EXTRINSIC COTTON EFFECTS OF ACRIDINE ORANGE BOUND TO NATIVE, DENATURED AND FORMYLATED DEOXYRIBONUCLEIC ACID*

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Summary. Optical rotatory dispersion curves and absorption spectra were obtained for acridine orange complexes formed by native DNA, heat denatured DNA and formaldehyde-treated DNA over a wide range of the ratio of anionic sites to dye. With regard to the controversy over whether induced optical activity does or does not occur when denatured DNA is used, the evidence appears to favor the view that denaturation, regardless of the method used, modifies but does not prohibit the appearance of extrinsic Cotton effects.

Extrinsic Cotton effects have been observed when acridine orange (AO) is bound to poly-α-L-glutamic acid in the random-coil conformation at pH 8 (1), or when either proflavine (2) or AO (3, 4, 5) is bound to heat denatured DNA. Consequently these results differ from the findings by Neville and Bradley that the helical conformation of DNA is requisite for induced optical activity (6). Confirming the result of these workers, however, Gardner and Mason (7) have reported that an induced Cotton effect appeared only if the DNA heated in the presence of AO was annealed.

In view of the lack of agreement concerning the necessity of the helical structure of the host polymer, we studied DNA and related polymers to determine the effects of their conformation on induced Cotton effects. For this purpose the optical rotatory dispersion (ORD) curves and absorption spectra were obtained for the AO complexes formed by native, heat denatured and formaldehyde-treated DNA.

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EXPERIMENTAL

All concentrations of DNA were expressed in terms of phosphorus. Phosphate analysis was by the method of Chen, et al (8). Unless specified, the pH was between 6 and 6.5 without buffer. The solvent was 0.001 N NaCl. The materials and procedures were the same as those described previously (3). Heat denaturation was carried out by heating the DNA solution (2×10^{-4} M) in a screw capped test tube, in a boiling water bath for 20 minutes and then quenching in an ice bath. AO was added to this denatured DNA at room temperature to form the heat denatured DNA-AO complex. This mixing order was observed throughout to avoid precipitation. Native DNA was also heated and quenched in the presence of AO. Formylated DNA was prepared by heating DNA in the presence of 2% formaldehyde. AO was then added at room temperature. Reagent grade formaldehyde was used according to the procedure of Grossman, et al (9). In these experiments the DNA was in 0.01 M Na_2HPO_4. An equal volume of AO in water was added to give final concentrations of 0.005 M Na_2HPO_4 and 1% formaldehyde. The molar rotation, $[R]^D_C$, and molar extinction coefficient, ϵ_M, were calculated according to previous procedures (3).

RESULTS

A--The ORD and absorption spectra of native DNA-AO.

The ORD curves and absorption spectra of complexes between AO and native DNA over a range of the ratio of DNA-phosphate to dye (P/D) from 0.666 to 5 were determined to extend the previous study (3). Between P/D 1.66 and 1, the ORD curves shown in Fig. 1 decrease in all regions except around 440 mμ and 450 mμ, but still show the positive peak around 520 mμ which is characteristic to those at higher P/D. As P/D decreases below 1 (dye molecules in excess of anionic sites), the longer wavelength region of the ORD curve changes drastically, the rotation becoming negative with shallow troughs and a peak. At the same time the shorter wavelength region predominates the whole curve. The peak at about 440-450 mμ intensifies becoming dominant with a very symmetric shape centered at 450 mμ. It should be noticed that the molar

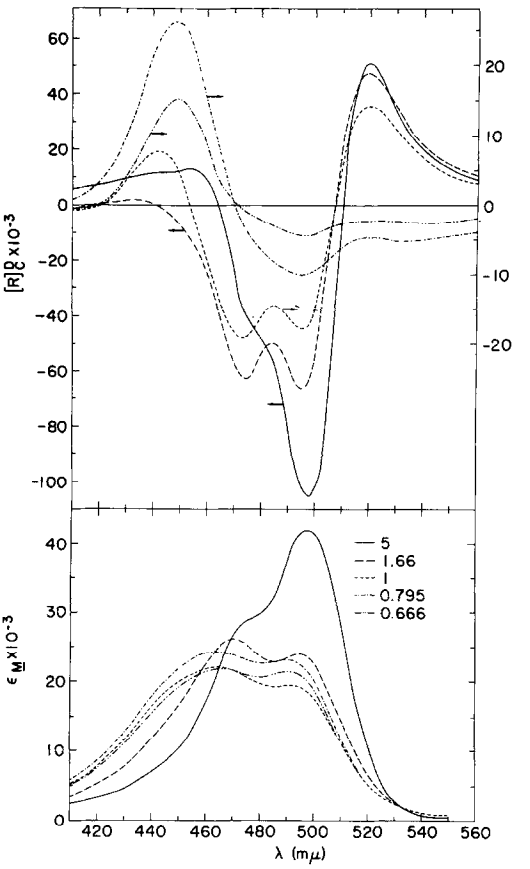

Figure 1. ORD curves (upper) and absorption spectra (lower) of native DNA complexes formed with acridine orange. The arrow on each curve indicates the ordinate scale to be referred to. P/D, in parentheses, is followed by the concentration of DNA phosphorus: (5) 1 x 10^{-4} M; (1.66) 4 x 10^{-5} M; (1, 0.795, 0.666) 1 x 10^{-5} M. A Cary Model 60 spectropolarimeter and a Cary Model 14 spectrophotometer were used at room temperature.

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rotation of the ORD for P/D less than 1 is plotted on an expanded scale (right ordinate). At low P/D, the absorption spectra of DNA-AO complexes are rather insensitive measures of the changes in contrast with the ORD curves. All of the spectra show two very broad maxima at about 495 mμ and 460-465 mμ (Fig. 1).

B--The ORD and absorption spectra of heated DNA-AO.

The ORD curves of heat denatured DNA-AO at P/D 5 shown in Fig. 2 are very similar in shape but not in magnitude to the curve for native DNA-AO at the

same P/D (Fig. 1). All key features are at the same wavelengths. The extrinsic Cotton effect for DNA heated before the addition of AO (filled circles in Fig. 2) is virtually identical with that of DNA heated in the presence of AO (open circles). The ORD curves of the heated DNA-AO at lower P/D (1.66 to 1) are markedly different from those for native DNA-AO at P/D less than 1 (i.e., 0.795-0.666) shown in Fig. 1. They are characterized by a symmetric, solitary peak around 462-463 mμ for denatured and at 450 mμ for native complexes. The absorption spectra of heated DNA-AO show one broad maximum at about 470 mμ, which steadily decreases in intensity and simultaneously shifts to the blue, and another maximum at about 496 mμ, which decreases more markedly and becomes a shoulder at low P/D.

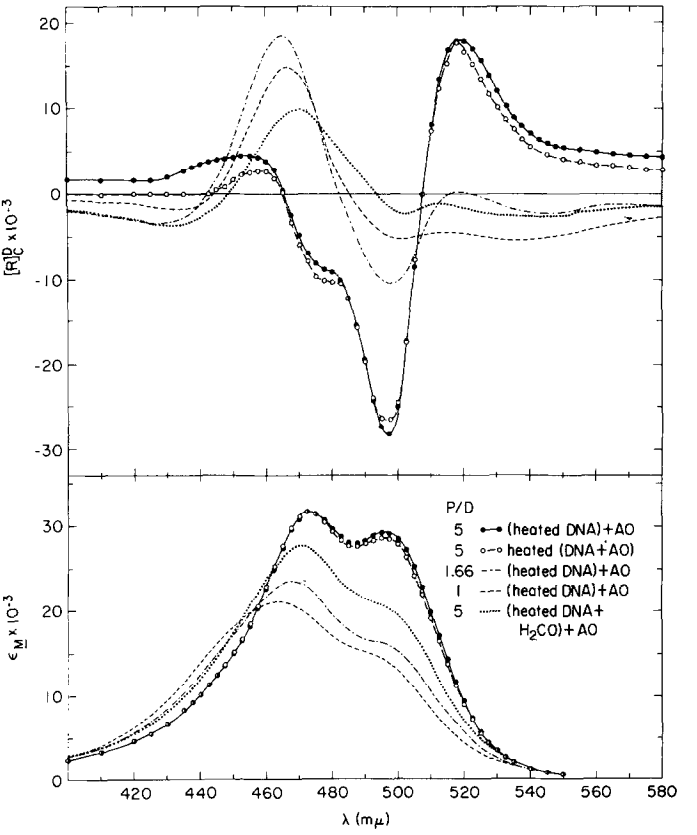

Figure 2. ORD curves (upper) and absorption spectra (lower) of heat denatured and formylated DNA complexes formed with acridine orange. The concentrations of DNA phosphorus are the same as those given in Fig. 1 at the indicated P/D.

C--The ORD and absorption spectra of formylated DNA-AO.

The ORD curve of solutions of formaldehyde-treated DNA plus AO differs both in shape and magnitude from those obtained with either native or heated DNA-AO at the same P/D of 5 (Figs. 1 and 2). It resembles those recorded for heated DNA-AO at lower P/D (Fig. 2) and native DNA-AO at P/D less than 1 (Fig. 1), showing a strong symmetric peak at 470 mμ with regions of negative rotation below 450 mμ and above 495 mμ (dotted curve Fig. 2). No change was observed in the ORD when formaldehyde was added to the native DNA-AO complex at room temperature. The absorption spectrum of the heated formylated complex has a shoulder at about 497 mμ and a maximum at 470 mμ.

DISCUSSION

Differences observed in the ORD curves and absorption spectra of heat denatured DNA-AO compared to comparable measurements on native DNA-AO suggest that heat denaturation causes irreversible changes in the conformation of DNA (Figs. 1 and 2). The changes noted are more quantitative than qualitative and suggest that some structure remains after heat denaturation. In the absence of hydrogen bonds which are responsible for base pairing, base stacking can have a role in the formation of secondary structure in DNA (10). The similarity in the shape of the Cotton effect observed for AO complexes of native and denatured DNA may be attributed to such base-base interactions. These should be prevented by chemically modifying the free amino groups of the bases. The marked changes in both the ORD curve and absorption spectrum of formylated DNA-AO complexes support this view.

The effect of decreasing P/D on the shape of the observed Cotton effect is worthy of note in relation to the environment of bound dye. As P/D decreases, there is a gradual change in shape and shift of peak positions. The net result is that ORD curves for native DNA-AO complexes assume a limiting shape represented by the curve for P/D 0.666 (Fig. 1) which is similar to that for formylated DNA-AO or heat denatured DNA-AO (Fig. 2). These curves are similar to the ORD curve obtained for complexes of poly- ,L-glutamic acid

(PGA), with AO both at pH 4, where PGA is helical, and at pH 8 where it is a random-coil (1).

The similarity of the extrinsic Cotton effects recorded for complexes formed by such diverse polymers with AO suggests that in both instances the bound dye is located in closely related environments. Although DNA and PGA in the absence of AO have vastly different conformations, the possibility exists that the bound dye can modify the conformation of the polymer in the region of its site of attachement.

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