Anomalous Rotatory Dispersion of Soluble Ribonucleic Acid and Its Relation to Amino Acid Synthetase Recognition*

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ABSTRACT: *Escherichia coli* soluble RNA (s-RNA), polyadenylic acid (poly-A), polyuridylic acid (poly-U), and the 1:1 complex of poly-A and poly-U exhibit characteristic anomalous dispersion in the ultraviolet region. Comparison of the Cotton effects near 260 mμ with those of the constituent monoribonucleotides shows that the optical rotatory dispersion of these macromolecules is attributable both to the configurational asymmetry of the nucleotide components and to the conformational asymmetry of their secondary structure. The latter contributions are abolished or reduced by alkaline hydrolysis, protonation of the amino groups, and exposure to heat, urea, or alkaline *p*H, resulting in marked alterations of the crossover points (λ_0) and amplitudes of the Cotton effects. The spectropolarimetric changes of s-RNA and of 1:1 complexes of poly-A and poly-U subsequent to heating or urea denaturation resemble one another closely, suggesting the participation of A-U base pairing in the stabilization of the secondary structure of s-RNA.

Changes of the Cotton effect of s-RNA also occur as a result of increasing bromination, a modification which alters the absorption of s-RNA and which has previously been found to result in loss of its acceptor function for activated amino acids. Highly brominated s-RNA exhibits a residual dispersion curve similar to that of the constituent purine nucleotides, supporting previous evidence that bromine adds preferentially to the pyrimidine bases of s-RNA.

Several lines of evidence indicate that RNA has an ordered structure in solution (Spirin, 1963). Transfer or soluble RNA, consisting of approximately only 80 nucleotide residues, has been most amenable to study in this regard. It appears that hydrogen bonding, base stacking, and hydrophobic interactions result in stabilization of the secondary structure of the single-stranded s-RNA molecule.

In recent years, optical rotatory dispersion has been employed to delineate the asymmetric structures of certain macromolecules in solution. The relationship of the Cotton effects of proteins and polypeptides in the absorption bands of peptide bonds to α-helical and other specific conformations has been well established (Blout, 1963). TMV[1] and rat liver (type unspecified) RNA and DNA have recently been reported to exhibit similarly distinctive Cotton effects in the absorption bands of the purine and pyrimidine bases (Simmons and Blout, 1961; Samejima and Yang, 1964). To examine the factors contributing to the formation of such Cotton effects in RNA, the optical rotatory dispersion of s-RNA,[2] its constituent nucleotides, and several synthetic homopolyribonucleotides have been measured. The data indicate that the characteristic Cotton effects of the polynucleotides superimpose upon and profoundly alter those of the constituent nucleotides. The position, shape, and magnitude of such Cotton effects are dependent upon temperature, *p*H, and solvent conditions and are altered by chemical modification of the bases through bromination.

Materials and Methods

s-RNA from *E. coli* and from yeast were purchased from General Biochemical Corp., Chagrin Falls, Ohio. RNA was stripped of attached amino acids by incubation in 0.1 M sodium carbonate, *p*H 10.1, at 37° for 30 minutes, followed by alcohol precipitation and extensive dialysis against cold distilled water. Ribosomal RNA from logarithmic phase *E. coli* cells was prepared by lysis and phenol extraction of the 100,000 × g pellet. Valyl-RNA ester (approximately 90% pure) was

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[1] Abbreviation used in this work: TMV, tobacco mosaic virus.

[2] When a mixed sample of s-RNA is purified so as to accept only a single amino acid, such as valine, this purified sample may be designated valyl-RNA. We designate as "valyl-RNA ester" such a purified sample which actually has valine esterified to it.

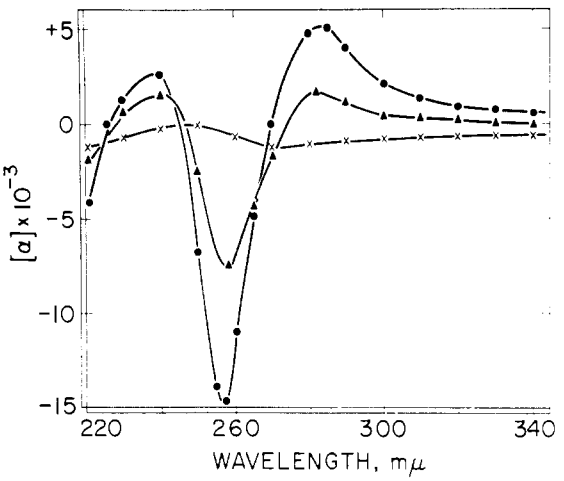

FIGURE 1: Optical rotatory dispersion of 2′(3′)-AMP and poly-A. Specific rotation at 22° is plotted against wavelength. AMP (0.75 mg/ml) was dissolved in 0.1 M succinate containing 5×10^{-3} M Mg^{2+}, *p*H 5.15 (x).[3] Poly-A (0.5 mg/ml) in the absence (●) and presence (▲) of 8 M urea was dissolved in 0.1 M Tris containing 5×10^{-3} M Mg^{2+}, *p*H 7.5. 2′(3′)-GMP (0.75 mg/ml) in 0.1 M succinate and 5×10^{-3} M Mg^{2+}, *p*H 5.15, exhibits a single negative Cotton effect in the same wavelength region as does AMP. $[\alpha]_{340} = -70$; $[\alpha]_{265} = -933$; $[\alpha]_{240} = 0$; $[\alpha]_{230} = +300$; $[\alpha]_{220} = -2300$.

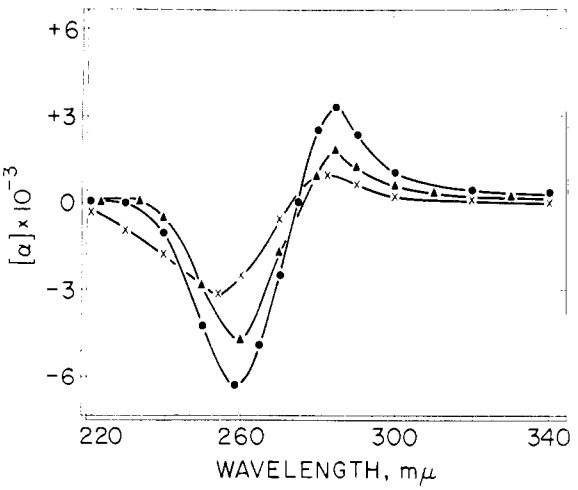

FIGURE 2: Optical rotatory dispersion of 2′(3′)-UMP and poly-U. Specific rotation at 22° is plotted against wavelength. UMP (0.75 mg/ml) was dissolved in 0.1 M succinate containing 5×10^{-3} M Mg^{2+}, *p*H 5.15 (x).[3] Poly-U (0.5 mg/ml) in the absence (●) and presence (▲) of 8 M urea was dissolved in 0.1 M Tris containing 5×10^{-3} Mg^{2+}, *p*H 7.5. 2′(3′)-CMP (0.75 mg/ml) in 0.1 M succinate and 5×10^{-3} M Mg^{2+}, *p*H 5.15, exhibits a single positive Cotton effect in the same wavelength region as does UMP. $[\alpha]_{340} = +130$; $[\alpha]_{290} = +1840$; $[\alpha]_{273} = 0$; $[\alpha]_{240} = -3660$; $[\alpha]_{220} = -1570$.

prepared according to Stephenson and Zamecnik (1962). AMP, UMP, GMP, and CMP were purchased from Calbiochem, Los Angeles, Calif. Polyadenylic acid and polyuridylic acid were purchased from Miles Laboratories, Elkhart, Ind. Urea was twice recrystallized from water before use. All other chemicals were reagent grade.

Hydrolysis of s-RNA was carried out in 0.3 N KOH at 37° for 24 hours. Bromination of RNA has been described (Yu and Zamecnik, 1963a). The concentration of the mononucleotides, polynucleotides, and RNA was measured spectrophotometrically using their known absorptivities. In 0.15 M NaCl–0.015 M Na citrate at *p*H 7.1, the absorptivity of s-RNA is 21.4 cm^2 mg^{-1} (Stephenson and Zamecnik, 1962).

Optical rotatory dispersion was measured from 350 to 215 mμ with a Cary Model 60 recording spectropolarimeter. The slit width of the instrument was programed to yield maximal and constant light intensity at all wavelengths. All measurements at room temperature (21°) were performed in a 1-mm path length cell having fused-quartz end plates (Opticell). For measurements at elevated temperatures, the temperature of the solution was maintained by circulating water from a heated external bath through a thermojacketed cell of identical design. The instrument was calibrated to give zero rotation for the buffer blanks at all wavelengths. In most instances, the concentration of the solutions was 1 mg/ml. The values for specific rotation were precise to ±200°. Comparison of the data on the basis of the reduced mean residue rotation, $([m']_\lambda)$ (Urnes and Doty, 1961), would incorporate variations in mean residue weight of the polymers and nucleotide composition. The mean residue weight of poly-A is 329, of poly-U, 306, and of *E. coli* s-RNA, 324. Specific rotations measured in the presence of urea were corrected for the change in refractive index (Harrington and Schellman, 1957).

Results

The purine mononucleotides 2′(3′)-AMP and 2′(3′)-GMP exhibit single *negative* Cotton effects in the 260-mμ absorption band of the bases (Figure 1). The amplitude of the Cotton effect of AMP is 1300° and that for GMP 900°. The pyrimidine nucleotides 2′(3′)-UMP and 2′(3′)-CMP exhibit single *positive* Cotton effects in this wavelength region (Figure 2). Their amplitudes are 4100° and 5300°, respectively.

Polymerization markedly alters the optical rotatory dispersion of the constituent nucleotides. Thus poly-A exhibits multiple Cotton effects in the wavelength region of the 260-mμ absorption band, with peaks at 284 and 240 mμ and a pronounced trough at 257 mμ (Figure 1). The total amplitude of the effects is 20,000°. In aqueous

[3] The optical rotatory dispersion of mononucleotides was measured at *p*H 5.15 in order to minimize ionization of the secondary phosphate group.

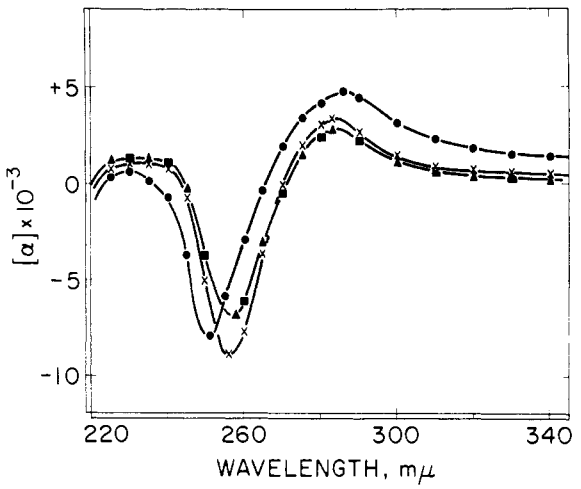

FIGURE 3: The effect of complex formation on the optical rotatory dispersion of poly-A and poly-U. Specific rotation is plotted against wavelength. Mixture of poly-A (0.5 mg/ml) and poly-U (0.5 mg/ml) in 0.01 M NaH_2PO_4, *p*H 6.9, at 35° (●) and 50° (▲) (complex is unstable at 50° [Fresco, 1963]). Algebraic sum of dispersion/curves of poly-A and poly-U, measured separately under the same conditions at 35° (x) and 50° (■).

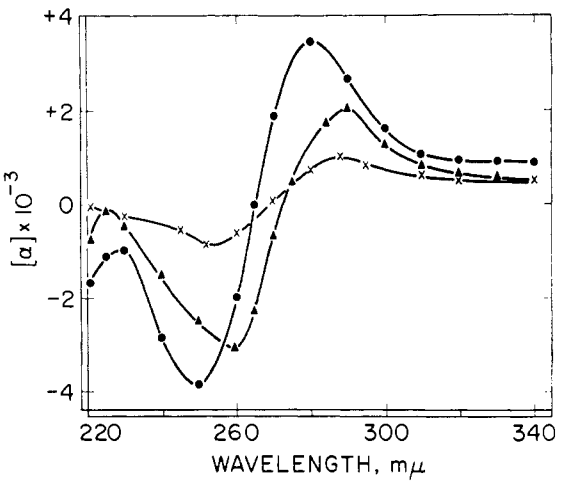

FIGURE 4: The effect of urea and alkaline hydrolysis on the optical rotatory dispersion of *E. coli* s-RNA. Specific rotation at 22° is plotted against wavelength. s-RNA (1.0 mg/ml) in 0.1 M succinate and 5×10^{-3} M Mg^{2+}, *p*H 5.15 (●), served as the control. An equivalent amount of s-RNA was dissolved in 8 M urea and the same buffer (▲) or hydrolyzed in alkali, extracted with perchloric acid, and dissolved in the succinate-Mg^{2+} buffer (x).[3]

solution, poly-A is known to have a considerable degree of secondary structure, which can be partially disrupted by urea and other H-bond breaking agents (Fresco and Doty, 1957; Steiner and Beers, 1959). In 8 M urea, the amplitudes of the Cotton effects of poly-A are reduced 50% and their crossover points (λ_0) at 270 and 245 mμ shift to 274 and 247 mμ, respectively, suggesting that these Cotton effects are in large measure dependent upon the conformation of the molecule.

In accord with this hypothesis, the optical rotatory dispersion of poly-U, which at room temperature is generally thought to be largely in a random configuration (Lipsett, 1960), resembles that of its constituent mononucleotide. The single Cotton effect is very similar in sign and shape to that of UMP; an increase in amplitude to 9500° is the only difference (Figure 2). In 8 M urea the amplitude decreases to 7000° and λ_0 shifts from 275 to 277 mμ. Thus urea produces a less pronounced alteration in the Cotton effect of poly-U than in that of poly-A.

At low ionic strength, poly-A and poly-U interact to form a 1:1 complex (Fresco, 1963). It is therefore important to examine whether or not the formation of the complex alters the optical rotatory dispersion of the component chains. The experimental rotatory dispersion curve of the 1:1 complex at 35°, and the theoretical rotatory dispersion curve which is obtained from the algebraic sum of the rotations of poly-A and poly-U, each measured separately under comparable conditions, are shown in Figure 3. The formation of the complex results in a hypsochromic shift of the crossover point from 271 to 266 mμ and in a slight increase in amplitude. As is to be expected, the shift does not occur under conditions where the complex is thermally dissociated. At 50° the two curves are superimposable. Similarly, on exposure to 8 M urea the rotatory dispersion curve of a 1:1 mixture of poly-A and poly-U becomes identical with the sum of the dispersion curves of poly-A and poly-U measured separately in 8 M urea, indicating the disruption of the complex.

In like manner, the optical rotatory dispersion of s-RNA, which contains a significant amount of secondary structure, would be expected to differ from that of its constituent mononucleotides and to undergo characteristic changes when its secondary structure is altered. The optical rotatory dispersion of the *E. coli* s-RNA hydrolysate, representing the sum of the rotational contributions of the component bases of s-RNA, is shown in Figure 4. The resultant positive Cotton effect is attributable to the greater contribution of the pyrimidine bases to the optical rotatory dispersion of the mixture. The amplitude of the effect is 1900° and the λ_0 is 269 mμ. Under the same conditions the intact s-RNA molecule has a positive Cotton effect whose amplitude is more than 3.5 times larger than that of the hydrolysate (e.g., 7000°). The peak of the s-RNA Cotton effect is at 280 mμ, the trough is at 250 mμ, and the λ_0 is at 265 mμ. A second peak appearing at 227 mμ suggests that a weak negative Cotton effect may be centered between 250 and 227 mμ, or that the dispersion at wavelengths lower than 220 mμ is also anomalous.

To ascertain whether the Cotton effect of *E. coli* s-RNA is dependent upon the conformation of the molecule, the effect of urea, temperature, and *p*H was

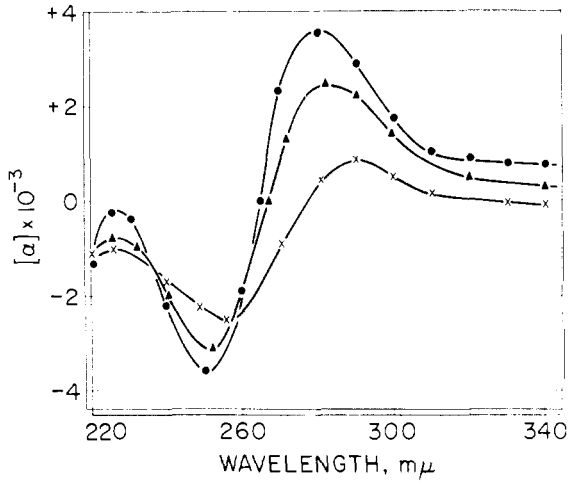

FIGURE 5: The effect of temperature on the optical rotatory dispersion of *E. coli* s-RNA. Specific rotation is plotted against wavelength. A solution of s-RNA (1.0 mg/ml) in 0.15 M NaCl, 0.015 M Na citrate, *p*H 7.1, and 22° (●) was heated to 65° (▲) and 85° (x). The decrease in amplitude began at 40°.

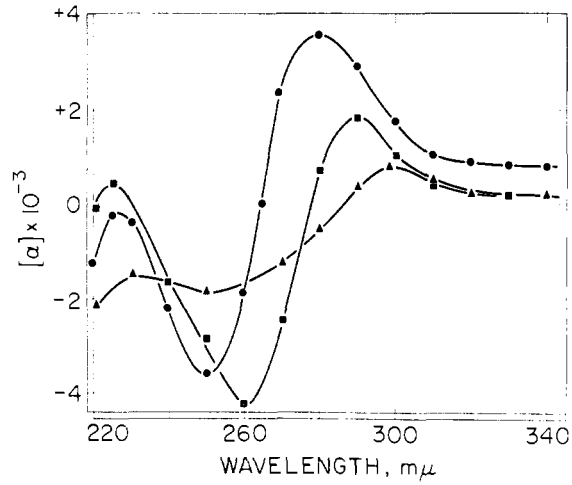

FIGURE 6: The effect of *p*H on the optical rotatory dispersion of *E. coli* s-RNA. Specific rotation at 22° is plotted against wavelength. s-RNA (1.0 mg/ml) is dissolved in 0.1 M sodium citrate at *p*H 2.5 (▲) and *p*H 7.1 (●). The *p*H of the latter solution was adjusted to 12.4 (■) and NaOH and the optical rotatory dispersion was measured immediately.

examined. The effects of these agents on the chromicity and specific rotations of RNA, DNA, and other polyribonucleotides have served as evidence for the presence of secondary structure in these macromolecules. In 8 M urea, the Cotton effect of s-RNA is reduced to 5600° and λ_0 undergoes a bathochromic shift of 8 to 273 mμ (Figure 4). Removal of urea by dialysis restores the rotatory dispersion to that characteristic of the native molecule, thus indicating reversibility of the process.

Similar changes occur as the temperature of a solution of s-RNA is raised gradually: the amplitude of the Cotton effect decreases, followed by a shift of λ_0 to longer wavelengths. The decrease in amplitude begins at 40° and the shift of λ_0 begins at 65°. At 85° the amplitude is 3320° and λ_0 is 275 mμ (Figure 5). When the solution is cooled to 22°, the curve is restored to precisely that obtained before heating. The changes in amplitude and λ_0 of the Cotton effect undergo a gradual transition upon heating which is in accord with the "melting curve" of s-RNA when its absorbance at 260 mμ is employed as the index of structural change (Doty *et al.*, 1959).

The Cotton effect of s-RNA is also altered when the *p*H of the solution is lowered or raised (Figure 6). Between *p*H 5 and 10 the rotatory dispersion curves are virtually identical. Protonation of the nitrogenous bases at *p*H 5 and lower progressively *decreases* the amplitudes of both the major peak and the trough, and λ_0 shifts toward the longer wavelengths. At *p*H 2.5, the amplitude is 2600° and λ_0 is 285 mμ. However, dissociation of the hydroxyl groups at *p*H 12.4 results in changes which are quite distinct from those which occur with low *p*H, upon heating or in urea. Notably, the trough of the Cotton effect *increases* in amplitude as does also the minor peak at 227 mμ, which becomes positive in sign. Concomitantly, there is a decrease in amplitude of the major peak at 280 mμ and a shift of λ_0 from 265 to 275 mμ. Similar rotational changes at alkaline *p*H have been reported for rat liver RNA (Samejima and Yang, 1964). The optical rotatory dispersion of s-RNA was measured immediately after adjustment of *p*H to 12.4, in order to minimize hydrolysis.

The Cotton effects arise, in part, from interactions of the purine and pyrimidine bases. Alterations in optical rotatory dispersion might also occur when the bases themselves are chemically modified. Bromination in aqueous media partially saturates the conjugated rings of U, C, and G, and alters the absorption of these bases between 240 and 320 mμ (Yu and Zamecnik, 1963a). In s-RNA, aqueous bromination also results in the loss of its amino acid–transfer function (Yu and Zamecnik, 1963b, 1964). Hence, *E. coli* s-RNA was brominated with increasing molar ratios of bromine to nucleotide to determine the effect on the optical rotatory dispersion of s-RNA. One mole or less of bromine/32 nucleotide residues does not alter the optical rotatory dispersion of s-RNA significantly. Bromination with 1 mole of bromine/16 residues of nucleotide decreases the amplitude of the Cotton effect slightly. At a molar ratio of 1 bromine/4 nucleotide residues, a bathochromic shift of λ_0 occurs in addition to a further decrease in amplitude (Figure 7). A concentration of bromine equimolar to that of the nucleotide brominates virtually all of U, C, and possibly a small fraction of G in s-RNA. At this juncture, the optical rotatory dispersion of s-RNA becomes negative, resembling that of AMP and GMP (Figure 7).

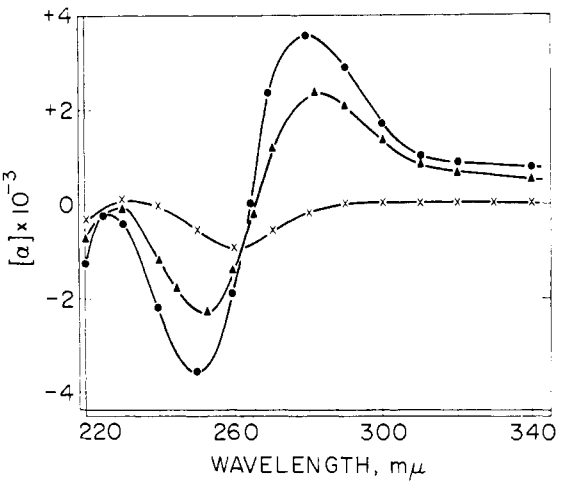

FIGURE 7: The effect of bromination on the optical rotatory dispersion of *E. coli* s-RNA. Specific rotation at 22° is plotted against wavelength. s-RNA (1.0 mg/ml) in 0.1 M Tris, 1×10^{-3} M Mg^{2+}, *p*H 7.6, served as the control (●). An equivalent amount of s-RNA was brominated with 1 mole bromine/4 nucleotide residues (▲) and 1 mole bromine/1 nucleotide residue (x) and dissolved in the Tris-Mg^{2+} buffer. From each molecule of bromine (Br_2) added, only one bromonium ion takes part in the actual addition reaction (Yu and Zamecnik, 1963a).

Discussion

The important discoveries of the last decade, demonstrating the pivotal function of ribonucleic acids in protein biosynthesis, have generated increasing interest in their physical and chemical properties. Some of the most significant functional features of RNA in amino acid activation and protein synthesis, however, have remained unexplained in terms of the chemical and physical details of these molecules. Except for the minor base constituents of s-RNA, analyses of the total composition of different RNA's show few distinguishing features which might account for the functional activities of this RNA. It has become reasonable to suspect that the structural properties of these molecules may be crucial in governing the specificities of their function.

In this regard, the role of transfer or soluble RNA in the protein synthetic process is particularly noteworthy. Amino acid specific s-RNA molecules react with each of the twenty amino acids and their respective synthetases to form aminoacyl s-RNA esters, which then react with the RNA template to promote peptide synthesis. While complementary pairing of nucleotide bases is a plausible mechanism in this RNA-RNA type of interaction, no comparable mechanism exists for the interaction of s-RNA with the synthetase enzyme. It may be postulated that a particular arrangement of amino acid side chains of the protein forms a unique conformational fit with a constellation of bases in the polynucleotide chain, and we regard this as the most likely type of interaction. The secondary and tertiary structure of s-RNA, which is still largely unaccounted for by objective physical measurements, assumes a critical role in such interactions. Hence knowledge of the structural details of the molecule such as can be obtained with optical rotatory dispersion measurements is crucial and fundamental to the understanding of the process.

Toward this end we have undertaken in the present study a systematic investigation of the optical rotatory dispersion of s-RNA, the purine and pyrimidine nucleotides, homopolymers of adenylic acid and uridylic acid, and the poly-A–poly-U complex as model systems of RNA in the 260-mμ absorption region of these molecules. This spectral region, which until very recently has not been accessible to study because of instrumental limitations, is of particular interest since formation of secondary structure in polynucleotides is thought to involve the interaction of the component bases. In addition, these systems have been modified by conventional physicochemical procedures known to be effective in altering secondary structure in order to ascertain their effects on optical rotatory dispersion. These data can thus be compared with those recently reported for DNA and other RNA's (Samejima and Yang, 1964) and correlated with other physicochemical parameters of the molecule. In this regard, much is already known about the structure of the synthetic polynucleotides and DNA which is not existent for s-RNA. While it is recognized that essential chemical differences must be taken into account, the similarities in the rotatory properties are nevertheless significant and indicative of the presence of similar structural features in all of these molecules.

Mononucleotides[3] exhibit single Cotton effects of small amplitude between 350 and 220 mμ. Similar results have been reported for the deoxyribomononucleotides (Yang and Samejima, 1963). The optical rotatory dispersion of the mononucleotides shows details which are different in kind from those discernible from their corresponding absorption spectra (Voets *et al.*, 1963). The Cotton effects demonstrate that the component bases are in an asymmetric environment, probably owing to their orientation by attachment to the optically active ribose residues. The effects differ in sign, location, and amplitude (Figures 1 and 2). Hence just as the spectral characteristics are sufficiently distinct to differentiate one nucleotide from another, so they can also be differentiated by their Cotton effects.

Synthetic polynucleotides have long served as model systems for RNA. The absorption spectrum of poly-A, in the region of the 260-mμ band, is closely similar to that of AMP, differing only in absorption. However, its optical rotatory dispersion in this wavelength region is strikingly different from that of AMP. In place of the single, small negative Cotton effect of AMP, the optical rotatory curve now consists of two Cotton effects of large amplitude, a positive one whose crossover point is at 270 mμ and a negative one with a crossover point at 245 mμ. Such pronounced changes in the optical

rotatory properties of the bases indicate an asymmetric ordering of the bases which is not accountable on the basis of the polymerization process. Rather the changes in rotation must be owing to the superimposed changes in conformation occurring through base stacking and hydrogen bonding between the bases. In accord with this interpretation, the amplitude of the Cotton effects decreases more than 50% on addition of urea. Furthermore, the optical rotatory dispersion curve corresponds in sign, position, and relative magnitude to the circular dichroism of poly-A at neutral *p*H (Brahms, 1963) and is in accord with the predictions from exciton theory for helices having absorption bands polarized perpendicularly to the helical axis (Tinoco, 1964). Thus it would appear that the asymmetric alignment of the bases in poly-A is related to the helical conformation of the molecule.

Studies of poly-U, which exists in aqueous solution as a random coil, give further support to these conclusions. In contrast to the optical rotatory dispersion of poly-A, which is entirely different from that of AMP, the single positive Cotton effect of poly-U is similar to that of UMP and, as expected, undergoes only a slight change in urea. Conclusions regarding the factors underlying the somewhat larger amplitude of the Cotton effect of the polymer cannot be drawn at this time. There may be some residual base-base interactions within the molecule, or alternatively the nucleotide residues in diester linkage may intrinsically exhibit a larger Cotton effect corresponding to the residual hypochromicity of oligonucleotides (Michelson, 1962).

Formation of the 1:1 complex of poly-A with poly-U produces a further change in optical rotatory dispersion which can be related to the known conformation of the resultant complex. The hypsochromic shift of 5 mμ of the optical rotatory curve and the increase in amplitude of the Cotton effect upon interaction are consistent with the planar stacking of bases and further ordering of the structures of poly-A and poly-U. Both these effects are reversed when the complex is dissociated at elevated temperature or in the presence of urea. The structure of the 1:1 complex of poly-A with poly-U has been characterized by X-ray diffraction to be a double-stranded helix (Rich and Davies, 1956). The A and U residues are hydrogen bonded and stacked so that the planes of the paired bases are approximately normal to the axis of the helix. Hence these rotatory changes can be attributed to the formation of the double-stranded helix and the interaction of A with U and their orientation within the helical framework of the molecule.

The bulk of the physicochemical data indicates that s-RNA is a flexible single-stranded chain, intracompacted through secondary coiling and tertiary folding of the molecule, and capable of undergoing reversible changes in conformation. These conclusions have been based largely on the hyperchromicity of s-RNA under varying conditions of *p*H, temperature, and solution, and by comparison with the behavior of DNA, which is known to be helical, under analogous circumstances (Doty *et al.*, 1959; Fresco *et al.*, 1960). The manner in which the helical segments are maintained in RNA, as well as the role of tertiary structure, has not been fully established. In this regard, optical rotatory dispersion offers a means of great promise to study these conformational features of s-RNA. As with poly-A and the poly-A–poly-U complex, the optical rotatory dispersion of s-RNA is different in shape, amplitude and location from that of its component bases. Upon exposure to urea or low *p*H, or upon heating, the amplitude of Cotton effect decreases and λ_0 shifts to a higher wavelength, changes analogous to those observed with poly-A and with poly-A–poly-U complex when their secondary structure is destroyed. These changes are fully reversible and occur over a wide range of temperature and *p*H. This is analogous to the broad melting curve of s-RNA at 258 mμ which led Doty *et al.* (1959) to suggest that s-RNA contains several helical segments of varying stability, maintained by hydrogen bonding and stacking of bases. In fact, the striking similarity of the optical rotatory dispersion curve of s-RNA with that of the poly-A–poly-U complex suggests that the helical regions are structurally similar to those of the double-stranded poly-A–poly-U complex and involve the specific pairing of A with U and hence also of C with G. Comparison of the spectral properties of DNA with those of the synthetic polynucleotides of DNA has led to similar conclusions concerning the types of base pairs that are present (Doty *et al.*, 1959; Fresco *et al.*, 1960).

It may well be that the magnitude and location of the Cotton effects in s-RNA may lend themselves to the determination of helicity of the molecule in a manner analogous to that employed for the calculation of helical content of proteins and polypeptides, based on the amplitude of their Cotton effect near 225 mμ (Blout, 1963).

The optical rotatory dispersion of s-RNA[4] bears only a qualitative resemblance to that of native DNA (Samejima and Yang, 1964). However it is of note that at *p*H 12.4 the 228-mμ peak of s-RNA of rat liver RNA becomes dextrorotatory, and the optical rotatory dispersion curves of RNA now more closely resemble that of alkaline denatured DNA (Samejima and Yang, 1964). The physical and chemical significance of such differences in the optical rotatory dispersion of RNA and DNA and the similarity of their rotatory properties at alkaline *p*H deserve further experimentation.

These studies indicate that the rotatory dispersion of s-RNA represents the combined rotational contributions of the mononucleotide subunits and the super-

[4] The dispersion curve of s-RNA is also closely similar to that reported for rat liver RNA (type unspecified) by Samejima and Yang (1964), suggesting that such Cotton effects may be a feature common to the conformational arrangement of RNA in general. The rotatory dispersions of yeast s-RNA, *E. coli* ribosomal RNA, and the purified valyl-s-RNA ester of *E. coli* in 0.1 M succinate, 0.001 M Mg^{2+}, *p*H 5.15 and 23°, have been measured and are indistinguishable from that of *E. coli* s-RNA. Thus it appears that in spite of the differences in the relative composition and linear sequence of the bases in these molecules, under these conditions, a similar amount of bases is associated in helical array.

imposed effect of the secondary structure of the polynucleotide. A major portion of the Cotton effect is attributable to the orderly stacking of bases, plus the hydrogen bonding of complementary bases in the polynucleotide structures, as previously suggested by other investigators (Moffitt, 1956; Simmons and Blout, 1961; Boedtker, 1960; Cox and Littauer, 1960; Fresco *et al.*, 1961; Kasha, 1961; Michelson, 1962; Ts'o *et al.*, 1962). That the greatest contribution to the Cotton effect is due to the secondary structure of s-RNA rather than to the primary structure is indicated by the great loss in Cotton effect as a consequence of hydrolysis of the polynucleotide and as the result of exposure to urea and high hydrogen ion concentration. In 8 M urea or at low *p*H and high temperatures, however, the Cotton effects are still considerably larger in amplitude than that of hydrolyzed s-RNA, suggesting that there are some residual asymmetric interactions within the molecule. The residual hypochromicity observed with RNA under similar conditions is consistent with this conclusion. Furthermore, it has been shown that ionic strength, monovalent and divalent cations, and in particular the intrinsically bound metals of the first transition series stabilize the secondary structure of RNA. These findings have been interpreted to denote the existence of tertiary structure in RNA, which is produced and maintained by metal to nucleotide bonds (Fuwa *et al.*, 1960). Thus tertiary folding is another factor which may influence the optical rotatory dispersion of the molecule. The present studies were conducted in high ionic strength media, and with added divalent metals. Delineation of the effects of these factors upon the optical rotatory dispersion of s-RNA should provide further understanding of the tertiary structure of this molecule.

Bromonium ions in aqueous solution form addition compounds with the nucleotide bases. Between *p*H 5.7 and 2.2, bromine reacts preferentially with U and C, more slowly with G, and not at all with A. Partial saturation of the conjugated rings ensues, and alters the absorption of the affected bases in the wavelength region between 320 and 240 mμ. At concentrations of 1 or more moles of bromine/16 nucleotide residues, the amplitude of the Cotton effect decreases, and λ_0 shifts toward the longer wavelengths, indicative of the disruption of secondary structure. When virtually all of the U and C and a small fraction of G have been brominated, the optical rotatory dispersion resembles that of AMP or GMP, since the modified bases themselves lose absorption in this wavelength region (Figure 7). The effect of bromination on the amino acid transfer function of s-RNA has been studied (Yu and Zamecnik, 1963a, 1964). Inhibition of enzymatic synthesis of aminoacyl RNA esters occurs when s-RNA is brominated with as little as 1 mole of bromine/40 nucleotide residues. This highly specific effect suggests that the inhibition of amino acid acceptance may be owing to the modification of one or two particularly sensitive residues in the molecule (in a nonrandom bromination) which are involved in the recognition of activating enzymes. Alternatively, bromination may also irreversibly alter the conformation of s-RNA and thus exert its inhibitory action indirectly. With 1 mole of bromine/40–32 nucleotide residues, where a significant degree of inhibition of esterification already occurs, optical rotatory dispersion changes are not detected. Decrease in amplitude of the Cotton effect is observed only when the bromination ratio exceeds 1:20. In addition a shift of the Cotton effect to longer wavelengths occurs when the bromination ratio is increased to 1:4.

Although the data are not yet sufficient to establish precise correlation between the changes in activity and in optical rotatory dispersion resulting from bromination, they would seem to favor the suggestion that the aminoacyl esterification is exquisitely dependent on correct structural conformation of s-RNA. Thus it may be sensitive even to very minute alterations at very low levels of bromination, the consequence of which may not be discernible by measurements of optical rotatory dispersion. The verification of this hypothesis must await further experimentation. Possibly titration of the system under different conditions of *p*H and temperature or alternate physicochemical methods may decide whether or not such localized changes accompany the functional effects of bromine.

Soluble RNA is reported to exist in cells mostly in the free state. This permits the conclusion that its conformation in solution may well reflect its state within the cell. However, the ability of this molecule to undergo reversible conformational changes suggests the possibility that it may assume different and specific conformations upon interaction with the activating enzymes. Whether the event involves change in conformation of the RNA molecule and of the protein at the time of mutual recognition is basic to the understanding of this enzymic reaction. In principle, optical rotatory dispersion is suited for the detection of such interactions since the Cotton effects characteristic of the secondary structures of both the protein and s-RNA can be measured simultaneously. This approach has already been employed successfully to study the interaction of TMV-RNA with protein (Simmons and Blout, 1961). In the present investigation we have carried out preliminary studies on the effect of adding a partially purified *E. coli* valine amino acid synthetase to the *E. coli* valyl s-RNA ester. No significant alterations in Cotton effects of s-RNA and in the amplitude of the 233-mμ trough of the protein could be detected. The limited availability of material has precluded an extensive and detailed examination of this problem at this time. However, efforts in this direction as well as the delineation of the optical rotatory dispersion properties of individual amino acid–specific s-RNA's are in progress.

Addendum

Since the submission of this manuscript, Fasman *et al.* (1964) have reported an optical rotatory dispersion study of polycytidylic acid. The Cotton effect of poly-C in the wavelength region of 350–225 mμ was measured under conditions in which the effect of hydrophobic

forces and hydrogen bonds upon secondary structure could be ascertained independently. It was concluded that at *p*H 4.1 the stabilization of helical structure of poly-C is dependent on hydrogen bonding while hydrophobic bonds are largely responsible for the maintenance of secondary structure at *p*H 7.0.

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