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Optical Rotatory Dispersion of DNA and RNA*

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Optical rotatory dispersion (ORD) of salmon sperm DNA was measured over a wavelength range of 190–600 mμ. The dispersion curve obeyed a one-term Drude equation at wavelengths above 350 mμ. Strong Cotton effects occurred in the ultraviolet region with three peaks at 290, 228, and 200 mμ, two troughs at 257 and 215 mμ, and cross-overs (zero rotations) at about 274 and 248 mμ. Heat (90°) or alkaline (*p*H 12.3) denaturation of DNA depressed the Cotton effects; the profile below 250 mμ appeared to be particularly sensitive to the breaking up of the secondary structure. The ORD of rat liver RNA, which followed a simple Drude equation down to about 310 mμ wavelength, showed a strong peak and trough at 280 and 252 mμ, with a cross-over at 265 mμ. A weak peak and trough occurred at 228 and 218 mμ, followed by another very strong peak at 195 mμ. In alkaline solution or at high temperatures the 280 mμ peak and 252 mμ trough shifted to 290 and 260 mμ.

The ORD[1] of nucleic acids follows a simple Drude equation

$$[\alpha]_\lambda = k/(\lambda^2 - \lambda_c^2) \qquad (1)$$

in the visible region (Fresco, 1961; Ts'o *et al.*, 1962). At wavelengths below 350 mμ, however, equation (1) is no longer applicable to DNA (Fresco, 1961). The presence of strong absorption bands in the ultraviolet region makes measuring optical rotations of nucleic acids very difficult. Nevertheless, Simmons and Blout (1960) were able to show a positive Cotton effect for the RNA isolated from TMV with an inflection point around 260 mμ, which shifted to 272 mμ in 8 M urea. Fresco *et al.* (1961) further demonstrated a multiple Cotton effect in calf thymus DNA and calf liver RNA between 230 and 300 mμ, which contained considerable detail not evident in the corresponding absorption spectra; their study was, however, done with 0.02% solutions ($A_{260} = 4$). Later, Urnes and Doty (1961) reported that rotatory artifacts simulating Cotton effects can be produced easily in regions of high absorbance, and they suggested a critical limit of 2 for the absorbance of solutions prepared for ORD experiments. In view of these findings we reinvestigated the rotatory properties of two DNA (salmon and calf thymus) and one RNA (rat liver) solutions using a spectropolarimeter that extends measurements down to 190 mμ.

Experimental Procedures

Materials.—The DNA and RNA solutions were clarified by filtering through a medium sintered-glass filter before being measured. Their concentrations (in the native state) were determined spectrophotometrically, assuming $A^{1\%}_{1\,cm} = 200$ at 260 mμ.

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[1] Abbreviations used in this work: ORD, optical rotatory dispersion; TMV, tobacco mosaic virus; dAMP, deoxyadenosine-5′-phosphate; dCMP, deoxycytidine-5′-phosphate; dGMP, deoxyguanosine-5′-phosphate; dTMP, deoxythymine-5′-phosphate.

Optical Rotatory Dispersion.—The early ORD measurements were made with a Rudolph manual spectropolarimeter (Model MSP 4), the calibration of which has been described elsewhere (Yang and Samejima, 1963a). We repeated most of the experiments in the ultraviolet range using a Cary Model 60 recording spectropolarimeter. This instrument was also calibrated with a sucrose solution (National Bureau of Standards grade), the rotations of which obey a one-term Drude equation between 220 and 600 mμ: our values were $[\alpha]_\lambda = 21.65/(\lambda^2 - 0.0217)$; Harris *et al.* (1932) found $[\alpha] = 21.676/(\lambda^2 - 0.0213)$. (Below 220 mμ the rotation of sucrose became more dextrorotatory than that calculated from the Drude equation; for example, it was 33% higher at 195 mμ.) Virtually no band-width dependence of the rotations was detected for the Cary instrument, thus eliminating the laborious extrapolation to zero slit width (on the monochromator). The rotations remained unchanged when the sample cell was placed in series with another cell containing an absorbing material such as formamide-water and K_2CrO_4 solution, indicating that there was no significant stray light (Yang and Samejima, 1963a). The polarimeter tubes used varied from 10 to 0.01 cm in light path, the latter being calibrated with a solution of known rotations. In regions of the absorption bands, the concentration of the solution was so adjusted that the absorbance against empty reference cell was always much less than 2. The experimental data were reproducible, and the specific rotations showed no concentration dependence beyond normal experimental error. Data obtained below 210 mμ were less precise than those at longer wavelengths, mainly because of the very dilute solutions used (to reduce absorbance) and the increase in the noise level of the instrument near its limit of wavelength range.

Spectrophotometry.—Absorption spectra were measured with an Optica spectrophotometer.

Results

DNA.—Figures 1 and 2 show the ORD of native and denatured DNA. To reduce the absorbance

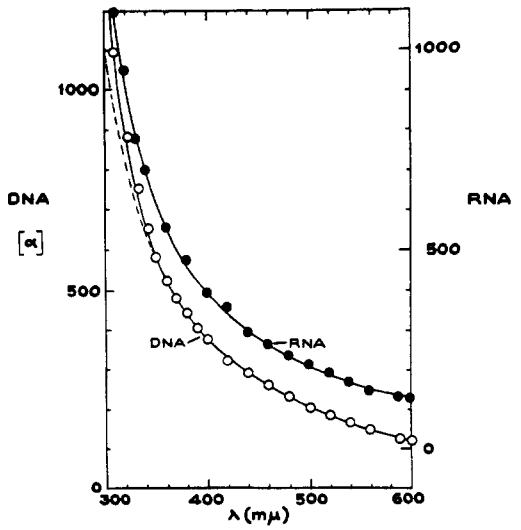

FIG. 1.—Optical rotatory dispersion of salmon sperm DNA and rat liver RNA in the visible region. ○, DNA in 0.15 M NaCl with 0.015 M sodium citrate (*p*H 7); ●, RNA in 0.14 M NaCl with 0.01 M phosphate buffer (*p*H 7). The broken line is the extension of the simple Drude equation. (With Rudolph MSP 4.)

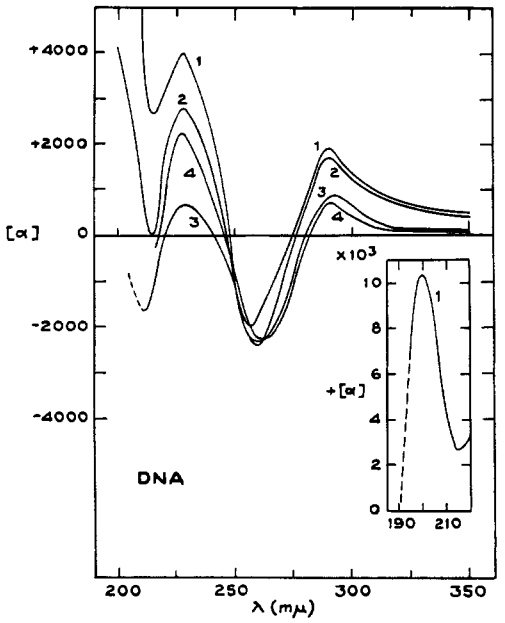

FIG. 2.—Optical rotatory dispersion of salmon DNA in the ultraviolet region. (1) In 0.15 M NaCl with 0.015 M sodium citrate (*p*H 7) (above 230 mμ) or in 0.15 M KF (*p*H 7) (below 240 mμ) at 27°; (2) in citrate-NaCl (boiled for 10 minutes and quickly cooled); (3) same as in (1) except at 90°; (4) in 0.15 M KF + NaOH (*p*H 12.3). (With Cary 60.)

due to the salts in the ultraviolet region, we used 0.15 M KF instead of the citrate-NaCl solution for measurements below 240 mμ. The dispersion curve in the visible region (Fig. 1) is featureless, as expected, and obeys equation (1) down to about 350 mμ, just as Fresco (1961) reported. Our results of k = 39.0 and λ_c = 0.234 μ agree fairly well with those of Fresco (1961) (k = 40.0; λ_c = 0.230 μ) and Ts'o *et al.* (1962) (k = 38.0; λ_c = 0.220 μ), although those investigators used calf thymus DNA rather than salmon DNA. Our $[\alpha]_D$ of +125 also agrees with that reported by the other laboratories but is somewhat lower than the value of +131 calculated from equation (1).

Native salmon DNA shows three peaks and two troughs of the Cotton effect in the ultraviolet region (Fig. 2, curve 1): the specific rotations were $[\alpha]_{290}$ = +1900, $[\alpha]_{257}$ = −2000, $[\alpha]_{228}$ = +4000, $[\alpha]_{215}$ = +2600, and $[\alpha]_{200}$ = +10,400; the cross-overs (zero rotations) occurred at approximately 274 and 248 mμ. The magnitude of the Cotton effect was greatly reduced when the *p*H of the solution was adjusted to 12.3 (curve 4) or, more strikingly, when the temperature was raised to 90° (curve 3). The Cotton effect was replenished when DNA denatured by 10 minutes' boiling was quickly cooled in an ice bath (curve 2). The 290 mμ peak shifted to about 292 mμ in alkaline solution, and the 257 mμ trough also showed a red shift upon denaturation by alkali or by heat. (In a preliminary communication [Yang and Samejima, 1963b] we reported that DNA shows two peaks at 290 and 223 mμ, two troughs at 225 and 192 mμ, and cross-overs at about 273, 243, and 210 mμ. These early data were obtained with a manual spectropolarimeter. Whereas the experimental points above 220 mμ were very close to those in Figure 2, we had not been able to detect another trough and peak below 220 mμ with the old instrument. Since the present recording spectropolarimeter performs better and thereby gives more refined detail than the manual instrument, we accordingly report the new data in Figure 2.)

For comparison we also measured the Cotton effects of calf thymus DNA (Fig. 3), using the same preparation studied by Fresco *et al.* (1961). (Because we had exhausted our supply of the sample sent to us by Professor Fresco, we were unable to repeat this experiment using the Cary 60 instrument. Nevertheless, we believe that the data reported in the figure are essentially correct.) The shape of the dispersion curve for calf thymus DNA was the same as that of salmon DNA, and the magnitude of their peaks and troughs (above 220 mμ) was roughly similar.

The peak at 290 mμ in Figures 2 and 3 resembles that reported by Fresco *et al.* (1961); however, instead of another peak at 257 mμ, we found a trough at 257 mμ. Fresco *et al.* (1961) reported dextrorotations only between 230 and 300 mμ, which again differ from our results in Figures 2 and 3. Those investigators could not measure the Cotton effect below 230 mμ because of the limitation of their instrument. On the other hand, their data for native DNA and for denatured DNA at 95° and at 30° (after heating at 95°) are comparable to our data in the same wavelength range.

The absorption spectra of DNA (not shown here) has the usual 260 mμ maximum. We also observed another much stronger peak at about 195 mμ. Both maxima exhibited hyperchromicity upon denaturation of DNA. These findings agree with the most recent findings of Voet *et al.* (1963).

RNA.—The ORD of rat liver RNA in the visible region is included in Figure 1. The shape and the magnitude of the curve were very similar to those for DNA, but the Drude equation is applicable to RNA down to about 310 mμ (350 mμ for DNA). The two constants for equation (1), k = 40.0 and λ_c = 0.244 μ, compared fairly well with the data of Ts'o *et al.* (1962) for pea RNA (k = 46.5 and λ_c = 0.247 μ). Our experimental $[\alpha]_D$ of +133 (+139 calculated from equation 1) is lower than their value of +171. The rotations of RNA dropped sharply in alkaline solution (*p*H 12.3). The ORD was anomalous; it was levorotatory above 430 mμ and dextrorotatory below that (not shown here). We also found a gradual change in rotation with time for moderately concen-

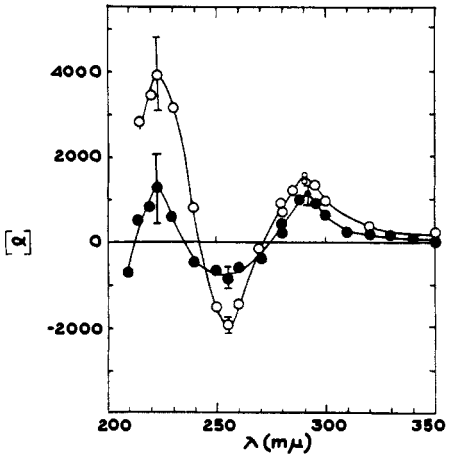

FIG. 3.—Ultraviolet rotatory dispersion of calf thymus DNA. ○, in 0.15 M NaCl with 0.015 M sodium citrate (*p*H 7); ●, in citrate + NaCl + NaOH (*p*H 12.3). (With Rudolph MSP 4.)

trated solutions of RNA presumably because of their instability at such high *p*H.

Figure 4 illustrates the Cotton effects of RNA in the ultraviolet region. For RNA in phosphate buffer (*p*H 7) above 230 mμ we observed a strong peak and trough: $[\alpha]_{280} = +3400$ and $[\alpha]_{252} = -3500$, with a cross-over at 265 mμ. A weak peak and trough occurred at 228 and 218 mμ, followed by another very strong peak, $[\alpha]_{195} = +11{,}600$. In alkaline solution (*p*H 12.3) or at 90° the 280 mμ peak and the 252 mμ trough shifted to 290 and 260 mμ, with a cross-over near 275 mμ. Note that the absorption maximum of rat liver RNA also shifted from 259 to 262 mμ when the *p*H of the solution was raised above 12. For the Cotton effects below 230 mμ the changes were more complicated; the 228 mμ peak turned levorotatory at high temperature, whereas it showed a blue shift with a much larger magnitude in alkaline solution. Because of experimental difficulties we could not follow the 195 mμ peak under these conditions. Our curves shown in Figure 4 are similar to those of Simmons and Blout (1960) for TMV-RNA, but the magnitudes of the data are much different. Our findings differ from those reported by Fresco *et al.* (1961) for calf liver microsomal RNA, except in the region above 270 mμ. We could not detect the 257 and 265 mμ peaks found by those authors in the wavelength range of 250–270 mμ. Their results may have been influenced by their use of solutions with too high absorbance. Our data obtained below 230 mμ were less precise than those obtained at longer wavelengths. Due to strong absorption of the RNA, which necessitated the use of very dilute solutions, the absolute magnitude of the rotations between 210 and 230 mμ was only in the order of ±0.001°.

DISCUSSION

Cotton Effect and the Secondary Structure of DNA.—We agree with Fresco *et al.* (1961) that the multiple Cotton effects of nucleic acids are closely related to their secondary structure. These conformation-dependent effects diminish when the ordered stacking of the base pairs is disrupted. This is especially true of the 228 mμ peak, which is highly sensitive to the extent of denaturation and almost disappears at 90° (Fig. 2, curve 3). That heat-denatured DNA that has been quickly cooled (curve 2) has stronger Cotton effects than the DNA solution at 90° may indicate that some

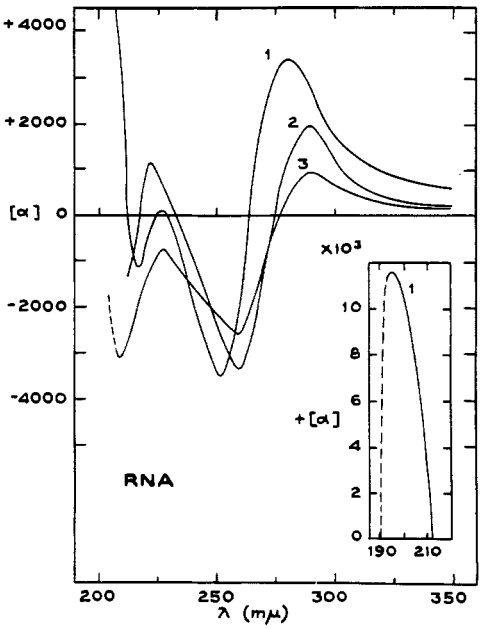

FIG. 4.—Optical rotatory dispersion of rat liver RNA in the ultraviolet region. (1) In 0.14 M NaCl with 0.01 M phosphate buffer (*p*H 7) (above 230 mμ) or in 0.14 M KF with 0.01 M phosphate buffer (below 240 mμ) at 27°; (2) at *p*H 12.3 (NaOH was added to the solvent used in 1); (3) same as in (1) but at 90°. (With Cary 60.)

secondary structure is reformed during quick cooling. In alkaline solution (*p*H above 12) or at high temperature (about 90°), however, DNA should lose its ordered structure; indeed, the ORD above 250 mμ seem to be nearly identical in both cases (curves 3 and 4), although the 228 mμ peak at *p*H 12.3 is still stronger than that at 90°. The 290 mμ peak does not disappear completely upon denaturation; it probably drops to a level representing the mean optical activity of the mononucleotides. Recently we have shown that dAMP, dCMP, dGMP, and dTMP all have a single Cotton effect (above 220 mμ) (Yang and Samejima, 1963b), which seems also to support the contention that the secondary structure of DNA is largely responsible for the 228 mμ peak. However, because the ORD of the mean residues in a polynucleotide chain could differ significantly from that of a mixture of mononucleotides, it is difficult to tell whether the residual 228 mμ peak at 90° represents the presence of some secondary structure.

Although our results (Figs. 2 and 3) differ in certain aspects from those of Fresco *et al.* (1961), their interpretation is nevertheless still valid. On the basis of recent theoretical developments (see, for example, Rich and Kasha, 1960; Kasha, 1961; DeVoe and Tinoco, 1963) Fresco *et al.* (1961) suggest that the 290 mμ peak very likely arises from n-π^* transitions and that the other Cotton effects at shorter wavelengths are attributable to π-π^* transitions. To what extent the π-π^* transitions may also contribute to the 290 mμ peak still remains to be investigated.

To understand the origin of the Cotton effects one must also study the circular dichroism and calculate the rotational strengths of the polymers. In a recent theory Tinoco (1964) states that the shape of the circular dichroism for DNA resembles a Cotton-effect curve. His theoretical calculations of ORD from circular dichroism fit the data given in Figure 3 for the native DNA. Tinoco's prediction of circular dichroism also explains the recent experimental data on polynucleotides (Brahms, 1963) and DNA (J. Foss, personal

communication, 1963). Thus we begin to have a better understanding of the Cotton effects of nucleic acids.

In a preliminary communication (Yang and Samejima, 1963b) we observed that dCMP will dominate the 290 mμ peak in a mixture of deoxyribonucleotides. Therefore, a close relationship may exist between the (cytosine + guanine) content and the 290 mμ peak for DNA when its secondary structure is destroyed; that is, the higher the (C + G) content, the larger the magnitude of the peak. Of course, the ORD of the mononucleotides could vary not only in magnitude but even in sign when they are incorporated into a polynucleotide chain. This hypothesis could be tested by studying the Cotton effects of DNA of various species. Furthermore, a comparative study of the Cotton effects of the DNA's in their native state might provide some information concerning the stacking interactions of the base pairs in the different species.

ORD of RNA.—Figure 4 suggests that RNA also possesses a certain degree of secondary structure and that its Cotton effects are significantly changed in alkaline solution or at high temperature. The red shift of the 280 mμ peak and 252 mμ trough (curves 2 and 3) might indicate the disruption of intramolecular hydrogen bonding between the stacking chromophores (bases). On the other hand, the weak 222 mμ peak actually increases its magnitude in alkaline solution or at high temperature. Why the peaks shift in opposite directions is not clear. Of interest is the similarity of the ORD curve for DNA (Fig. 2, curve 4) and RNA (Fig. 4, curve 3) in alkaline solution. DNA and RNA also appear to have very similar strong peaks at 200 mμ and 195 mμ, respectively, with nearly identical magnitude (Figs. 2 and 4). Since we are still unable to measure this Cotton effect at high temperature because of experimental difficulties, it is not certain whether this peak is closely related to the secondary structure or not. Note also that the measurements below 200 mμ approach the instrument limit, and therefore should be viewed with some reservation until we have more concrete evidence by studying the Cotton effects of various nucleic acids.

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Species Variation of the RNA Methylases*

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The complex of enzymes which methylate t-RNA at the polynucleotide level were found to be species specific. Therefore a preparation of t-RNA (transfer ribonucleic acid) from a given source, while fully methylated with respect to its homologous enzymes, will receive methyl groups *in vitro* from a heterologous enzyme source. Some closely related organisms exhibit a similarity in their pattern of methylation of foreign t-RNA's. A study of amino acid–specific t-RNA's with heterologous enzymes revealed that the different t-RNA's do not behave uniformly as substrates for methylation. Therefore the species variation may be restricted only to some of the t-RNA's.

It has recently been demonstrated (Borek *et al.*, 1962; Fleissner and Borek, 1963) and confirmed in several laboratories (Svensson *et al.*, 1963; Starr, 1963; Gold *et al.*, 1963; Comb, 1963) that the methylated bases of t-RNA are acquired by the methylation of the preformed polynucleotide chain by a complex of enzymes. The demonstration of the sequential nature of t-RNA synthesis was made possible by the discovery of a mutant, *Escherichia coli* $K_{12}W6$, in which, as a result of two independent mutations, the primary synthesis and the methylation of the product are uncoupled (Borek *et al.*, 1955). During methionine starvation this organism produces a species of t-RNA devoid of methylated derivatives of the component bases (Mandel and Borek, 1963). The availability of a naturally produced nonmethylated t-RNA as a substrate made possible the demonstration of the existence of a t-RNA-methylating enzyme. The enzyme system proved to be a complex whose individual members are endowed with highly restricted specificities (Fleissner and Borek, 1963; Gold *et al.*, 1963).

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