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LINEAR DICHROIC SPECTRA OF DNA-CRYSTAL VIOLET COMPLEXES UNDER FLOW FIELDS

Kiwamu YAMAOKA and Hideko HASHIMOTO
Faculty of Science, Hiroshima University,
Higashisenda-machi, Hiroshima, Hiroshima 730

The linear dichroic spectra of DNA-Crystal Violet complexes under flow were measured in the visible absorption region of Crystal Violet at three mixing ratios. The reduced dichroism was both positive (>620 nm) and negative (620-400 nm). The average angles between the transition moments and the orientation axis were estimated.

For the full understanding of the metachromatic behavior of a dye bound to polyelectrolyte, the visible absorption spectrum of a polymer-dye solution should be studied with an equal emphasis on the wavelength regions both shorter and longer than the absorption peak of the dye itself. This is because the long wavelength absorption of the solution also changes, as the hypso- and hypochromic effects become appreciable, and because the possible existence of a long wavelength band in the isotropic spectrum of the dye bound to polymer has been suggested.[1,2] The flow linear dichroism of a solution is the difference in the absorptions by solute chromophores of the incident light polarized parallel and perpendicular to the direction of flow field along which the solutes tend to orient.[3,4] Linear dichroism methods are useful for detecting a weak absorption band which is overlapped by strong bands and also for determining the average angle between the direction of the transition moment of an absorption band and the orientation axis of the individual solute.

Preliminary results of the linear dichroic spectra of the DNA-Crystal Violet (CV) solution under the orienting flow are presented. The DNA-CV system was found to be the first case in which the bound dye gave rise to the flow linear dichroic spectrum whose sign was positive in the long wavelength (>620 nm) but reversed to be negative at about 620 nm, while a single absorption band at 592-600 nm was observed in the isotropic spectrum. This change in sign of the reduced dichroism of the DNA-CV solution in the wavelength region where the isotropic spectrum was monotonic should be direct evidence that a long wavelength band is genuine and associated with the metachromatic behavior of CV which showed a short wavelength band or shoulder at about 500-550 nm when bound to DNA in aqueous solutions.

EXPERIMENTAL

Materials. CV was purified as described before.[1] The dialyzed calf thymus DNA stock solution was diluted with 1mM or 0.2mM NaCl to an appropriate concentration. To this DNA solution (90ml), the CV solution containing 1mM or 0.2mM NaCl (30ml) was added dropwise under the slow but constant stirring in an iced water bath. The mixing ratio of the DNA phosphate residue to dye (P/D) was 27, 10, and 3.

The hyperchromicity of the DNA solution in 1mM NaCl was 32-33% at 25°C.

Apparatus. The flow linear dichroic spectra were measured on a Hitachi Model EPS-3T double beam recording spectrophotometer with a flow cell attachment designed and constructed in this laboratory. The flow cells, which were made of high grade quartz, are rectangular in cross section and of a parallel plate type. The DNA-CV solution was circulated in a closed system at 23-25°C with use of two externally driven continuous-flow rotary pumps. The highest flow rate of the DNA-CV solution was about 9ml/sec at the full pump speed with silicone tubing.

Calculation. For an ensemble of like molecules of ellipsoid of revolution each possessing a transition dipole moment which makes an angle of θ relative to the molecular axis that is assumed to coincide with the orientation axis of the molecule, the reduced dichroism under flow fields is defined as,[3]

$$\frac{A_{\parallel} - A_{\perp}}{A} = \frac{\varepsilon_{\parallel} - \varepsilon_{\perp}}{\varepsilon} = \frac{3}{2}(3\cos^2\theta - 1)\cdot\Phi(\Theta,G). \quad (1)$$

$A_{\parallel}$ and $A_{\perp}$ are the anisotropic absorbances of a sample solution in flow which are associated with the monochromatic light polarized parallel and perpendicular to the flow direction, respectively. A is the isotropic absorbance of the same solution in the absence of flow fields. $\varepsilon_{\parallel}$, $\varepsilon_{\perp}$, and ε are the corresponding molar absorption coefficients. All $A_{\parallel}$, $A_{\perp}$, and A are experimentally determinable at any given wavelength. $\Phi(\Theta,G)$ is called the orientation function and is a complicated function of the rotary diffusion constant of the solute molecule, Θ, and the velocity gradient of flow, G, which is defined as $4Q/b^2d$, where Q is the volume of a sample solution delivered through the flow cell per unit time, b the path length, and d the width of the cell.[5] At the limiting high value of G, $\Phi(\Theta,G)$ approaches unity for the molecule represented by a prolate ellipsoid of revolution. Then, Eq. (1) reduces to

$$\left(\frac{A_{\parallel} - A_{\perp}}{A}\right)_{G\to\infty} = \left(\frac{\varepsilon_{\parallel} - \varepsilon_{\perp}}{\varepsilon}\right)_{G\to\infty} = \frac{3}{2}(3\cos^2\theta - 1). \quad (2)$$

Thus, the experimental determination of the reduced dichroism at the infinite G, $[(\varepsilon_{\parallel} - \varepsilon_{\perp})/\varepsilon]_{\infty}$, allows the evaluation of the absolute value of the angle θ.

RESULTS AND DISCUSSION

Flow Dichroic Spectra of the DNA-CV Solution. As shown in Fig. 1, where the numbers indicate P/D values, the isotropic spectra (dashed) of the native DNA-CV solutions are all changed bathochromically and hypochromically relative to the spectrum (dotted) of pure CV. This spectral behavior indicates that binding occurs between DNA and CV. It is also clear that the DNA-CV complex can be oriented along the flow direction as evidenced by the dichroic spectra (solid) of those same DNA-CV solutions under a flow field of ca. 17,000 sec^{-1} in G. The dichroic spectrum, $(\varepsilon_{\parallel} - \varepsilon_{\perp})$, is always positive at the long wavelength regardless of the P/D value and then turns to be negative to 460 nm. There is a crossover in the dichroic spectrum at 630 nm (P/D=27), 625 nm (P/D=10), or 620 nm (P/D=3), the wavelength longer than the principal maximum of the corresponding isotropic spectrum. It is evident from these data that, while the broad, visible absorption band of CV slightly shifts to the red as a result of binding to DNA, a new absorption band definitely develops in the longer wavelength side where each isotropic spectrum descends monotonically

indicating no hidden absorption band. The fact that this new long wavelength band is always associated with the DNA-CV complex whose P/D value ranges between 27 and 3 in either 1mM or 0.2mM NaCl solution is quite significant, because a pending question is now possibly clarified on whether or not a new long wavelength band develops when a dye is bound to polyelectrolyte to show metachromasy.[1)]

The reduced dichroic spectrum should be flat throughout an absorption band, if the band originates from a single electronic transition. This is apparently not the case for DNA-CV complexes with P/D 27-3, since the reduced dichroic spectra (closed circles) do not remain constant between 700 and 450 nm. The spatial arrangement of the bound CV relative to the orientation axis of the DNA-CV complex appears to be independent of the P/D, as judged from the general similarity of the reduced dichroic spectra, although the isotropic spectra are remarkably dependent on the P/D. Visual inspection of the $(\varepsilon_{\parallel}-\varepsilon_{\perp})/\varepsilon$ plots suggests that the absorption band of the CV bound to DNA involves three or possibly four component bands whose approximate locations are at 650-620 nm, 600-580 nm, 560-530 nm, and below 500 nm. The transition moment of the longest wavelength band should make an average angle less than 54.7° with respect to the orientation axis of the DNA-CV complex, while the moment of the remainder should each make an angle more than 54.7°.

The Velocity Gradient Dependence of the Reduced Dichroism. The specific value of the above angle can be determined from Eq. (2), once the value of

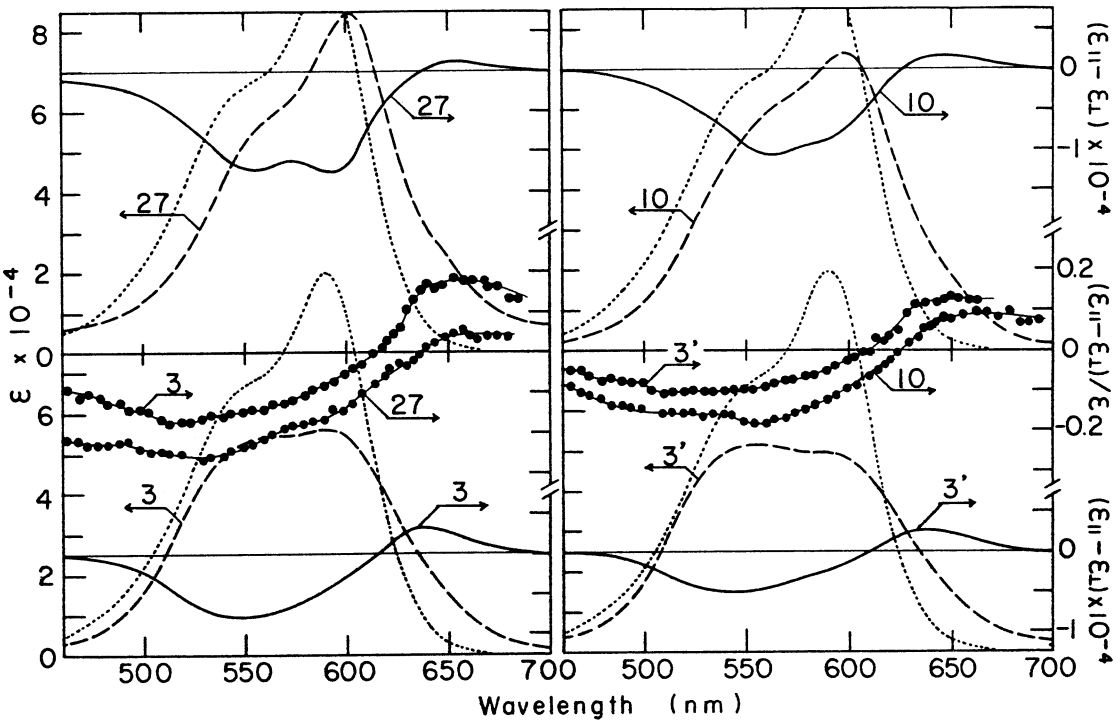

Fig. 1. The flow linear dichroic (————), reduced dichroic (-●-●-●-), and isotropic (— — —) spectra of the DNA-CV solutions at three P/D values. The absorption spectrum of CV (-------) is shown for comparison. The arrows indicate the ordinates to be referred to. The unprimed and primed numbers correspond to the P/D in 1mM and 0.2mM NaCl, respectively. The velocity gradient of flow is ca. 17,000 sec^{-1}. The dye concentration ranges between 10 and 50 μM.

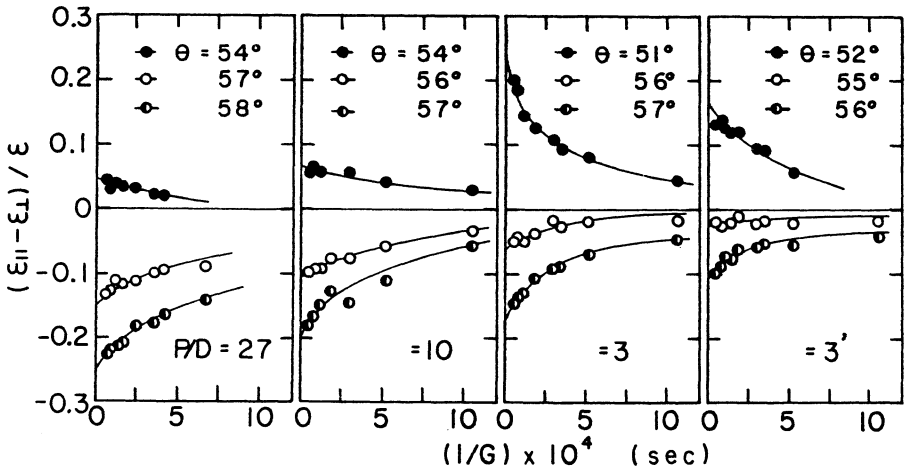

Fig. 2. The dependence of the reduced dichroism of DNA-CV solutions on velocity gradient at three wavelengths (650 nm —●—; 600 nm —○—; 550 nm —◐—).

$[(\varepsilon_{\parallel}-\varepsilon_{\perp})/\varepsilon]_{\infty}$ is obtained. The dependence of $(\varepsilon_{\parallel}-\varepsilon_{\perp})/\varepsilon$ on velocity gradient at three selected wavelengths is shown in Fig. 2. The reduced dichroism of the DNA-CV complex increases, as G increases. The $[(\varepsilon_{\parallel}-\varepsilon_{\perp})/\varepsilon]_{\infty}$ may be estimated by extrapolating 1/G to zero. Such an extrapolation may introduce some ambiguity, unless measurements are carried out at sufficiently high values of G. Nevertheless, the average angles estimated at each wavelength indicate that the transition moments responsible for the broad, visible absorption band of the CV bound to native DNA are inclined to the orientation axis of the complex by 51-58°, as shown by the most probable values in Fig. 2. Whether or not the orientation axis of the DNA-CV complex under flow can be taken as the long axis of the double-stranded helical DNA remains to be resolved. Since it is unlikely that a native DNA molecule of about $4\sim5\times10^6$ Daltons maintains a rigid rod-like conformation, the average angles of 51∿58° do not necessarily imply that the CV molecules are bound to each DNA at those angles. It is also indeterminable at present if the values given in Fig. 2 indicate that the bound CV and the DNA base pairs are not in parallel. Detailed work on other DNA-dye systems is in progress and will be reported.

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