A Spectrophotometric Study on Binding of Acridine Orange with DNA

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SUMMARY

The interaction of acridine orange (AO) with calf thymus DNA was studied by the ordinary and difference absorption spectra methods. The spectral changes of AO produced with DNA were found to be due to binding of AO with the deoxyadenosine and deoxyguanosine moieties of DNA. It was also found that the contribution of interaction with the deoxycytidine and deoxythymidine moieties to the above spectral changes was less remarkable. A comparative study was made on a typical metachromatic system of AO and heparin.

INTRODUCTION

Previous investigators have obtained much information about the binding of acridine orange (AO) with DNA. Armstrong and Niven (1) reported that AO formed a green fluorescent complex with DNA of tissues and bacterial cells and a red phosphorescent complex with RNA. Detailed spectrophotometric studies in the visible and ultraviolet regions were reported by Steiner and Beers (2) and Bradley and Wolf (3). From data on viscosity, sedimentation, and the patterns of X-ray diffraction, Lerman (4) proposed a model in which an AO molecule was intercalated between adjacent nucleotide-pair layers of DNA. Another possibility, originally suggested by Bradley and Wolf, is that the dye molecules are associated with the phosphate groups on the outside of the double helix of DNA. This model was recently refined by Mason and McCaffery (5), who measured the optical rotation of streaming solutions. In the former model, external attachment of the dye molecules, as in the latter model, is also possible. From thermal denaturation experiments and free energy calculations, Gersch and Jordan (6) supported the former model. Neville and Davies (7) carried out X-ray diffraction and optical investigations and reported that although their results were well consistent with the intercalation model, a fraction of AO was bound on the outside of the DNA chain and the size of this fraction depended on the experimental conditions. More recently, Peacocke *et al.* (8, 9) measured the viscosity and optical rotatory dispersion of amino acridines bound to DNA and correlated their results with the two types of binding, stacking and intercalation.

The present paper has a twofold objective: (a) to confirm the mode of binding of AO with DNA by the difference absorption spectra method which has not hitherto been applied to AO-DNA systems and (b) to elucidate the nature of binding of AO with DNA from a comparison with metachromasy of AO-heparin systems.

MATERIALS AND METHODS

AO was a reagent grade compound purchased from E. Merck Co. and was recrystallized before measurement. DNA was a highly polymerized preparation isolated from calf thymus, purchased from Sigma

Chemical Co. Deoxyadenosine (AdR), deoxyguanosine (GdR), and deoxythymidine (TdR) were purchased from Sigma Chemical Co. Deoxycytidine (CdR) was purchased from Tokyo Kasei Industries. Heparin was purchased from Wako Pure Chemicals.

All DNA concentration was expressed in molarity with respect to DNA phosphorus (DNA-P). All heparin concentration was expressed in percentage. Absorption spectra in the visible region were obtained with a Hitachi recording spectrophotometer. All measurements were carried out at 23.0°.

RESULTS AND DISCUSSION

Difference Absorption Spectra of AO-DNA Systems

Figure 1 shows the ordinary absorption spectra in the visible region of the AO-DNA systems at pH 7.0. In the case of AO alone, the α-band at 492 mμ was quite clear while the β-band at 465 mμ appeared only as a shoulder. This indicates that the equilibrium is displaced largely to the monomer side under the present experimental conditions.

When increasing amounts of DNA were added to the solution, the absorption spectra varied from curve *b* to *e*, as shown in Fig. 1. At concentrations of DNA-P lower than 1.11×10^{-5} M, that is, in the region of AO/DNA-P ratio close to 1.0, there was a marked decrease in the α-band which was accompanied by the appearance of the β-band. This apparently corresponds to a change due to the formation of the complex I of Steiner and Beers (2). These authors thought that in this case almost all the binding sites of DNA were occupied by dye molecules, and interactions between adjacent dye molecules occurred. In this (I) region, the mechanism of binding of AO to DNA is considered to be that of stacking or self-association of AO molecules along the DNA helix.

At concentrations of DNA-P higher than 1.11×10^{-4} M, that is, in the region of AO/DNA-P $\ll 1$, there occurred an apparent increase in intensity of the α-band, but the peak shifted to 502 mμ. This corresponds to a change due to the formation of the complex II of Steiner and Beers. In this (II) region, a red shift occurred, indicating a strong interaction between AO and DNA, and the mechanism of binding is considered to be that of intercalation of AO molecules inside the DNA helix.

Figure 2 shows the difference absorption spectra of the same systems as in Fig. 1. In these spectra the above-mentioned effects

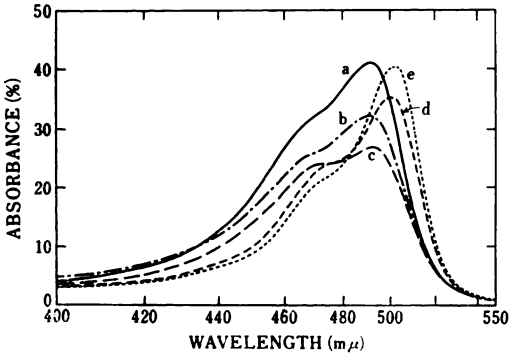

FIG. 1. *Ordinary absorption spectra of the AO-DNA systems*

The concentration of AO was 1.25×10^{-5} M in pH 7.0 phosphate buffer of 10^{-2} M. The concentration of DNA was varied as follows: (a) AO alone, no DNA; (b) DNA 1.11×10^{-6} M; (c) DNA 1.11×10^{-5} M; (d) DNA 1.11×10^{-4} M; (e) DNA 1.11×10^{-3} M.

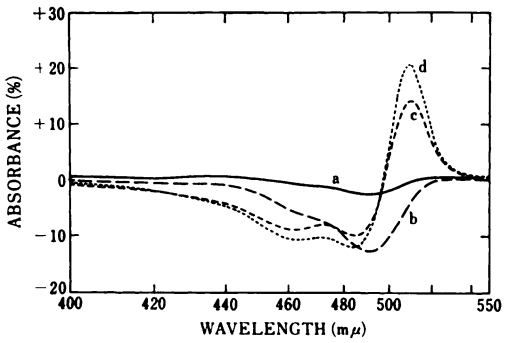

FIG. 2. *Difference absorption spectra of the AO-DNA systems*

The concentration of AO was 1.25×10^{-5} M in both the sample and reference cell. The concentration of DNA in the sample cell was varied as follows: (a) DNA 1.11×10^{-6} M; (b) DNA 1.11×10^{-5} M; (c) DNA 1.11×10^{-4} M; (d) DNA 1.11×10^{-3} M.

of DNA upon AO are more distinctive. In the (I) region, the β-band as well as the α-band were weaker but showed no red shifts. In the (II) region, on the other hand, a red shift of the α-band was more clearly observed, whose peak was at 510 mμ. These results indicate that there is an essential difference in nature between the mode of binding of AO to DNA in the (I) region and that in the (II) region.

Difference Absorption Spectra of AO-AdR, AO-GdR, and AO-CdR Systems

Figures 3 and 4 show the difference absorption spectra of the AO-AdR and AO-GdR systems, respectively. It is interesting that the same spectral changes of AO depending on the concentration of AdR or GdR were observed for these systems as

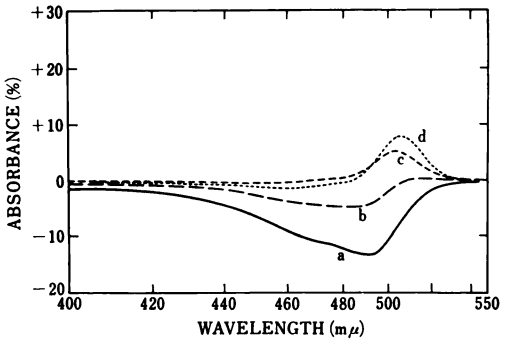

FIG. 3. *Difference absorption spectra of the AO-AdR systems*

The concentration of AO was 1.25×10^{-5} M in pH 7.0 phosphate buffer of 10^{-2} M. The concentration of AdR was varied as follows: (a) AdR 6.25×10^{-4} M; (b) AdR 1.25×10^{-3} M; (c) AdR 2.5×10^{-3} M; (d) AdR 5.0×10^{-3} M.

for the AO-DNA systems, though the concentration of purine nucleosides necessary to produce the spectral changes was much higher in the former systems. In the (I) region of AO/purine nucleoside ratio greater than 1.0×10^{-2}, the β-band as well as the α-band were weaker but showed no red shifts. In the (II) region of AO/purine nucleoside ratio less than 0.5×10^{-2}, a red shift of the α-band was clearly observed, whose peak was at 506–508 mμ. However, no remarkable spectral changes were observed for the AO-TdR and AO-CdR sys-

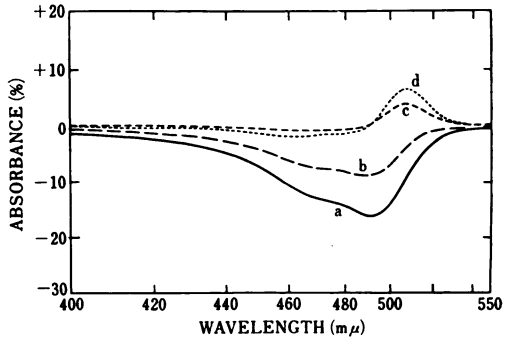

FIG. 4. *Difference absorption spectra of the AO-GdR systems*

The concentration of AO was 1.25×10^{-5} M in pH 7.0 phosphate buffer of 10^{-2} M. The concentration of GdR was varied as follows: (a) GdR 6.25×10^{-4} M; (b) GdR 1.25×10^{-3} M; (c) GdR 2.5×10^{-3} M; (d) GdR 5.0×10^{-3} M.

tems, as shown in Fig. 5. Therefore, it is concluded that the spectral changes of AO shown in Fig. 2 were mainly the result of binding with the AdR and GdR moieties of DNA. For such a binding to be stabilized, it may be necessary for AO molecules to face the molecular planes of the AdR and GdR moieties of DNA.

Thus the present differential spectra of the AO-purine nucleoside systems provide convincing data about the intercalation mechanism of AO-DNA in the (II) region. The physical chemical principles behind this observation is that of the hydrophobic-stacking interaction of the purine bases, as reported by Ts'o *et al.* (10–13). This association process did not proceed simply to the dimer stage, but continued to form

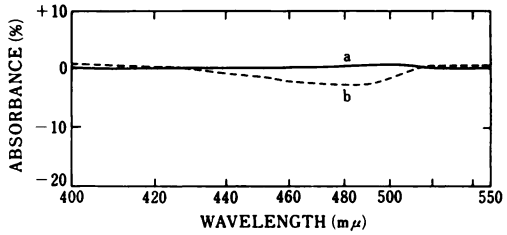

FIG. 5. *Difference absorption spectra of the AO-CdR systems*

The concentration of AO was 1.25×10^{-5} M in pH 7.0 phosphate buffer of 10^{-2} M. The concentration of CdR was varied as follows: (a) CdR 6.25×10^{-4} M; (b) CdR 5×10^{-3} M.

higher polymers. Because of this type of interaction, AO molecules associated with the purine nucleosides by stacking. Pyrimidine nucleosides associated much less than purine nucleosides in water, thus the stacking of AO molecules with pyrimidine nucleosides was much reduced.

A Comparison of the Metachromasy of AO-DNA with That of AO-Heparin

Figure 6 shows the ordinary absorption spectra of the AO-heparin systems. As was extensively studied by Appel and Zanker (14), these systems showed no bathochromic shifts. Therefore, it seems likely

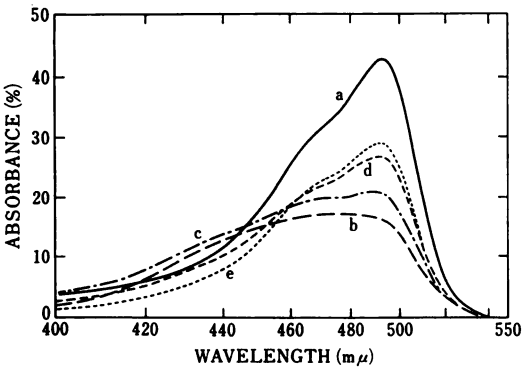

FIG. 6. *Ordinary absorption spectra of the AO-heparin systems*

The concentration of AO was 1.25×10^{-5} M and that of heparin was 0.002%. The concentration of pH 7.0 phosphate buffer was varied as follows: (a) AO alone, no buffer and no heparin; (b) buffer 2.5×10^{-2} M; (c) buffer 5.0×10^{-2} M; (d) buffer 1.25×10^{-1} M; (e) buffer 2.5×10^{-1} M.

that the nature of the spectral changes or metachromasy observed for the AO-heparin (or other acid polysaccharides) systems is not essentially different from that for the above-mentioned AO-DNA systems in the (I) region. That is, the mechanism of binding of AO to heparin is considered to be stacking or self-association. However, there are some quantitative differences in metachromatic effect between heparin and DNA. As pointed out by many investigators (15–18), the metachromatic activity of heparin is higher than that of DNA in the (I) region. This is thought to be due to a more regular arrangement of AO molecules on a heparin molecule, because of stronger interaction with the sulfonate groups of heparin.

For the occurrence of metachromasy, it is important that the molecular weight of acid polysaccharides is high and therefore degradation of the macromolecule does not produce metachromasy. It is also important that the addition of inorganic salts reverses the above metachromasy (salt

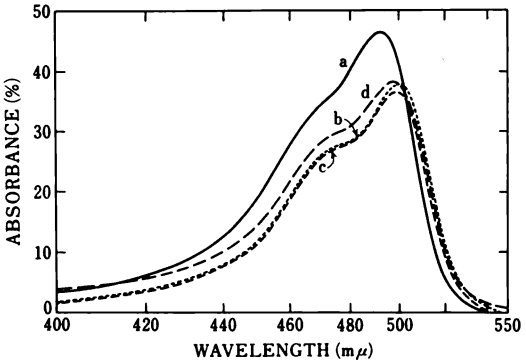

FIG. 7. *Ordinary absorption spectra of the AO-DNA systems*

The concentration of AO was 1.25×10^{-5} M and that of DNA was 1.11×10^{-4} M. The concentration of pH 7.0 phosphate buffer was varied as follows: (a) AO alone, no DNA; (b) buffer 5×10^{-2} M; (c) buffer 1.25×10^{-1} M; (d) buffer 2.5×10^{-1} M.

effect), as shown in Fig. 6. On the other hand, metachromasy for AO-DNA systems was produced with either DNA or its basic components of low molecular weight, and this metachromasy was unaffected by addition of inorganic salts, as shown in Fig. 7. These quantitative differences in spectra are thought to correspond to those in character of binding sites on heparin and DNA.

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