EXTRINSIC COTTON EFFECTS OF ACRIDINE ORANGE BOUND TO APURINIC ACID*

Robert A. Resnik and Kiwamu Yamaoka

Research Analysis and Evaluation Branch, Division of Research
Grants, National Institutes of Health, Bethesda, Maryland, 20014
and
Department of Chemistry, Faculty of Science, Hiroshima University
Hiroshima 730, Japan

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Summary. Optical rotatory dispersion curves and absorption spectra were obtained for acridine orange complexes formed by apurinic acid which was prepared from calf thymus DNA. The effects of ionic strength, of dilution and of the variation of the ratios of anionic sites to dye were examined. The analogies between the Cotton effect of the apurinic acid complexes and that of the complexes formed by native or denatured DNA and poly-α,L-glutamic acid were pointed out.

In the preceding paper (1) it was reported that as the ratio of the DNA phosphate to dye (P/D) decreases, the optical rotatory dispersion (ORD) curves of complexes of acridine orange (AO) with native and heated DNA approach a limiting shape which is similar to that obtained with formylated DNA. Although the heating of DNA has been shown to result in strand separation with the conformation greatly altered (2), it is possible that some short (or local) helical segments within a single-stranded DNA molecule remain. These helical portions could then give rise to an extrinsic Cotton effect when AO is bound to the polymer. Hydrogen bonding in denatured DNA is effectively prevented by formaldehyde (3, 4), yet the complexes of AO with formylated DNA are able to exhibit induced Cotton effects (1).

In our attempt to find out the basic requirements for the appearance of induced optical activity in the complex of metachromatic dyes with optically active, biologically important polymers, we prepared apurinic acid, in which

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purine-pyrimidine base pairing is virtually non-existent, and investigated its interaction with AO using both ORD and absorption methods.

EXPERIMENTAL

Apurinic acid was prepared from calf thymus DNA by the methods of Tamm, et al (5) and of Petersen and Burton (6). Several preparations were used in this work and all gave similar results. The molar extinction coefficient in dilute sodium phosphate buffer at pH 7 was 4600 to 4700 in terms of phosphorus at 268 mμ. The concentration of apurinic acid was expressed in terms of phosphorus. Complexes were prepared by adding AO to polymer. This mixing procedure was not reversed because of a tendency of the complex to precipitate at low P/D. Other experimental procedures and conditions were all the same as in the preceding paper (1).

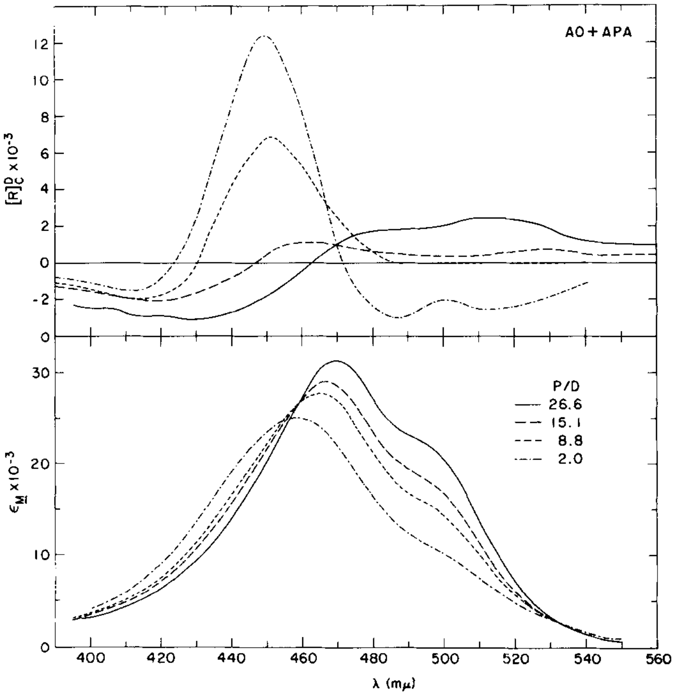

Figure 1. The effect of variations in the ratio of anionic sites to dye (P/D) on the ORD curves (upper) and absorption spectra (lower) of acridine orange (AO) complexes of apurinic acid (APA) at constant polymer concentration (4.25 x 10^{-4} M). Other details were described in references (1) and (7).

RESULTS

A--The effect of P/D on the ORD curves and absorption spectra of complexes.

In determining the effect of P/D on the Cotton effect induced in the apurinic acid-AO complex, the concentration of apurinic acid was held constant (4.25×10^{-4} M) for reasons previously noted (7). The salt concentration was 0.01 N NaCl to take advantage of the enhancing effect of ionic strength on the extrinsic Cotton effect. The induced Cotton effect at P/D 2 has a positive peak at 450 mμ with negative regions above and below it (Fig. 1). Further increase in P/D produces an entirely different ORD pattern. Thus at P/D 15 and 26 the curves have a single crossover point and broad regions of positive and negative rotation above and below the point. The absorption spectra all have a single maximum and a shoulder, and are very different from that of free AO. A steady blue shift of the maximum accompanied with hypochromism is also noticeable.

B--The effect of salt concentration on the ORD curves and absorption spectra.

As shown in Fig. 2, the Cotton effect induced in apurinic acid-AO at a P/D of 8.7 has a peak at 450 mμ and two troughs in 0.001 N NaCl. In 0.1 N NaCl, however, the size of the induced Cotton effect was doubled with the peak shifted to 445 mu. As for the absorption spectra of these solutions, the increase in salt concentration results in a lowering of the molar extinction of the maximum at 465 mμ and an increase in that of the shoulder around 495 mμ.

C--The effect of the dilution on solutions of apurinic acid-AO complexes.

Upon diluting the solutions tenfold with the respective solvent (solvent was always added to a solution of the complex), the extrinsic Cotton effect in 0.001 N NaCl was considerably reduced, wherease no induced rotation was detectable in 0.1 N NaCl between 350 - 540 mμ (Fig. 2). At the same P/D and ionic strength, however, the concentration of the polymer is also a factor which can modify the shape of the induced ORD curve of the complex (not shown here).

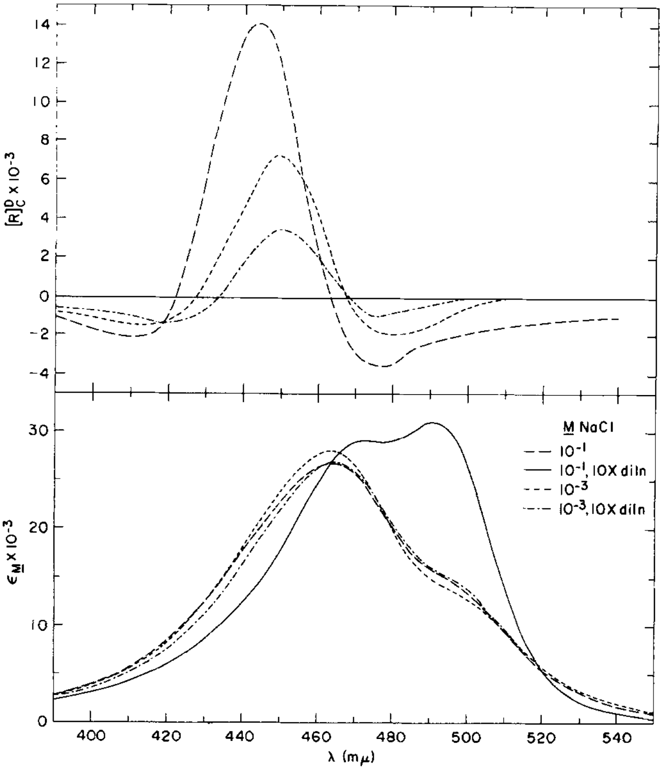

Figure 2. The effects of ionic strength and of dilution on the ORD curves (upper) and absorption spectra (lower) of AO complexes of apurinic acid. Before dilution, apurinic acid was 1.6×10^{-4} M in terms of phosphorus; AO was 1.85×10^{-4} M. P/D was 8.7 for all curves.

DISCUSSION

Apurinic acid is a single-stranded polydeoxyribonucleotide with virtually all of the purine bases removed (5). Although there is little possibility for the existence of significant amounts of purine-pyrimidine interaction, optical activity of considerable magnitude was induced in the visible spectrum of the bound AO (Figs. 1 and 2). In this regard, it should be pointed out that there are clear analogies between the ORD curves obtained for apurinic acid-AO complexes at all but high P/D (greater than about 10) and for native DNA-AO complexes at very low P/D. Furthermore, similar Cotton effects were obtained for heated DNA-AO complexes at low P/D and for formylated DNA-AO complexes (1).

The spectral features associated with the extrinsic Cotton effects of those systems noted above are also present in the induced Cotton effect of the complexes of AO with both helical (7) and non-helical (8) poly-α,L-glutamic acid (PGA). All of these complexes give rise to multiple Cotton effects showing a positive, symmetric peak at 450 mu in certain circumstances. The detailed analysis of similar ORD curves has shown that such an apparent Cotton effect can result from the complicated interplay of several Cotton effects (7).

AO complexes with mucopolysaccharides like heparin (9) and chondroitin sulfate C (10, 11) also show extrinsic Cotton effects analogous to those reported here. Preliminary experiments with apyrimidinic acid-AO complexes show that the extrinsic Cotton effect resembling those obtained for apurinic acid-AO complexes can be observed in the absence of the normal complement of pyrimidine bases. In addition, extrinsic Cotton effects were detected with AO bound to polydeoxyribosephosphate, the backbone chain of DNA which lacks most of the purine and pyrimidine bases. These polymers were prepared by the method of Takemura (12).

All of these aforementioned optically active polymers are related to one another only in the sense that they are negatively charged polyelectrolytes, whose ionized groups or anionic sites are either phosphates, carboxylates or sulfates. Thus, the origin of the extrinsic Cotton effect commonly observed for complexes formed between AO and such diverse polymers, regardless of their chemical composition and conformation, is best attributed to some kind of polymer-induced dissymmetry in the proximity of the bound dye. This leads to the view that the stacking of the bases or base-base pairings in DNA plays no primary role toward the induction of optical activity in the ORD curve of bound AO over a certain range of P/D.

Three factors which can affect the appearance and the shape of the extrinsic Cotton effect are: (i) the competitive interaction between anionic sites and counter ions, (ii) interaction between bound AO and/or between bound and unbound AO to the site and (iii) the type of acridine dye bound to the polymer, i.e., no induced optical activity was detected when proflavine was added to apurinic acid, although it binds to this polymer (13). Finally, studies of DNA, polymers derived from it and of poly-α,L-glutamic acid indicate that the occurrence of an extrinsic Cotton effect does not necessarily imply the presence of helicity in a polymer.

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