Induced Optical Activity of Nucleic Acid-Toluidine Blue O Complex

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The absorption, circular dichroism (CD) and optical rotatory dispersion spectra of DNA–toluidine blue O complexes were observed over the visible absorption spectral region. Effect of temperature on CD was examined at the temperatures 6—86°C. For the complex of a low DNA phosphate to dye ratio, the Krönig-Kramers transform was carried out and one positive and four successive negative CD bands were resolved. It is concluded that the shorter wavelength CD bands, one positive and one negative, are due to an exciton splitting of the dye dimers and that the rest may be induced by an asymmetric environment of the DNA deoxyribose residue.

In a previous paper,[1] we reported that the DNA- and RNA-thionine (Th) complexes show Cotton effects in the visible absorption region of the dye and that these Cotton effects may have two origins; one induced from the dye monomers bound to DNA (Optical Activity I) and the other caused by an interaction between the dimerically bound dyes (Optical Activity II). In a preliminary report on the study of the DNA-toluidine blue O (TBO) complexes,[2] we demonstrated that the usual exciton theory fails to predict the longer wavelength Cotton effects of the high P/D (DNA phosphate residue/dye concentration) complexes. In Optical Activity I, the dye monomers seem to be involved in a complicated interaction with the asymmetric environment due to deoxyriboses of DNA.

The most striking feature of the DNA-TBO complex at low P/D is the sign of each partial circular dichroism (CD) spectrum, which is completely the reverse of that found in the DNA-acridine orange (AO) complex. On the other hand, the general feature of the CD spectra of the DNA- and RNA-Th complexes resembles that of the DNA-AO complex including their signs.[1] In this paper, we report the absorption spectra and Cotton effects of the DNA-TBO complexes at low as well as at high P/D values, with particular reference to their temperature dependence. CD band resolution is carried out for the low P/D complex by means of the Krönig-Kramers transform. It is shown that both effects of exciton splitting and asymmetric field are necessary for inducing the longer wavelength Cotton effects.

Experimental

Commercial calf-thymus DNA (Sigma Type I) and toluidine blue O (3-amino-7-dimethylamino-2-methylphenazathionium chloride) (Merck) were used without further purification. Stock aqueous solutions of DNA and TBO were dessolved in 0.01M phosphate buffer (pH 6.84). Concentration of TBO was chosen to be 5×10^{-5}M. A Jasco ORD/UV-5 recording spectropolarimeter with CD attachment was used. Further experimental details were the same as given previously.[1]

Results

The intensity of the absorption spectrum of TBO in water changes with concentration. According to Rabinowitch and Epstein,[3] the absorption peak appearing at low concentration is called M-band (monomer band) and at high concentration D-band (dimer band). In our experiment, the M-band and the D-band of TBO are located at 637 nm ($\varepsilon=2.9\times10^4$) and 562 nm ($\varepsilon=1.1\times10^4$), respectively. Using the same method as theirs, we obtained the average equilibrium constant for dimerization $K=2.6\times10^{-4}\,l\cdot mol^{-1}$ at room temperature, the value being nearly equal to that of methylene blue.[3]

An analogous change in the visible absorption spectrum of the dye can be seen when DNA is added to the dye solution. This has been reported by Huse, Miyake, and Tsuboi.[4] Apart from a detailed charac-

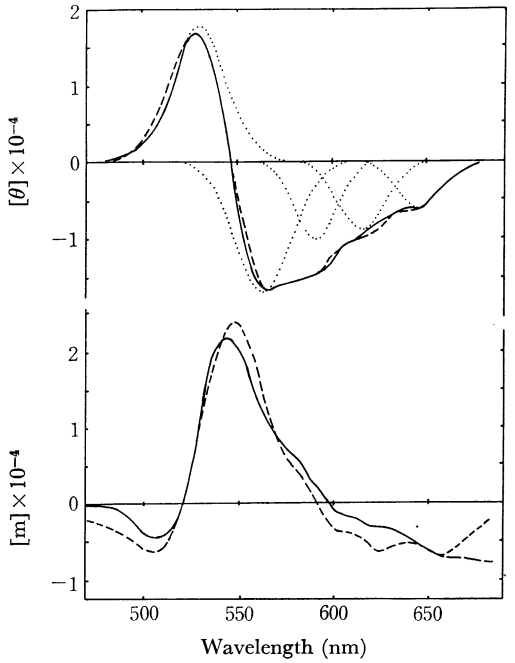

Fig. 1. The observed and calculated CD (upper) and ORD (lower) spectra of DNA-TBO $P/D=0.26$ complex; solid curve: observed; dotted curve: resolved components; broken curve: sum of the resolved components.

1) Y. J. I'Haya and T. Nakamura, This Bulletin, **44**, 951 (1971).

2) Y. J. I'Haya, T. Nakamura, Y. Yagi, T. Sano, and H. Ito, *Intern. J. Quantum Chem. Symposium*, **5**, 361 (1971).

3) E. Rabinowitch and L. F. Epstein, *J. Amer. Chem. Soc.*, **63**, 69 (1941).

4) Y. Huse, K. Miyake, and M. Tuboi, This Bulletin, **38**, 1039 (1965).

teristic, the changing profile of the absorption spectrum of DNA-TBO is similar to that of DNA-AO.[5] On the other hand, the optical rotatory dispersion (ORD) and CD spectra of DNA-TBO are quite different from those of DNA-AO.[6-8] The representative CD and ORD spectra of DNA-TBO are shown in Fig. 1 with their CD band components resolved by the Krönig-Kramers transform.[9] At low P/D values, one positive and four successive negative CD bands appear with maxima located at 529, 563, 590, 615, and 645 nm, respectively. They are hereafter referred to as Bands I, II, III, IV, and V. Band V (at 645 nm) is somewhat doubtful. At high P/D values, the positive CD maximum (Band I) disappears and the successive negative CD maxima (Bands II—V) shift a little to the shorter wavelength side, a newly developed positive CD band then coming out at about 650 nm (Fig. 2). The features of CD bands change with both temperature and P/D values (Fig. 3).

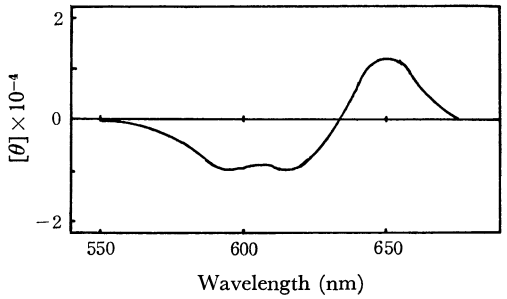

Fig. 2. CD spectrum of DNA-TBO P/D=2.6 complex.

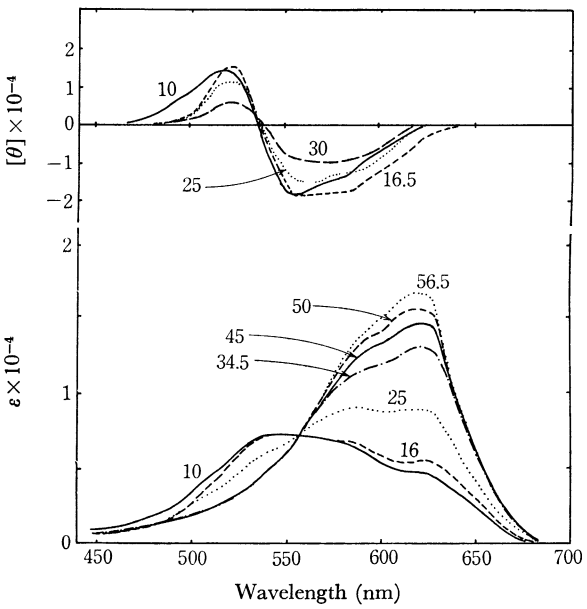

Fig. 3. Effect of temperature on CD (upper) and absorption (lower) spectra of DNA-TBO P/D=0.46 complex. Each figure shows temperature (°C).

5) H. Ito and Y. J. I'Haya, *Intern. J. Quantum Chem.*, **2**, 5 (1968).
6) B. J. Gardner and S. F. Mason, *Biopolymers*, **5**, 79 (1967).
7) K. Yamaoka and R. A. Resnik, *J. Phys. Chem.*, **70**, 4051 (1966).
8) M. Zama and S. Ichimura, *Biopolymers*, **9**, 53 (1970).
9) C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill, New York (1960), p. 159.

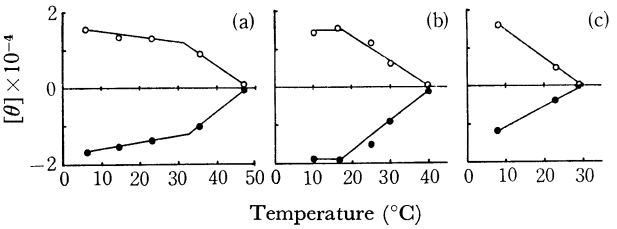

Fig. 4a. Temperature dependence of $[\theta]_{max}$ of DNA-TBO complexes; ○: positive maxima at 520—530 nm, ●: negative maxima at 555—560 nm.
(a): P/D=0.22, (b): P/D=0.46, (c): P/D=0.93

The change in molar ellipticity $[\theta]$ is plotted against temperature in Fig. 4a. It should be noted that the $[\theta]$ values of the positive CD maximum at about 524 nm (Band I) and the negative one at about 560 nm (Band II) diminish linearly with increasing temperature. Furthermore, the $[\theta]$ values of these CD bands do not change very much even at sufficiently low temperature when P/D is considerably low (<0.5), although a slight shift of the whole spectrum to the shorter wavelength side is observed (Fig. 3). It was difficult to observe a positive CD band located at about 650 nm, as the sensitivity of our photomultiplier was very low in the spectral region. The temperature effect of this band, therefore, was not examined. The temperature dependence of the negative CD band of the P/D=2.6 complex is shown in Fig. 4b. The effect of temperature on the absorption curves of TBO dissolved in 0.01 M phosphate buffer is given in Fig. 5a.

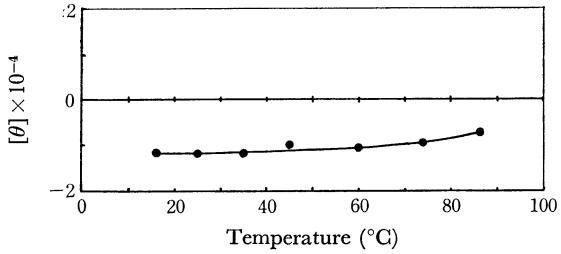

Fig. 4b. Temperature dependence of negative CD maximum (600—604 nm) of DNA-TBO P/D=2.6 complex.

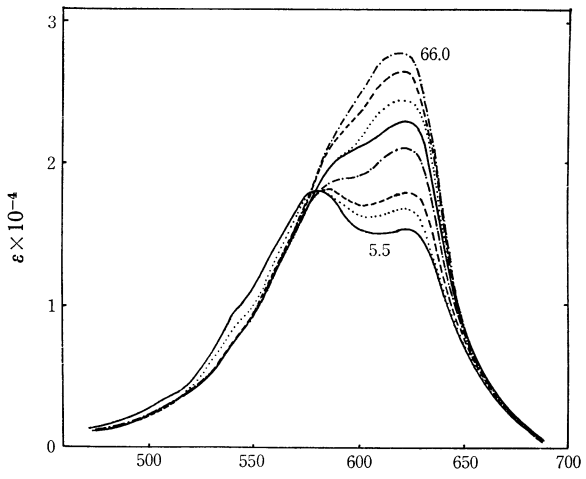

Fig. 5a. Absorption spectrum of TBO in 10^{-2} M phosphate buffer at various temperatures. Concentration of TBO, 5×10^{-5} M. Temperatures, from lower to upper, 5.5, 10, 15, 25.5, 36, 45.5, 56 and 66°C.

Discussion

According to Gardner and Mason,[6] dyes contributing to the optical activity of nucleic acid-dye complexes can be divided into two species, those bound to the nucleic acid helix monomerically and those bound dimerically. In our previous study on the DNA- and RNA-Th complexes, we confirmed the validity of their interpretation.[1] The present study on the DNA-TBO complexes also supports this conclusion. As shown in Fig. 1, the two resolved CD bands located at 529 and 563 nm are of nearly equal ellipticity and have signs opposite to each other (*vide infra*). Moreover, their $[\theta]_{max}$ wavelengths (Bands I and II) correspond to the shoulders, which may be thought to be split absorption bands due to a dye-dye interaction of each absorption spectrum of the complexes taken at various temperatures (Fig. 3).

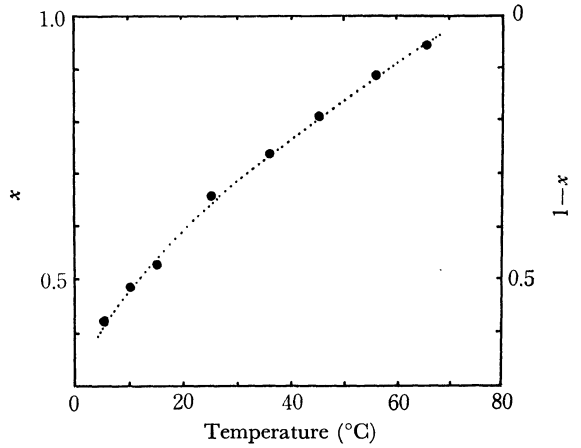

Fig. 5b. Plot of monomer (x) and dimer ($1-x$) fraction of TBO in aqueous solution against temperature.

These facts suggest that the optical activity appearing at 500—550 nm is induced by an exciton interaction in line with the works of Moffitt[10] and of Tinoco.[11] In addition to this, a comparison of the change in the dimer fraction ($1-x$ in Fig. 5b) and that in the maximum values of molar ellipticity with increasing temperature ($[\theta]$ in Fig. 4a) suggests that the intensities of these CD bands depend upon the amount of the dye dimers bound to the DNA helix. The numerical value of molar ellipticity should be constant if there is no conformational change in the dye-dye steric conformation. Actual molar ellipticity should then be evaluated using the concentration of the *dye dimers* bound to the DNA helix at appropriate sites. The actual molar ellipticities for bands I and II located at 529 and 563 nm, respectively, are thus evaluated and listed in Table 1. Here the following assumptions are made: (i) in the temperature range where $[\theta]_{max}$ is constant, there is no free dye[12] and the fraction of the bound dye dimers is constant; (ii) the fractions of monomer and dimer are estimated from the absorption spectrum of the dye in aqueous solution measured at the corresponding temperature.

Assumption (i) seems to be valid since in the temperature range where the $[\theta]$ value is constant (10—16°C for the complex of $P/D=0.46$) the absorption spectrum of the complex does not change its profile very much as seen in Fig. 3. Assumption (ii) is not justified but requisite, since estimation of monomer and dimer fractions from the absorption spectrum of the complex is not possible due to the shift in the absorption spectrum and the strong hyper- and/or hypo-chromisms. In order to evaluate actual $[\theta]$ values under Assumptions (i) and (ii), the dimer fraction of the dye must be estimated at the temperature where slope of the plot $[\theta]_{max}$ *vs.* temperature changes (Fig. 4a).

The rotational strengths for the resolved CD bands are also calculated by means of the relationship[9]

$$R_k \simeq 0.696 \times 10^{-42} \sqrt{\pi}\,[\theta_k] \frac{\Delta_k}{\lambda_k} \quad (k = \text{I—V})$$

where $[\theta_k]$, Δ_k, and λ_k are the molar ellipticity, the half band width and the wavelength of $[\theta_k]_{max}$ of the k-th CD band, respectively. The results are given in Table 1. The calculated $[\theta]_{max}$ and R for bands I and II are comparable with observed values. The absolute values of R_I and R_{II} are nearly equal to each other within experimental error. This suggests that the optical activity appearing in the neighborhood of 550 nm is due to just one origin as has been stated.

Table 1. Molar ellipticities and rotational strengths for resolved CD bands of DNA-TBO $P/D=0.26$ complex[a]

Band	λ_{max} (nm)	$\lambda_{shoulder}$ (nm)	$[\theta]_{max}\times 10^{-4}$ Obsd	$[\theta]_{max}\times 10^{-4}$ Calcd	$R\times 10^{-40}$cgs Obsd	$R\times 10^{-40}$cgs Calcd
I	529.1	~532	1.75	3.85	7.18	15.81
II	563.4	~554	−1.72	−3.78	−6.67	−14.73
III	590.7	~582	−1.05		−2.85	
IV	615.3	~620	−0.9		−2.60	
V	645.2	—	−0.6		−1.60	

a) Calculations of $[\theta]_{max}$ and R for Bands III—V have not been carried out since the origins of the bands are not due to the TBO dimers.

Three causes seem to be possible for Bands III, IV, and V. (1) They might be induced from an interaction between the monomerically bound dyes, since the wavelengths of the CD band maxima are located around the monomer absorption band. (2) A contribution from some higher energy levels may induce such successive CD bands as reported by Bush and Brahms in the case of oligonucleotides.[13] (3) They might be induced by an asymmetric environment attributed to the asymmetric carbons of deoxyriboses. It should be noted that for high P/D values a positive CD band located at about 650 nm appears as shown in Fig. 2. Previous results indicated that this band (called M(+)

10) W. Moffitt, *J. Chem. Phys.*, **25**, 407 (1956).

11) I. Tinoco, Jr., "Advances in Chemical Physics," Vol. 4, ed. by I. Prigogine, Interscience Publishers, New York (1962), p. 113.

12) An equilibrium dialysis experiment carried out in our laboratory shows that the value of P/D becomes less than one below 24°C.

13) C. A. Bush and J. Brahms, *J. Chem. Phys.*, **46**, 79 (1966).

in reference 2) results from the dye monomers, the type of interaction being probably not of an exciton type.[2] Thus, Bands III—V cannot be attributed to (1) alone, *i.e.* to the monomerically bound dyes.

The maxima of Bands III and IV correspond well with the absorption shoulders of the complex (Table 1). The bands can thus be attributed to a combination of (2) and (3), *i.e.* to vibrational levels of the dye (probably in its dimerically bound state), which become optically active by the influence of the asymmetric carbons of deoxyriboses.

We might conclude as follows. (1) The Cotton effects of the low *P*/*D* complexes in the wavelength region 500—580 nm (Optical Activity II) are induced from an exciton-type interaction between the dye dimers. (2) The Cotton effects of the high *P*/*D* complexes in the wavelength region 620—670 nm (Optical Activity I) result from the dye monomers through the asymmetric perturbation of deoxyriboses. (3) Three negative CD bands developed in the wavelength region 550—650 nm correspond to vibrational components of the dye, which become optically active due to the asymmetric environment of DNA. Characteristics of the DNA–TBO system are the signs of the CD bands of its low *P*/*D* complex, which are opposite to the corresponding CD bands of DNA–AO and DNA–Th complexes. This suggests that the steric conformations of DNA-dye complexes are not always the same, contrary to the conventional interpretation. Since AO, Th, and TBO have a similar molecular framework, there seems to be no big difference between the binding sites of the dye molecules to the DNA helices. The major factor determining the CD band signs, therefore, can not be the whole helical arrangement of the bound dyes but is probably the local arrangement and/or relative orientation of the neighboring dye molecules that form dimers.

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