insoluble proteins were detected in the interfacial layer after centrifugation. The aqueous phase was then treated with 3 volumes of 95% ethanol, and the precipitated polymers were washed with ethanol and ether and dried *in vacuo*.

The purified poly-A was the same sample that was reported previously (Sarkar and Yang, 1965a). Poly-(G + C) was a gift from Professor R. Haselkorn, and was used without further purification.

The concentrations of the polymers in 0.1 M salt concentration were determined spectrophotometrically, using the following molar extinction coefficients: for poly-I, E_p (248 mμ) = 10.2 × 10³; for poly-C, E_p (268 mμ) = 6.3 × 10³; and for poly-A, E_p (257 mμ) = 9.9 × 10³. These values were obtained on the basis of phosphorus analyses (Allen, 1940). The concentration of poly-I in 1 M NaCl was obtained by hydrolyzing the polymer with NaOH, comparing its absorbance with that of the control similarly treated with alkali, and using E_p (248 mμ) = 12.3 × 10³ (Warner, 1957). The concentration of poly-(G + C) was calculated from the absorbance by assuming 20% hypochromicity of the polymer with respect to the corresponding mononucleotides (the mean E_p at 260 mμ = 9.0 × 10³ for the equimolar mixture of the mononucleotides [R. Haselkorn, private communication]).

Poly-I in high salt solution was prepared by dialyzing successively the aqueous solution against 0.1 M, 0.5 M, and finally 1 M NaCl solutions each containing 0.01 M EDTA. The complexes poly-(I + C) and poly-(A + 2I) were prepared by mixing the appropriate homopolymers in the desired molar ratios (on a monomer basis). The formation of the complexes was further checked by the appearance of an absorption minimum in an absorption–molar ratio plot (Rich, 1958a; Davies and Rich, 1958). IMP was bought from California Corp. for Biochemical Research. All chemicals used were of reagent grade.

ORD. All measurements were done with a Cary Model 60 recording spectropolarimeter, the calibration of which has been described elsewhere (Sarkar and Yang, 1965a). Water-jacketed cells with light paths of 10 and 1 cm were used; the solutions used always had an absorbance well below 2 to minimize any possible artifacts. The data were expressed in terms of mean residue rotation, or molar rotation for mononucleotide, $[m] = 10\alpha/d_{MP}$, where α is the observed rotation in degrees, d the light path in decimeters, and M_P the molar concentration on the basis of phosphorus analyses.

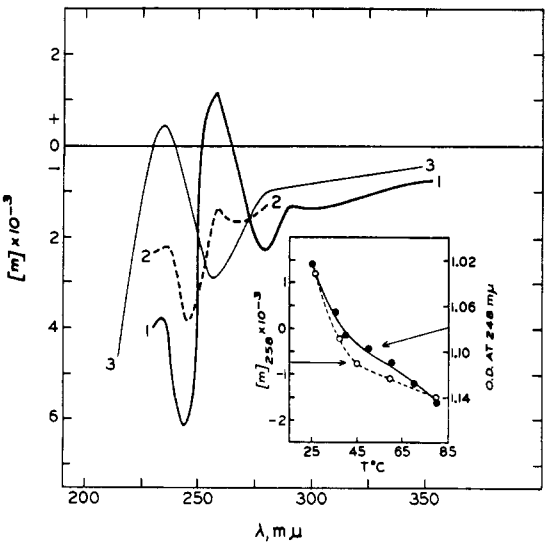

FIGURE 1: Ultraviolet rotatory dispersion of polyinosinic acid in low salt concentration at *p*H 7.4. Solvent: 0.1 M NaCl and 0.01 M glycylglycine. Curve 1, 27°; curve 2 (broken line), 80°. For comparison, curve 3, inosine monophosphate in water (*p*H 6) at 27°. Insert: variation of the mean residue rotation at 258 mμ peak and the absorbance at 248 mμ maximum with temperature.

Spectrophotometry. The absorption spectra of polynucleotides were measured with a Cary Model 14 spectrophotometer.

Results

Poly-I. Since the conformation of poly-I is known to be markedly dependent on the ionic strength of the solution (Haselkorn, 1959; Rich, 1958b), we measured its ORD in (a) 0.1 M NaCl with 0.01 M glycylglycine buffer (*p*H 7.4), where poly-I is believed to be in the coiled form, and (b) 1 M NaCl with 0.01 M EDTA (*p*H 7.0), where poly-I assumes a triple-stranded helical conformation (Haselkorn, 1959). Like other polynucleotides and nucleic acids (Sarkar and Yang, 1965a; Samejima and Yang, 1964, 1965), poly-I shows mutliple Cotton effects below 300 mμ (Figures 1 and 2). But poly-I is the first polynucleotide studied that distinguishes itself from the others by exhibiting two troughs and one peak between 240 and 300 mμ instead of two peaks and one trough in the same wavelength range; furthermore, the magnitude of the peak and troughs of poly-I is relatively small (Table I).

In low salt solution (Figure 1) poly-I has its first

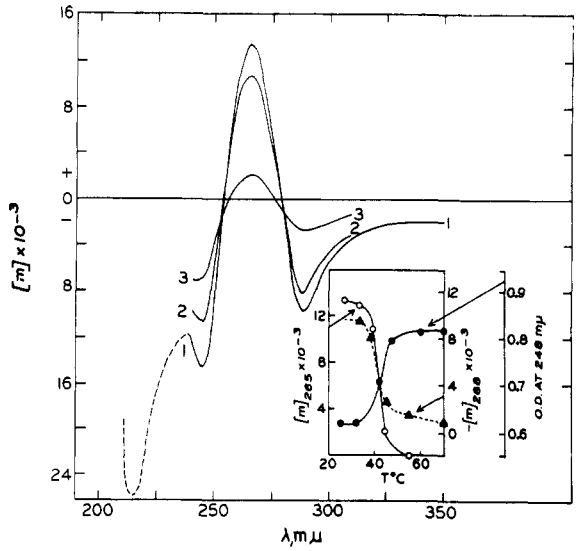

FIGURE 2: Ultraviolet rotatory dispersion of polyinosinic acid in high salt concentration at *p*H 7.0. Solvent: 1 M NaCl and 0.01 M EDTA. Curve 1, 27°; curve 2, 39°; curve 3, 45°. Broken line: poly-I in 1 M $NaClO_4$ at 27°. Insert: variation of the mean residue rotation at 265 mμ peak and at 288 mμ trough and the absorbance at 248 mμ maximum with temperature.

trough, t_1, from the longer-wavelength side at 280 mμ, a first peak, p_1, at 258 mμ, a second trough, t_2, at 244 mμ. The fact that poly-I shows multiple Cotton effects indicates the presence of base interactions and therefore some secondary structures. For comparison, like other purine mononucleotides (Yang and Samejima, 1963). IMP shows only a single negative Cotton effect above 230 mμ with an inflection point close to the absorption maximum of 248 mμ (Figure 1). At elevated temperatures the Cotton effects gradually diminish. This is more easily shown by following the variation of p_1 with temperature (Figure 1, insert) which parallels the hyperchromic effect of poly-I under the same conditions. The broad temperature profile, the small hyperchromicity (about 10%), and the small magnitude of p_1 (cf. p_1 in Figure 2) all suggest that poly-I has little, but not negligible, secondary structure in low salt solutions. Figure 1 also shows a second peak, p_2, near 235 mμ. In 0.1 M NaCl measurements were limited to above 230 mμ because of strong solvent absorption; however, replacement of NaCl with $NaClO_4$ extended the wavelength range beyond 230 mμ, and the levorotation in this case increased sharply to about −10,000 at 210 mμ (not shown in Figure 1).

Figure 2 shows the ORD of poly-I in high salt solution (1 M NaCl or $NaClO_4$). Below 240 mμ only $NaClO_4$ was used to minimize the solvent absorption. The solution was prepared first in 1 M $NaClO_4$ containing 0.01 M EDTA, followed by removal of EDTA, which absorbs, through dialysis. A first look at the profiles in Figures 1 and 2 seemed to indicate a significant difference between them; actually, Figure 2 also shows

TABLE I: Parameters of the Cotton Effects of Polynucleotides.

Compound	Solvent	Temp (°C)	λ_{t_1} (mμ)	$[m]_{t_1} \times 10^{-3}$	λ_{p_1} (mμ)	$[m]_{p_1} \times 10^{-3}$	λ_{max} (mμ)	λ_{t_2} (mμ)	$[m]_{t_2} \times 10^{-3}$	λ_{p_2} (mμ)	$[m]_{p_2} \times 10^{-3}$
Poly-I	0.1 M NaCl + 0.01 M glycylglycine (*p*H 7.4)	27	280	− 2.2	258	+ 1.2	248	244	− 6.2	235	− 3.8
		80	270	− 1.6	258	− 1.4	248	245	− 3.8	237	− 2.2
Poly-I	1 M NaCl + 0.01 M EDTA (*p*H 7.0)	27	288	− 9.7	265	+13.5	248	245	−14.5		
		45	288	− 2.6	265	+ 2.1	248	242	− 7.1		
IMP	Water (*p*H 6)	27	255	− 2.9	235	+ 0.5					
Poly-C	0.1 M NaCl + 0.01 M cacodylate (*p*H 7.0)	27	268	−45	293	+35	268	250	−26		
		80	268	−19	293	+17	269	250	−19		
Poly-(I + C)	Same as for poly-C	27	270	−11	293	+17	248, 268			254	+16
		80	272	−15	302	+ 6	243, 268			257	− 9
Poly-(A + 2I)	0.1 M NaCl + 0.01 M glycylglycine (*p*H 7.4)	27	270	−16	294	+ 8	254			252	+20
		80	257	− 6.5	284	+ 1.8	250			240	0
Poly-(G + C)	0.1 M Tris (*p*H 7.5)	27–85	245	−37	276	+33	260			220–230	−23
		95	270	−26	294	+ 9				240–245	− 1.3

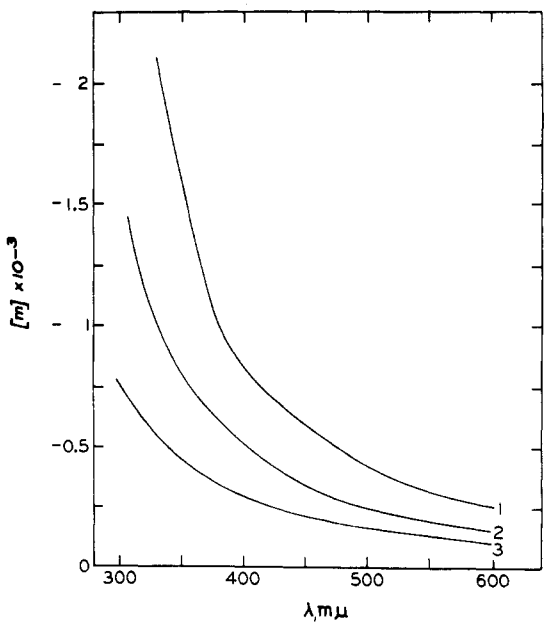

FIGURE 3: Visible and near-ultraviolet rotatory dispersion of polyinosinic acid. Curve 1, same as in Figure 2 at 27°; curve 2, same as in Figure 1 at 27°. For comparison, curve 3, inosine monophosphate in water at 27°.

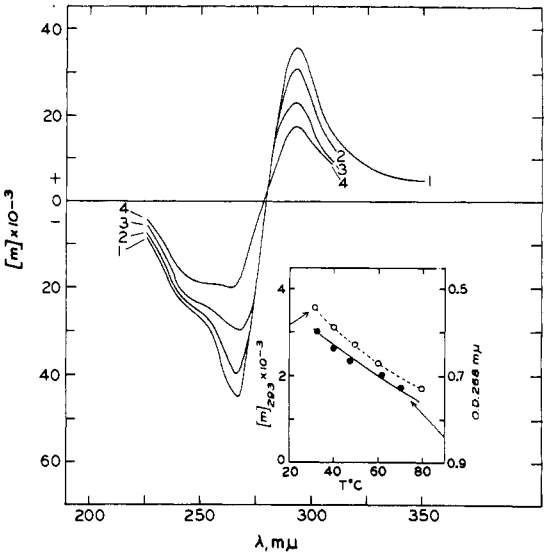

FIGURE 4: Ultraviolet rotatory dispersion of polycytidylic acid at *p*H 7.0. Solvent: 0.1 M NaCl and 0.01 M Na cacodylate. Curves 1–4, at 27, 40, 60, and 80°. Insert: variation of the mean residue rotation at 293 mμ peak and the absorbance at 268 mμ maximum with temperature.

two troughs at 288 and 245 mμ and one peak at 265 mμ, although the rotation of the peak is an order of magnitude higher than that in Figure 1. In the insert we show the parallel of variation of the rotations with temperature and the hyperchromic effect. The temperature curves reveal a sharp transition between 40 and 45°, which reflects the cooperative melting of a highly ordered secondary structure of the polymer. This is consistent with the T_m obtained from the hyperchromic effect by Doty *et al.* (1959).

In the visible region the rotations of poly-I in both 0.01 M and 1 M NaCl, as well as those of IMP, are all levorotatory and their profiles are featureless and monotonic. But the magnitude of the rotations of poly-I is larger than that of IMP rotations, and poly-I in high salt solution is more levorotatory than that in low salt solution. The data in Figure 3 obey a one-term Drude equation, $[m] = k/(\lambda^2 - \lambda_c^2)$, between 580 and 310 mμ, and the calculated λ_c and k are: for poly-I in 1 M NaCl, $\lambda_c = 270$ mμ and $k = -21.4 \times 10^6$; for poly-I in 0.01 M NaCl, $\lambda_c = 258$ mμ and $k = 13.3 \times 10^6$; and for IMP, $\lambda_c = 187$ mμ and $k = -10.7 \times 10^6$.

Poly-C. While this work was in progress, Fasman *et al.* (1964) reported their ORD study of poly-C at *p*H 4.1 (0.1 M acetate buffer) and 7.0 (buffer, 0.15 M Na citrate + 0.015 M NaCl); we therefore shall not present our results in detail. These authors detected a single Cotton effect between 255 and 350 mμ, which is confirmed by our results in 0.1 M NaCl plus 0.01 M cacodylate (*p*H 7.0) (Figure 4). We have, however, extended our measurements to 255 mμ, and observed another shoulder around 250 mμ. Thus one can visualize that poly-C also shows multiple Cotton effects with two peaks at 293 and 250 mμ, and one trough at 267 mμ. The latter value actually is very close to the absorption maximum of 268 mμ. The broad temperature curves in the insert are indicative of the destruction of some secondary structure which is not highly ordered. On quick cooling from high temperature, both the peak and trough in Figure 4 were reversed to an extent of about 85%. Note also that the magnitude of the peak and trough for poly-C is much larger than that found for poly-I and also for other polynucleotide complexes to be described later.

Poly-(I + C). Complex formation of poly-I with poly-C in 0.1 M NaCl plus 0.01 M Na cacodylate (*p*H 7) changed the ORD profile from two troughs and one peak to that observed with other polynucleotides, that is, two peaks and one trough between 240 and 330 mμ (Figure 5). The change in the magnitude of the peaks with temperature (see insert) indicates that the T_m based on the data of p_1 (at 293 mμ) differed slightly from that obtained from p_2 (at 254 mμ). Poly-(I + C) shows two absorption maxima at 268 and 248 mμ (Davies and Rich, 1958); correspondingly, we found that the T_m obtained from p_1 was close to that based on the hyperchromicity at 268 mμ, whereas T_m from p_2 agreed with that at the 248-mμ absorption maximum. Whether this is merely a fortuitous coincidence remains to be determined. At elevated temperatures both the peaks and the trough in Figure 5 underwent a red shift. Upon quick cooling, the magnitude of p_1 was essentially reversed to its original value, but that of p_2 was only partially recovered.

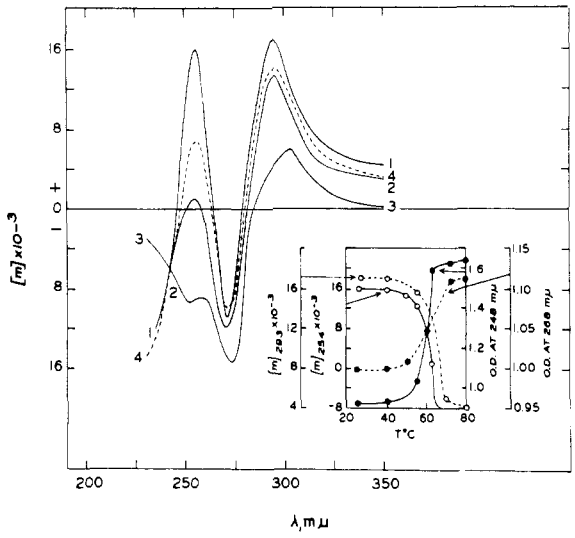

FIGURE 5: Ultraviolet rotatory dispersion of equimolar mixture of polyinosinic and polycytidylic acids at *p*H 7.0. Solvent: 0.1 M NaCl and 0.01 M Na cacodylate. Curves 1–4, at 27, 62, 70, and 27° (after heating). Insert: variation of the mean residue rotations at 293 and 254 mμ peaks and the absorbances at 268 and 248 mμ maxima with temperature.

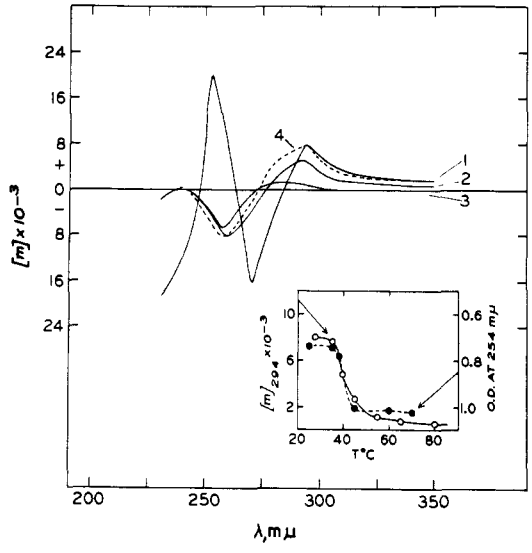

FIGURE 6: Ultraviolet rotatory dispersion of 1:2 molar mixture of polyadenylic and polyinosinic acids at *p*H 7.4. Solvent: 0.1 M NaCl and 0.01 M glycylglycine. Curves 1–4, at 27, 40, 80, and 27° (after cooling back from 80°). Insert: variation of the mean residue rotation at 294 mμ peak and the absorbance at 254 mμ maximum with temperature.

Poly-(A + 2I). Like other polynucleotides (except poly-I), the ORD of poly-(A + 2I) in 0.1 M NaCl plus 0.01 M glyclyglycine (*p*H 7.4) also shows two peaks and one trough (p_1, p_2, and t_1 at 294, 252, and 270 mμ)

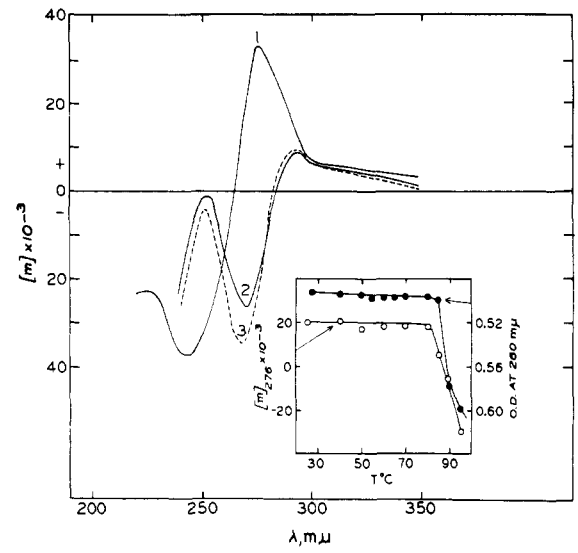

FIGURE 7: Ultraviolet rotatory dispersion of equimolar mixture of polyguanylic and cytidylic acids at *p*H 7.5. Solvent: 0.1 M Tris buffer. Curve 1, at 27 to 85°; curve 2, at 95°; curve 3, at 27° after cooling back from 95°. Insert: variation of the mean residue rotation at 276 mμ and the absorbance at 260 mμ maximum with temperature.

(Figure 6). But unlike other polynucleotides this complex has a second peak much larger than the first one, for reasons not clear to us. Above 40° p_2 underwent an abrupt change, and its magnitude dropped almost to zero; this was accompanied by a blue shift of t_1 and p_2. The magnitude of p_1 also decreased with increasing temperatures but not as drastically as that of p_2. The change in p_1 (see insert) and p_2 (not shown here) with temperature paralleled the hyperchromic effect. On quick cooling from 80 to 27°, the magnitude of p_1 was completely reversed, but that of p_2 was irreversible.

Poly-(G + C). Figure 7 shows the Cotton effects of poly-(G + C) with two peaks at 276 and 220–230 mμ, and one trough at 245 mμ. This polymer complex had a much larger p_1 than the corresponding one of poly-(I + C), and also a very sharp transition near 90° (see insert) as compared with about 60° for poly-(I + C) (Figure 5) and also for poly-(A + U) (Sarkar and Yang, 1965a). Upon melting, both the peaks and the trough underwent a large red shift together with a sharp, irreversible change in magnitude, which was also confirmed from the absorbance-temperature study. That the disruption of the secondary structure of double- or triple-stranded helices such as poly-(I + C), poly-(A + 2I), and poly-(G + C) is often partially reversible or completely irreversible is not too surprising since the homopolymers, poly-I and poly-G, have a tendency to interact with themselves. Poly-G is a classical example; it is difficult to prepare and highly insoluble in aqueous solution because of its ease of aggregation.

Discussion

In previous papers (Samejima and Yang, 1964, 1965; Sarkar and Yang, 1965a) we have shown that all nucleic acids and polynucleotides exhibit multiple Cotton effects in their absorption bands below 300 mμ. This general finding is once again confirmed in the present study. Furthermore, in all cases the decrease in magnitude of the Cotton effects at elevated temperatures parallels the hyperchromic effect of these polymers under the same conditions. But the salient observation in this communication is that poly-I shows two troughs and one peak in the wavelength range of 240–300 mμ instead of two peaks and one trough for those reported previously. Qualitatively, our results still agree with the theoretical prediction of Tinoco (1964) that such multiple Cotton effects arise from the base stackings and interactions of polynucleotides regardless of the inversion in the profiles reported herein. However, the present state of theoretical developments does not permit us to speculate whether such inversion of profiles necessarily involves any change in the screw sense of the ordered structure. (This is different from the polypeptides, where the Cotton effects of a helical conformation are produced by the amide chromophore, so that the Cotton effect of a right-handed helix becomes the mirror image of that of the left-handed helix.) Specific interactions of the stacking bases or even interactions of bases across the strands in a superstructure and their geometrical relationships, which cannot yet be calculated or predicted from theories or experiments, might cause such reversal of the Cotton effects without involving the handedness of the helices. Note that X-ray diffraction data of poly-I are actually incompatible with a left-handed, three-stranded helix (Rich, 1958b), just as the complexes of other polynucleotides are currently believed also to form right-handed helices. It should also be pointed out that the stereochemical arrangements of the triple-stranded poly-I and poly-(A + 2I) are quite different from each other, as evidenced by model building based on X-ray diffraction and infrared spectra studies (Rich, 1958b; Miles, 1964). Poly-(A + 2I) has a bonding arrangement rather similar to that for poly-(A + 2U). In both cases the amino group on C6 of adenine is hydrogen-bonded to the carbonyl group on C6 of the two inosines and on C2 in one uridine and on C4 in the other; the N1 and N7 of the adenine are hydrogen bonded to N1 of the inosines and N3 of the uridines. On the other hand, the three hypoxathine bases of the three-stranded poly-I helix are arranged about a 3-fold rotation axis held together by three hydrogen bonds between N1 and C6. This perhaps is related to the inversion of the Cotton effects, if such triple-stranded helices remain intact in solution.

Studies of hydrodynamic properties, light scattering (Haselkorn, 1959), electron microscopy (Hall, 1959), hyperchromic effect (Steiner and Beers, 1961), and infrared spectroscopy (Miles, 1964; Sigler *et al.*, 1962) of poly-I all suggest that it possesses a highly ordered secondary structure only in high salt concentrations (e.g., 1 M), which agrees with a three-stranded hydrogen-bonded helical conformation. Our ORD results fully support this conclusion; the larger magnitude of the Cotton effects in 1 M NaCl, as compared with that in 0.1 M salt, and its sharp T_m curve (Figure 2, insert) do reflect an ordered structure in high salt solutions. In 0.1 M NaCl, while the infrared spectra indicate the absence of any hydrogen bonding (Miles, 1961), the multiple Cotton effects, as contrasted with a single Cotton effect for IMP (above 230 mμ), and the reduction of their magnitude with increasing temperature suggest some residual base interactions and stackings for poly-I even in low salt solutions.

Our data on poly-C at *p*H 7 are essentially identical with those of Fasman *et al.* (1964), except that we have extended the measurements down to 225 mμ and detected a shoulder near 250 mμ (Figure 4). We concur with these workers that the secondary structure of poly-C at neutral *p*H is probably a single-stranded helix stabilized by base stackings. We also note that the magnitude of p_1 dropped from 35×10^3 at *p*H 7.0 to 27×10^3 at *p*H 4.0 (Fasman *et al.*, 1964), where poly-C is believed to be in a double-stranded helical form (the lowering of *p*H was also accompanied by a hyperchromic effect near 270 mμ). This is quite different from the *p*H effect of poly-A, where the magnitude of p_1 was about 30×10^3 at *p*H 7.5 (single stranded) and 53×10^3 at *p*H 4.85 (double stranded) (Sarkar and Yang, 1965a), and a hypochromic effect was observed upon lowering the *p*H. Note that the added proton for partially protonated poly-C helix at *p*H 4 participates directly in hydrogen bonding between the two N3's of the cytosine bases (Akinrimisi *et al.*, 1963), whereas protonation of poly-A in acidic *p*H involves no additional hydrogen bonding, but stabilizes the two-stranded helix through the formation of inner salt (Rich *et al.*, 1961). While protonation of AMP does not alter its absorption spectrum to any extent, the spectrum of CMP is significantly changed in acidic *p*H. Thus the change in ORD of poly-A with *p*H could be substantially attributed to the development of secondary structure, but that for poly-C could be due to both protonation and change in polymer conformation (Ts'o *et al.*, 1962; Akinrimisi *et al.*, 1963). However, we have reason to believe that protonation of poly-C is at least partially responsible for its reduced magnitude in acidic *p*H, since the same phenomenon is now observed for several DNA's and RNA's studied (Sarkar and Yang, 1965b).

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References

Akinrimisi, E. O. Sander, C. and Ts'o, P.O.P. (1963), *Biochemistry 2*, 340.

Allen, R. J. L. (1940), *Biochem. J. 34*, 858.

Davies, D. R., and Rich, A. (1958), *J. Am. Chem. Soc. 80*, 1003.
Doty, P., Boedtker, H., Fresco, J. R., Haselkorn, R., and Litt, M. (1959), *Proc. Natl. Acad. Sci. U.S. 45*, 482.
Fasman, G. D., Lindblow, C., and Grossman, L. (1964), *Biochemistry 3*, 1015.
Fresco, J. R., and Su, D. F. (1962), *J. Biol. Chem. 237*, PC 3304.
Hall, C. (1959), *Ann. N.Y. Acad. Sci. 81*, 723.
Haselkorn, R. (1959), Ph.D. dissertation, Harvard University.
Miles, H. T. (1961), *Proc. Natl. Acad. Sci. U.S. 47*, 798.
Miles, H. T. (1964), *Proc. Natl. Acad. Sci. U.S. 51*, 1104.
Rich, A. (1958a), *Nature 181*, 521.
Rich, A. (1958b), *Biochim. Biophys. Acta 29*, 502.
Rich, A., Davies, D. R., Crick, F. H. C., and Watson, J. D. (1961), *J. Mol. Biol. 3*, 71.
Samejima, T., and Yang, J. T. (1964), *Biochemistry 3*, 613.
Samejima, T., and Yang, J. T. (1965), *J. Biol. Chem. 240* (in press).
Sarkar, P. K., and Yang, J. T. (1965a), *J. Biol. Chem. 240* (in press).
Sarkar, P. K., and Yang, J. T. (1965b), *Federation Proc. 24*, 538.
Sigler, P., Davies, D. R., and Miles, H. T. (1962), *J. Mol. Biol. 5*, 709.
Steiner, R. F., and Beers, R. F. (1961), Polynucleotides, Amsterdam, Elsevier.
Tinoco, I., Jr. (1964), *J. Am. Chem. Soc. 86*, 297.
Ts'o, P. O. P., Helmkamp, G. K., and Sander, C. (1962), *Biochim. Biophys. Acta 55*, 584.
Warner, R. C. (1957), *J. Biol. Chem. 229*, 711.
Yang, J. T., and Samejima, T. (1963), *J. Am. Chem. Soc. 85*, 4039.

Helix-Coil Transition of Poly-L-glutamic Acid and Poly-L-lysine in D_2O*

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ABSTRACT: The specific rotations at 233 mμ of poly-L-glutamic acid and poly-L-lysine in D_2O were measured as a function of *p*D and compared with those in H_2O. In both cases the helix-coil transition shifted toward the alkaline side; i.e., $(pD - pH)_{tr} = \sim 0.6$. Thus, deuteration appeared to favor the formation of the helix for poly-L-glutamic acid but the coil for poly-L-lysine. This apparent contradiction was resolved by studying the titration behavior of the two polypeptides. Just as in simple acids, both the glutamic acid and lysine side groups in the polypeptide chains became weaker Brønsted acids upon deuteration. The difference in the intrinsic dissociation constant, $pK_i(D_2O) - pK_i(H_2O)$, was about 0.5 for poly-L-glutamic acid and 0.7 for poly-L-lysine. When the rotations were replotted against the degree of dissociation instead of against *p*D or *p*H, the experimental data in D_2O and H_2O coincided. Thus the deuterated helices of the two polypeptides in D_2O seem to have the same stability as the protonated ones in H_2O.

The deuterium isotope effect on protein structure and function has been extensively investigated. Several protein molecules have shown a change in the stability of their helical conformation in a D_2O medium; these results have been variously interpreted in terms of hydrogen bonds (Hermans and Scheraga, 1959; Maybury and Katz, 1956), the structure of water (Von Hippel and Harrington, 1960), hydrophobic forces (Berns *et al.*, 1963), or internal rotation (Berns, 1963) being responsible for maintaining the native conformation of the protein molecules. One important factor not considered is the weakened acidity of ionizable groups in deuterium systems. Deuterated weak acids (Brønsted) have smaller dissociation constants than the corresponding protonated ones (LaMer and Chittum, 1936; Lumry *et al.*, 1951; Li *et al.*, 1961). We anticipated that polyelectrolytes, both acidic and basic, would show similar changes in their dissociation behavior when ionizable hydrogen atoms were replaced by deuterium atoms. We chose poly-L-glutamic acid and poly-L-lysine as model polymers in this study because their conformations have been well characterized.

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