Electric Dichroism Study of a Sonicated DNA and Its Complex with an Acridine Dye in Aqueous Solutions: Field-Strength Dependence and Linear Dichroic Spectra

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ABSTRACT: Electric dichroism of sonicated DNA (sDNA, $M_r = 22.5 \times 10^4$ daltons) and its complex with 3,6-bis(dimethylamino)-10-methylacridinium chloride (MeAO) in aqueous solutions containing 1 mM NaCl and sodium cacodylate buffer was studied at 25 °C with an apparatus which can deliver a single rectangular-wave pulse whose voltage and duration are variable (0–8 kV and 5 ms to 5 μs). The dependence of dichroism signals on the applied field strength was examined at peak wavelengths: 260 nm for sDNA and 262 and 505 nm for sDNA–MeAO at a P/D value of 8, where P and D are the concentrations of DNA-phosphate and MeAO in solution, respectively. In the low-field range (0–4 kV/cm), the signals were proportional to the square of the field strength, obeying the Kerr law. In the high-field range, the signals showed a saturating trend and the limiting values of reduced dichroism were evaluated. The apparent angles of DNA bases and bound MeAO relative to the orientation axis were about 69–70°. Linear dichroic (LD) spectra were measured at fixed field strengths in three wavelength regions, i.e., 215–300 nm for sDNA and 215–305 and 415–525 nm for sDNA–MeAO. The visible LD spectrum of sDNA–MeAO was wavelength independent, indicating no out-of-plane transition, while the LD spectrum of sDNA was strongly wavelength dependent, indicating the presence of several such transitions. Similarities and differences between sDNA and high molecular weight DNA samples are discussed.

In a previous paper,[1] detailed descriptions were given of an apparatus and the method of measurements of electric linear dichroism and electric birefringence together with some results on the dichroic spectra and field-strength dependence of DNA and its complex with an acridine dye, 3,6-bis(dimethylamino)-10-methylacridinium chloride (MeAO), in aqueous solutions. A high molecular weight calf thymus DNA (hDNA) was peculiar in the sense that its electric dichroism signals were nearly dependent on the first power of the externally applied electric field strength (E) over the wide range 0–8 kV/cm. This result is a contradiction to the well-known Kerr law, which predicts that such signals depend on the square of E in the limit of low fields.[2] If the native hDNA in solution were a double-stranded rigid rod representing the B form of the Watson–Crick model, the reduced dichroism at saturation, $(\Delta A/A)_s$, should be −1.5.[1,2] This value could not be verified experimentally because of instrumental difficulties.

Since then, some reports have been published on the electric dichroism and related subjects of hDNA and sonicated or enzymatically digested low molecular weight DNA in order to clarify their electrooptical properties and secondary structure in aqueous solutions.[3-11] (A monograph[12] and extensive reviews[13] have since appeared in which numerous references are cited to date of publication.) The diverse results have indicated that the dependence of the reduced dichroism on field strength is quite complex, being affected by such experimental factors as molecular weights, ionic strengths, and the species of counterions, and that the estimated $(\Delta A/A)_s$ values of the 260-nm band of the DNA bases are in the range −1.0 to −1.5. These values led to various conclusions on the apparent angle between DNA base pairs and the axis of the double-stranded helix. They were in the range 70–90°.[3,4,6,11] The linear dichroic (LD) spectra of various DNA–dye complexes have also been studied in the absorption region of bound dyes.[1,4,7,14-17] However, it has become clear that no precise structural determination of such complexes can be made unless the conformation of the DNA backbone, the electric field dependence of the complexes, and the direction of the optical transition moments of the dyes per se are all available.

In this paper we report the LD spectra and the field-strength dependence of a sonicated rodlike DNA (sDNA) and its complex with MeAO (sDNA–MeAO) in 1 mM NaCl solutions over a wide electric field strength range of 0–22 kV/cm, which is attained with a newly built apparatus.[18] The aims of the present work are (1) to compare the two results by performing experiments with sDNA under conditions nearly identical with those of the previous case for hDNA, (2) to evaluate the saturation value at the limiting high field from the field-strength dependence of reduced dichroism, (3) to test the applicability of the theory,[2,25] which is based on the interaction between permanent dipole and/or covalent polarizability anisotropy and the external electric field, to DNA in aqueous solutions, (4) to estimate the transition moments involved in the UV absorption band of DNA from the LD spectrum between 215 and 300 nm, and (5) to know the configuration of MeAO bound to DNA from the LD spectra in the visible and UV regions.

Experimental Section

Materials and Preparations. A calf thymus DNA sample, purchased from Worthington Biochemical Corp., was sonicated according to the procedure described elsewhere in detail.[11] The weight-average molecular weight of this sonicated DNA was determined to be 22.5×10^4 daltons from equilibrium sedimentation. A stock sDNA solution (ca. 0.6 mg/mL) containing 1 mM NaCl and 0.1 mM sodium cacodylate buffer was dialyzed against the same solvent at 4 °C for 48 h. This dialyzed solution was diluted with a 1 mM NaCl–0.1 mM cacodylate solvent in an ice bath. The concentration of sDNA was determined at 25 °C with a molar absorption coefficient ϵ of 6400 M^{-1} cm^{-1} at 258.5 nm.[19,20] The hyperchromicity of the thermally denatured DNA solution was larger than 31% after shock cooling.[1,11,19] 3,6-Bis(dimethylamino)-10-methylacridinium chloride (MeAO) was the same sample as used previously.[1,20] It was dried in vacuo at 56 °C for 6 h. An sDNA–MeAO solution in a 1 mM NaCl–0.2 mM cacodylate buffer was prepared by dropwise addition of the dye solution to the dilute sDNA solution at a mixing ratio (P/D) of 8, where P/D is defined as the ratio of the molar concentration of DNA-P to the molar concentration of MeAO in a solution.[1,19] The final concentration of MeAO was 22.9 μM.

Measurements. Isotropic UV and visible absorption spectra were measured on a Hitachi EPS-3T recording spectrophotometer at 25 °C with a matched pair of 1-cm cells. Electric linear dichroism of the sDNA and sDNA–MeAO solutions was measured at 25 °C on an apparatus which was built in our laboratory.[18] The previous design was basically followed for both the optical and electric systems of this apparatus.[1,21] An R106UH photomultiplier

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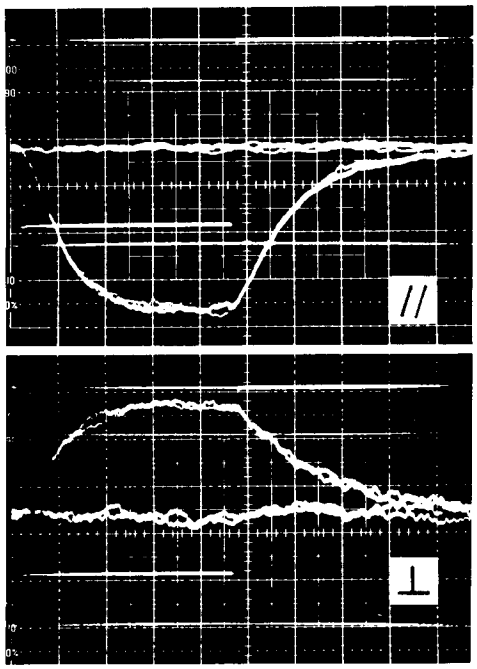

Figure 1. Photographs of pulsed electric field and parallel (||) and perpendicular (⊥) dichroism signals. A single rectangular pulse of 10 kV/cm was applied twice to each sDNA–MeAO solution containing 1 mM NaCl–0.2 mM sodium cacodylate at 25 °C and at a pH of 6.25 and at 505 nm. Sweep time is 10 μs/division and voltages are 20 mV/division for || and 10 mV/division for ⊥.

(Hamamatsu TV Co.) was used to cover a wavelength region between 540 and 215 nm, the shorter wavelength end being limited by the characteristics of the Glan–Taylor calcite polarizing prism. The tube was operated in such a way that the deviation from linear response between the input photon flux and the output photocurrent was less that 1%.[18] The half-intensity bandwidth was kept better than 1.5 nm throughout. The "Kerr" cell was of cylindrical shape with an optical path length of 1.00 cm and an electrode gap of 0.207 cm.[1,22] Each transient dichroism signal reached the steady state after an external single rectangular voltage pulse was applied, with a duration of 40–200 μs and an intensity of 0.5–4.6 kV. The voltage pulse, dichroism, and stationary signals were displayed on the CRT of a Tektronix 7623A storage oscilloscope and photographed (Figure 1). A fresh sample solution was used each time when a high pulse field was applied in order to avoid denaturation of the sample DNA. Dielectric breakdown of the solution occurred by a single pulse of ca. 22 kV/cm or higher with a pulse width of 45 μs.

Data Analysis. The procedure for acquisition and processing of dichroism data has been described elsewhere.[1,18,22] The parallel (specific) dichroism ($\Delta A_{\parallel}/A$) and the perpendicular (specific) dichroism ($\Delta A_{\perp}/A$) were calculated from the photographed signals, together with the isotropic absorbance (A) of the same solution in the absence of an electric field, as follows:

$$\frac{\Delta A_{\parallel}}{A} = \frac{A^{E}_{\parallel} - A}{A} = -\frac{1}{A}\log\left(1 + \frac{\Delta V_{\parallel}}{V_{\parallel,0}}\right) \quad (1)$$

$$\frac{\Delta A_{\perp}}{A} = \frac{A^{E}_{\perp} - A}{A} = -\frac{1}{A}\log\left(1 + \frac{\Delta V_{\perp}}{V_{\perp,0}}\right) \quad (2)$$

where $A^{E}_{\parallel}$ is the absorbance of a sample solution for the incident monochromatic light beam polarized parallel to the field and $A^{E}_{\perp}$ is the same for the perpendicularly polarized light. $\Delta V_{\parallel}$ is the difference ($V_{\parallel,E} - V_{\parallel,0}$) of the voltages which are generated across a photomultiplier load resistor with and without applied field and are proportional to the intensities of the transmitted parallel-polarized light. $\Delta V_{\perp}$ is the difference ($V_{\perp,E} - V_{\perp,0}$), which are the corresponding quantities for the intensities of the transmitted perpendicular-polarized light. The reduced dichroism at any wavelength λ is calculated from eq 1 and 2 as $\Delta A/A = (A^{E}_{\parallel} - A^{E}_{\perp})/A$. It should be noted that, if there is no electrochromism involved, the following relation should hold:[1,2]

$$A = \tfrac{1}{3}(A^{E}_{\parallel} + 2A^{E}_{\perp}) \quad \text{or} \quad \Delta A_{\parallel} = -2\Delta A_{\perp} \quad (3)$$

When a system consists of rigid rodlike macroions of more than one kind ($j > 1$), the reduced dichroism may be written in general as[7,12,23,24]

$$\frac{\Delta A}{A} = \frac{\frac{3}{2}\sum_j \Phi_j(\beta_j,\gamma_j,\rho_j)\sum_i (3\cos^2\theta_{ij} - 1)A_{ij}}{\sum_i\sum_j A_{ij}} \quad (4)$$

where θ_{ij} is the angle between the ith transition moment of the chromophoric group of the jth macroionic species and the longitudinal axis of symmetry of this macroion, A_{ij} is the corresponding absorbance at λ, and $\Phi_j(\beta_j,\gamma_j,\rho_j)$ is the orientation function which describes the orientation behavior of the jth macroionic species in solution under an external electric field.[1,2,10,25,26] The variables β_j, γ_j, and ρ_j are the interaction terms between the field strength E and the electric properties of the jth macroionic species, i.e., the apparent permanent dipole moment along the long axis μ_3, the covalent polarizability anisotropy $\Delta\alpha \equiv (\alpha_{33} - \alpha_{11}) \geq 0$, and the induced dipole moment along the long axis caused by the ionic atmosphere polarization σ_3, respectively. These variables are expressed as[2,25,26]

$$\beta = \mu_3 E/kT \qquad \gamma = \Delta\alpha E^2/2kT \qquad \rho = \sigma_3 E/kT \quad (5)$$

where k is the Boltzmann constant and T is the absolute temperature. The exact form of the counterion-induced dipole moment is under active investigation.[27,28] It depends on the specific model; e.g., σ_3 is given equal to $(ezL/2)n^{1/2}$ according to the Kikuchi–Yoshioka model.[9-11,26] At low electric fields ($E \to 0$), the orientation function is given as

$$\Phi = \frac{E^2}{15}\left[\left(\frac{\mu_3}{kT}\right)^2 + \frac{\Delta\alpha}{kT} + \frac{1}{3}\left(\frac{\sigma_3}{kT}\right)^2\right] \quad (6)$$

which indicates that the reduced dichroism is proportional to the square of applied field.

For a dye–polyion solution which contains a single complex species and some unbound dye ions, the reduced dichroism of the complex, $(\Delta A/A)^{b}$, is given from eq 4 as

$$\left(\frac{\Delta A}{A}\right)^{b} = \left(\frac{\Delta A}{A}\right)\left(\frac{A}{A - A_u}\right) = \left(\frac{\Delta A}{A}\right)\left(\frac{A}{A_b}\right) \quad (7)$$

where A_b and A_u are the absorbances of bound and unbound dyes at λ, respectively, since the latter cannot be oriented by an electric field.

Results

Electric Field Dependence of Dichroism. The field-strength dependence of the dichroism signals of sDNA and sDNA–MeAO is shown in Figure 2. The values of $\Delta A_{\parallel}/A$ were measured at the absorption band maxima over a wide field strength range (0–22 kV/cm). The similarities and differences between the present sDNA and sDNA–MeAO and the previous hDNA and hDNA–MeAO systems are as follows:[1] (1) The sign of $\Delta A_{\parallel}/A$ is negative in all cases, (2) the field dependence of $\Delta A_{\parallel}/A$ shows a saturating trend at high fields for the sDNA and sDNA–MeAO systems but not for the hDNA system, and (3) the Kerr law holds for the sDNA system at low fields but not for the hDNA system. It should be noted that heat-denatured sDNA samples showed no dichroism signals over the entire range of field strengths and that the native sDNA showed no anomalous transient signal.[29]

Quadratic vs. Linear Dependence at Low Fields and the Saturating Trend at High Fields. The dependence of $\Delta A_{\parallel}/A$ on field strength in the low-field range is shown in Figure 3. The $\Delta A_{\parallel}/A$ values of sDNA and sDNA–MeAO are clearly proportional to the second power of the field strength and not the first below ca. 4 kV/cm; hence, the Kerr law is obeyed. This result is contrary to

Table I
Electrooptical Parameters of sDNA and sDNA-MeAO in 1 mM NaCl-Sodium Cacodylate Buffer Solutions at 25 °C

	λ/nm	$\mu_3 \times 10^{-3}$/D	$(\mu_3)_r$[a]/D	$\Delta\alpha \times 10^{17}$/cm^3	$(\Delta A_\parallel/A)_s$	$\lvert\theta\rvert$/deg
sDNA	260	4.9	14	7.2	−0.62	69
sDNA-MeAO	262	4.4	13	4.0	−0.66	70
	505	4.4	13	4.0	−0.65	70

[a] μ_3 per base pair which is 340.

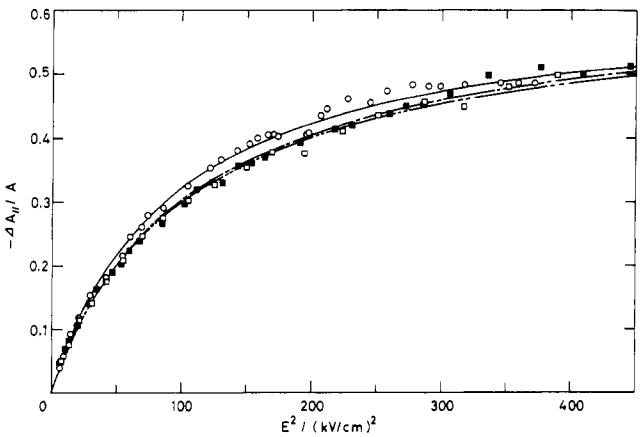

Figure 2. Dependence of the parallel specific dichroism ($\Delta A_\parallel/A$) of sDNA at 260 nm (○) and sDNA–MeAO at 262 (■) and at 505 nm (□) on the square of electric field strength, E^2. The points were experimentally determined and the curves were calculated by using the theoretical orientation function $\Phi(\beta,\gamma)$. The values of the parameter $\beta^2/2\gamma$ are 8 (—) for (○) [$\beta^2 + 2\gamma = 10$ at E^2 = 64.2 (kV/cm)^2], 12 (– – –) for (■) [$\beta^2 + 2\gamma = 10$ at E^2 = 72.5 (kV/cm)^2], and 12 (– · – · –) for (□) [$\beta^2 + 2\gamma = 10$ at E^2 = 72.5 (kV/cm)^2].

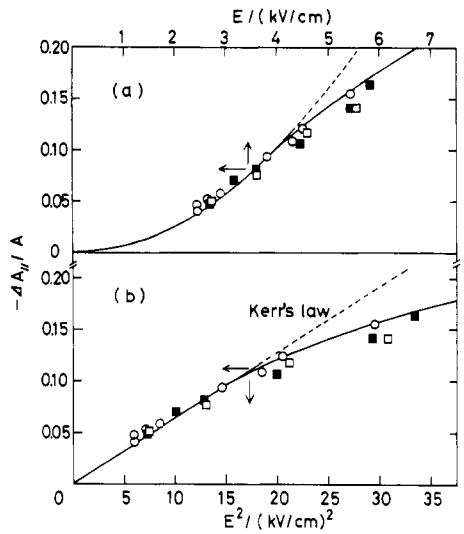

Figure 3. Field-strength dependence of $\Delta A_\parallel/A$ of sDNA and sDNA–MeAO in the low-field range. $\Delta A_\parallel/A$ values are plotted (a) against E and (b) against E^2. The quadratic dependence of $\Delta A_\parallel/A$ is clearly seen. Symbols and conditions are the same as in Figure 2.

the previous findings for hDNA and hDNA–MeAO,[1] in which the $\Delta A_\parallel/A$ values were dependent on nearly the first power of field strength below 8 kV/cm. However, the present finding on sDNA is in agreement with results on smaller polyelectrolytes[30-32] and nonionized polymer.[33] The estimation of the saturated value of reduced dichroism at infinitely high fields, $(\Delta A/A)_s$, or the intrinsic reduced dichroism is necessary for evaluating the angle θ from eq 4. The electric field strength dependence of $\Delta A/A$ values may be analyzed quantitatively if the orientation function $\Phi(\beta,\gamma,\rho)$ is available. Unfortunately, no rigorous and tractable form of Φ has been completed for polyelectrolytes as yet.[9,10,26-28] Therefore, use has been limited to the classical orientation function $\Phi(\beta,\gamma)$ for the nonionic cylindrical model with the permanent dipole moment and/or the polarizability anisotropy given in eq 5. In the case of polylectrolytic DNA, the orientation was considered to be solely due to either the permanent dipole (β only)[3] or the induced dipole (γ only) mechanism.[4,29] It has also been shown that the orientation of some sDNA samples can be best described by the mixed-dipole mechanism in which both β and γ are nonzero.[11] These parameters may be determined by a *matching procedure*, a curve-fitting method,[11,21] and also by an extrapolation procedure.[3,4,6,11] Figure 2 shows that the experimental points of sDNA fit reasonably well to one of the theoretical curves over a wide range of E^2 with a $\beta^2/2\gamma$ value of 8, the $(\Delta A_\parallel/A)_s$ being −0.62. The two sets of experimental points of sDNA–MeAO also fit to a curve with a $\beta^2/2\gamma$ value of 12.

Values of Orientation Parameters. The matching procedure allows determination of both β and γ values separately and, hence, μ_3 and $\Delta\alpha$, besides the $(\Delta A_\parallel/A)_s$ value, which are all given in Table I. Since the antiparallel double-stranded DNA is unlikely to possess any intrinsic permanent dipole moment contributing to field orientation, the value of β or μ_3 should be zero. Yet, there are a number of reports that ionized DNA behaves as if it possessed a permanent dipole.[3,34-36] Therefore, it would be permissible to use the values of β and γ with the understanding that they are parameters which characterize the field-strength dependence of dichroism for each sDNA solution. Since they are useful in describing the relative magnitude of orientability of the various samples, we shall term the constant μ_3 the pseudopermanent dipole moment.[11] The reasonable agreement between the theoretical $\Phi(\beta,\gamma)$ function and the experimental points (Figure 2) suggests that the counterion-induced dipole moment σ_3 (eq 5) is saturated at the very weak field strength and becomes independent of field strength in the medium-to-high fields.[26] Hence, the moment σ_3 may behave as if it were the permanent dipole moment μ_3 (eq 6). (It should be noted that the permanent dipole moment μ_3 as such is an apparent quantity which requires an internal field correction.[37])

The results in Table I show that (1) the values of μ_3 are large, (2) the $(\Delta A_\parallel/A)_s$ values are −0.62 to −0.66, differing from −1.0, and (3) the apparent angles, θ, between the orientation axis and the transition moments of both the base pairs of sDNA and the bound MeAO are all about 69–70°, far less than 90°.

LD Spectrum of sDNA in the UV Region. Figure 4 shows absorption spectra of a native sDNA solution in the presence ($A^E_\parallel$ and $A^E_\perp$) and in the absence (A) of applied field in the lower half and the parallel dichroism ($\Delta A_\parallel/A$), the perpendicular dichroism multiplied by −2 ($-2\Delta A_\perp/A$), and the reduced dichroism ($\Delta A/A$) in the upper half. The dichroism in the 215–300-nm region is negative with no reversal of the sign. Both $\Delta A_\parallel/A$ and $-2\Delta A_\perp/A$ agree with each other within experimental uncertainty. Thus, eq 3 holds for them (A_{calcd}), which indicates that no detectable electrochromism is involved in the sDNA solution. The wavelength dependence of the

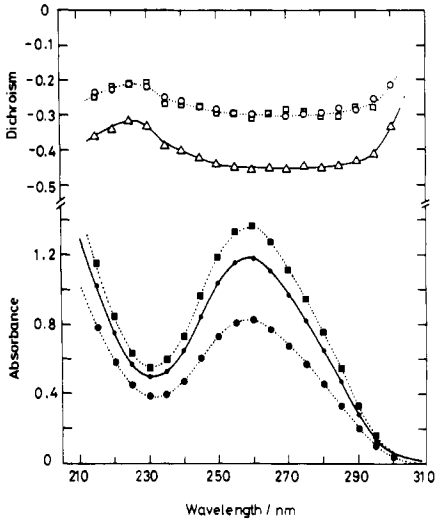

Figure 4. Linear dichroic spectra of sDNA in a 1 mM NaCl–0.1 mM sodium cacodylate solution at a constant field strength of 9.71 kV/cm. In the lower half, $A^E_{\parallel}$ (●) and $A^E_{\perp}$ (■) are the absorbances of the solution for light polarized parallel and perpendicular to the direction of the electric field. The isotropic spectrum A (solid curve) shows a maximum at 258.5 nm and a minimum at 231.5 nm with an intensity ratio $A_{258.5}/A_{231.5}$ of 2.36. The spectrum A_{calcd} (-·-) was calculated from the relation $A_{calcd} = (A^E_{\parallel} + 2A^E_{\perp})/3$. In the upper-half, $\Delta A_{\parallel}/A$ (○) is the specific parallel dichroism and $\Delta A/A$ (△) is the reduced dichroism. The specific perpendicular dichroism $\Delta A_{\perp}/A$ (□) was multiplied by -2 in order to indicate that the relation $\Delta A_{\parallel}/A = -2\Delta A_{\perp}/A$ holds for the present system.

$\Delta A/A$ values of sDNA, i.e., the LD spectrum, is in good agreement with a previous result of hDNA.[1] The LD spectrum is constant only in the 250–270- and 275–290-nm regions. It decreases gradually above 290 nm and below 250 nm, reaching a saddle point near 230 nm. No anomalous behavior of the LD spectrum near 283 nm could be observed, contrary to the report by Ding et al.[3]

LD Spectrum of sDNA–MeAO in the Visible Region. Figure 5 shows absorption spectra of a native sDNA–MeAO solution in the presence ($A^E_{\parallel}$ and $A^E_{\perp}$) and in the absence (A) of applied field in the lower half and the parallel dichroism ($\Delta A_{\parallel}/A$), the perpendicular dichroism multiplied by -2 ($-2\Delta A_{\perp}/A$), and the reduced dichroism ($\Delta A/A$) in the upper half. The isotropic spectrum shows an absorption peak at 505 nm, a distinct shoulder near 480 nm, and another weak shoulder near 445 nm, as in the case of hDNA–MeAO.[1] The wavelength dependence of dichroism indicates that (1) the dichroism of sDNA–MeAO is negative in the visible region, (2) the reduced dichroism is almost constant throughout this region [$\Delta A/A = -(0.42 \pm 0.02)$], and (3) no electrochromism is involved because $\Delta A_{\parallel}/A$ is equal to $-2\Delta A_{\perp}/A$. Interestingly, these features are nearly identical with the previous findings for hDNA–MeAO (Figure 11 of ref 1). Since the DNA moiety of DNA–MeAO complex makes no contribution, the observed LD spectrum in the visible region is solely due to the MeAO molecule bound to sDNA.

The directions of transition moments of MeAO are shown in Figure 5. Film dichroism studies of MeAO and other acridine dyes have clarified that the simple and broad absorption band in the visible region consists of two mutually perpendicular electronic transitions (the ^1L_a and ^1L_b bands), each with a vibrational structure.[38-40] Contrary to the result observed for sDNA- or hDNA–MeAO complex, the wavelength dependence of the reduced dichroism of MeAO in a stretched film shows a rather complex variation (Figure 4 of ref 38). The long-axis polarized A_z

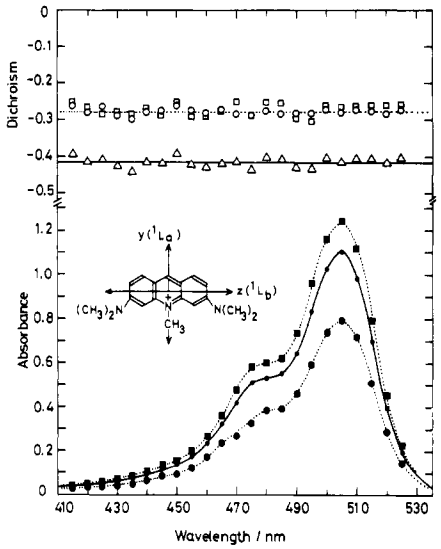

Figure 5. Linear dichroic spectra of sDNA–MeAO in a 1 mM NaCl–0.2 mM sodium cacodylate solution at a P/D value of 8 at a constant field strength of 9.63 kV/cm in the visible absorption region. Symbols are the same as in Figure 4.

spectrum (^1L_b band) is dominant over the short-axis polarized A_y spectrum (^1L_a band) in the 530–430-nm region (Figure 6 of ref 38).

If an sDNA–MeAO solution contains some free, unbound MeAO molecules, the dichroism data must be corrected according to eq 7. Since the bound fraction of MeAO was estimated to be more than 99% in a 1 mM NaCl-containing DNA solution at a P/D of 8,[41-43] the amount of unbound MeAO in the present solution should be negligibly small. Therefore, the observed LD spectra in Figure 5 can be considered the LD spectra of bound MeAO, $(\Delta A/A)^b$. Then, the nearly constant values of either $\Delta A/A$ or $\Delta A_{\parallel}/A$ throughout the visible band may be interpreted as being due to the nearly equal orientation of two transition moments (the y and z axes) of MeAO relative to the orientation or helix axis of sDNA–MeAO complex, provided that the number and the directions of the transition moments remain unaltered when MeAO is bound to the DNA site. This assumption is still open to rigorous questioning and must be verified. Nevertheless, the present result strongly indicates that no transition moment would be polarized along the orientation axis of sDNA–MeAO complex.

LD Spectrum of sDNA–MeAO in the UV Region. Figure 6 shows spectra of a native sDNA–MeAO solution in the presence ($A^E_{\parallel}$ and $A^E_{\perp}$) and in the absence (A) of applied field in the lower half and the parallel dichroism ($\Delta A_{\parallel}/A$), the perpendicular dichroism multiplied by -2 ($-2\Delta A_{\perp}/A$), and the reduced dichroism ($\Delta A/A$) in the upper half. The maximum of the isotropic spectrum was at 262 nm, differing slightly from the 259 nm of sDNA. The dichroism in the UV region is again negative throughout. Both $\Delta A_{\parallel}/A$ and $-2\Delta A_{\perp}/A$ agree with each other, indicating no electrochromism.

The observed reduced dichrosim $(\Delta A/A)'$ of DNA–MeAO complex results from DNA bases and bound MeAO. (The prime denotes the quantity of the complex.) It may be expressed from eq 4 as

$$\left(\frac{\Delta A}{A}\right)' = \frac{3}{2}\Phi'\left[(3\cos^2\theta'_{DNA} - 1)\frac{A'_{DNA}}{A'} + (3\cos^2\theta'_{MeAO} - 1)\frac{A'_{MeAO}}{A'}\right] \quad (8)$$

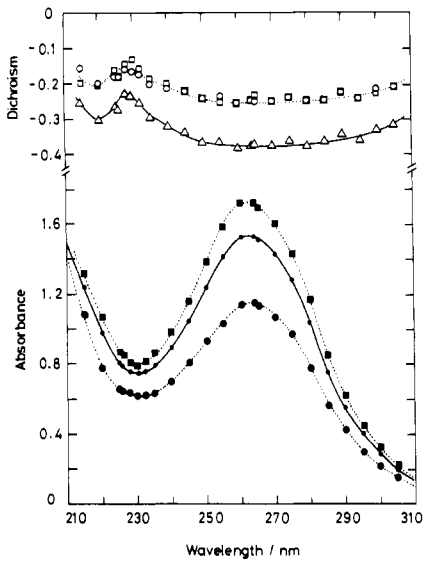

Figure 6. Linear dichroic spectra of sDNA–MeAO in a 1 mM NaCl–0.2 mM sodium cacodylate solution (P/D = 8) at a constant field strength of 9.08 kV/cm in the UV absorption region. Symbols are the same as in Figure 4.

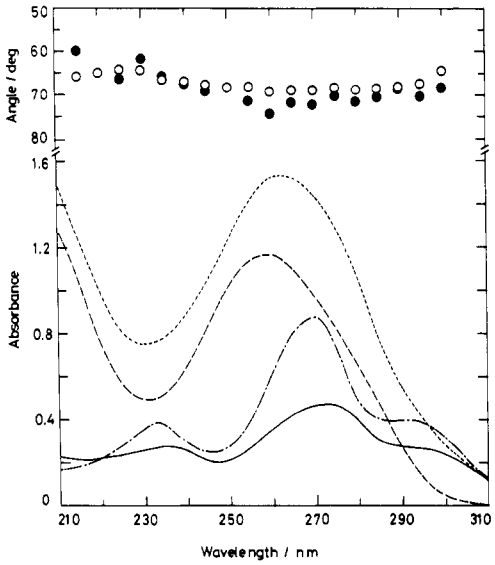

Figure 7. Apparent angles of sDNA and sDNA–MeAO solutions and the isotropic absorption spectra of sDNA, MeAO, and sDNA–MeAO solutions, together with the spectrum of the bound MeAO. Upper half: (○) is the apparent angle of sDNA itself, while (●) is the apparent angle of bound MeAO in the sDNA–MeAO complex, at the perfect degree of orientation (see Table I; e.g., $(\Delta A_{\parallel}/A)_s = -0.62$ at 260 nm and $\Phi = 1$ for sDNA). Lower half: (– – –) is the absorbance of the sDNA whose concentration is 183 μM and is the same as that of the sDNA–MeAO solution. (– · –) is the absorbance of the MeAO solution whose concentration is 22.9 μM and is the same as that of the sDNA–MeAO solution. (– · · –) is the observed absorbance of the sDNA–MeAO solution. (—) is the contribution of the bound MeAO to the absorbance of the sDNA–MeAO solution. Note that the absorbance of the bound MeAO is hypochromic and bathochromic relative to that of MeAO itself.

where Φ' is the orientation factor of the complex at 9.08 kV/cm, θ' is the average angle between the transition moments of bases and the orientation axis of the complex, θ'_{MeAO} is the average angle between the transition moments of bound MeAO and the orientation axis, and A'_{DNA} and A'_{MeAO} are the absorbances contributed by the bases and the bound MeAO moiety, respectively. It is reasonable to assume that the conformation of the DNA backbone remains unchanged in the complex in which the bound MeAO molecules (eight per DNA-phosphate) are probably distributed apart from one another. Consequently, $\theta'_{DNA} = \theta_{DNA}$ and $A'_{DNA} = A_{DNA}$ at the same DNA concentration, where θ_{DNA} and A_{DNA} are the quantities for pure sDNA. Thus, the angle θ'_{MeAO} may be calculated from eq 8 in the UV region.

Figure 7 shows that the values of θ'_{MeAO} of bound MeAO are only slightly larger than the values of θ'_{DNA} of sDNA between 250 and 300 nm. The absorption spectrum of free MeAO in Figure 7 consists of two transitions in this region: the short-axis (y axis) polarized band (^1B_a) and the long-axis (z axis) polarized band (^1B_b).[39,44,45] The ^1B_b band dominates over the ^1B_a band near 270 nm, but this trend is reversed in the 305–350-nm region (Figure 6 of ref 38). Again as above, with the assumption that the number and the directions of the transition moments of bound MeAO remain the same as those of the free MeAO (this is probably correct as shown in Figure 7), it is concluded that at least the transition moment of the ^1B_b band is inclined to the helical axis of the DNA backbone at 70 ± 2.5°. This angle is about the same value obtained for the visible band of bound MeAO, which is reasonable because all four transition moments of MeAO are in the molecular plane.

Discussion

Electric Field Dependence of sDNA and sDNA–MeAO. The steady-state dichroism of sDNA and sDNA–MeAO clearly obeys the Kerr law (Figure 3). This quadratic dependence of reduced dichroism on field strength has been predicted by the theories of electric dichroism for molecules with cylindrical symmetry, which may be a rigid rodlike or a stiff (or extended) wormlike configuration. The molecular weight of the present sDNA sample is 22.5 × 10^4 daltons, corresponding to 340 base pairs as an average. Its contour length is about 1150 Å as the B form or 870 Å as the A form. Since the persistence length of DNA is about 1.5 × 10^3 Å (5 mM NaCl)[46] or 1.3 × 10^3 Å (30 mM NaCl),[47] both sDNA and sDNA–MeAO in 1 mM NaCl solutions are probably rigid rods with little flexibility; hence, they may be treated with the theories for the rigid rod. In fact, the present results are consistent with those already reported for sDNA samples of various chain lengths.[6,11] Consequently, the linear dependence of dichroism and birefringence on field strength, which has been often observed for high molecular weight DNA with sodium ion as the counterion, must be considered rather anomalous or exceptional.[1,48] Although the origin of the linear dependence is still under investigation,[8] it appears to be affected by several factors such as the molecular flexibility which makes the segmental motion of the backbone vary with applied field strength, the kind of counterion which contributes to the ionic atmosphere polarization, and the ionic strength which suppresses charge repulsion between the ionized groups.

The dichroism signals of sDNA definitely show a saturating trend at high fields even in a 1 mM NaCl solution. If sDNA in aqueous solution is in the B form, the intrinsic parallel and reduced dichroism values are probably −1.0 and −1.5, respectively (Figure 15 of ref 1). These values are difficult to obtain directly from experiments since (1) dielectric breakdown or electrolysis occurs due to electric conduction of the solution[18] and (2) DNA may be partially denatured by high electric fields.[49] The use of the classical orientation function $\Phi(\beta,\gamma)$ for the curve-fitting procedure for the data of polyionic DNA might be questioned because $\Phi(\beta,\gamma)$ was derived for nonionic polymer systems.[25] Nevertheless, the experimental points fit well to a curve of $\Phi(\beta,\gamma)$ over a wide field range, particularly in the medium-to-high fields. This is probably because the electric

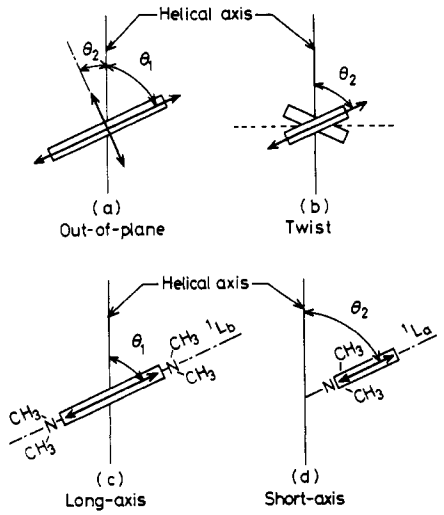

Figure 8. Schematic illustration of possible orientation angles of DNA base pairs and bound MeAO plane with respect to the helical axis (orientation axis) of sDNA. In a and b, θ_1 and θ_2 are the assumed angles of two mutually orthogonal transitions (1 and 2, respectively) of DNA bases relative to the helical axis. For sDNA–MeAO in c and d, θ_1 and θ_2 are assumed to be the angles of the ^1L_b and ^1L_a transitions relative to the helical axis of the complex, respectively.

moment due to ionic atmosphere polarization is already saturated at weak fields.[8,26] Fitting of the points was attempted by using an orientation function which was proposed by Kikuchi and Yoshioka for ionic atmosphere polarization of counterions,[26] but the result was not particularly good over a wide range of E^2.

Since the reversing-pulse electric birefringence signal of sDNA in 1 mM NaCl showed no extremum upon reversal of pulse polarity, the sDNA possesses neither the permanent nor slow-induced dipole moment to any appreciable extent.[48,50] Therefore, the electric orientation of sDNA is mostly due to the fast-induced dipole moment probably resulting from the migration of condensed counterions on the DNA surface.[37,48] The behavior of this ionic dipole moment probably resembles that of the permanent dipole moment and polarizability anisotropy closely, as the result of curve-fitting indicated (Figure 2). Together with the known antiparallel helical structure, the apparent permanent dipole moment μ_3 noted previously should actually be a "pseudopermanent" dipole moment whose magnitude is large enough to orient the whole sDNA molecule (Table I).[1,11]

Wavelength Dependence of Reduced Dichroism of sDNA and sDNA–MeAO. The angle of 69° at 260 nm, estimated from the intrinsic reduced dichroism (Table I), is in excellent agreement with the values of 73 ± 3° reported by Hogan et al.[6] and 68–72° by Yamaoka and Charney.[11] The interpretation of these values is rather complex because the 260-nm absorption band of DNA is actually composed of several electronic transitions of the constituent bases. The apparent angles in the vicinity of 225 and 300 nm are about 64 and 64.5°, respectively; both are less than the value of 69° (Figure 7). These values are all incompatible with the original B form of DNA, suggesting that sDNA may deviate from it in solution under external electric fields.[6,49,51] It seems, however, to be unlikely that the electric field itself alters the conformation of DNA under the present experimental condition which satisfies eq 3.[49]

If an observed value of $(\Delta A/A)_s$ results from two overlapping transitions (1 and 2) whose absorbances are A_1 and A_2 and whose angles are θ_1 and θ_2 relative to the axis of orientation (say, the helical axis of sDNA) (see Figure 8), eq 4 may be written as

$$\left(\frac{\Delta A}{A}\right)_s = \frac{3}{2}\,\frac{(3\cos^2\theta_1 - 1)A_1 + (3\cos^2\theta_2 - 1)A_2}{A_1 + A_2} \quad (9)$$

Some values of $(\Delta A/A)_s$ were calculated for such a two-transition band system, which will be adopted as a simplified model for DNA, and are given in Table II. If, schematically, the transition moments along the long and short axes of a DNA base pair, denoted by 1 and 2 respectively, are in the same plane, the values of θ_1 and θ_2 at a given wavelength, say 260 nm, may be approximately 90° for a B-DNA and 70° for an A-DNA with a tilt angle of 20°. The twist of each base pair may introduce another complication (Figure 8b). In an A-DNA the θ_2 value would be 70° if the bases are twisted from each other by 20°: hence, the value of $(\Delta A/A)_s$ is −0.974. If there is no twist (θ_2 = 90°), it would depend on the relative intensity of the two transitions (1 and 2) (Table II). If the bases are not twisted in B-DNA ($\theta_1 = \theta_2 = 90°$), the $(\Delta A/A)_s$ value is −1.50. If they were twisted by, say 20°, the absolute magnitude of $(\Delta A/A)_s$ should be less than 1.50, depending on the ratio of A_1/A_2. If, in a slightly different case, an out-of-plane n–π^* transition (2) is overlapped by an in-plane π-π^* transition (1), the angle θ_2 equals $(\theta_1 - 90)°$ and the absorbance A_2 may be about one-fiftieth of A_1. The values of $(\Delta A/A)_s$ calculated with these parameters are −1.41 for a B-DNA and −0.906 for an A-DNA (Table II), differing from −1.50 and −0.974 for a single-transition (A_2 = 0) band system (see Figure 8a).

Table II
Values of $(\Delta A/A)_s$ Calculated for a Hypothetical Two-Transition Band System

θ_2/deg	A_2	θ_1 = 90°, A_1: 50	10	2	θ_1 = 70°, A_1: 50	10	2
90	50		−1.50		−1.24	−1.41	−1.48
	1				−0.984	−1.02	−1.15
70	50	−1.24	−1.06	−0.994		−0.974	
	1	−1.49	−1.45	−1.32			
50	50	−0.570	0.049	0.288	−0.307	0.137	0.308
	1	−1.46	−1.33	−0.880	−0.947	−0.852	−0.524
0	50	0.750	2.25	2.83	1.01	2.34	2.85
	1	−1.41	−1.09	0	−0.896	−0.612	0.351
−20	50	0.487	1.81	2.32	0.750	1.90	2.34
	1	−1.42	−1.14	−0.175	−0.906	−0.660	0.175

The scheme of the binding of MeAO to the DNA site is controversial in that the planar dye may insert itself between two base pairs (intercalation)[52] or its positively charged nitrogen (probably the quaternized nitrogen at the 10-position) may interact electrostatically with the negatively charged phosphate of the DNA backbone (external binding).[53] The arrangement of MeAO relative to the DNA site depends on the model we choose. Since the apparent angle θ of the 505-nm band of MeAO in the MeAO–sDNA does not differ much from that of the 260-nm band of sDNA itself (70 vs. 69°) and since the wavelength dependence of $\Delta A/A$ in the visible region is nearly constant, we cannot exclude either of the two possible binding modes unequivocally. Both the long-axis (^1L_b) and the short-axis (^1L_a) transitions of MeAO would be in the radial direction according to the intercalation model, whereas the ^1L_b transition would be tangential and the ^1L_a transition would be radial according to the external binding model (Figure 8c,d). If the transition moment directions and the corresponding band intensities of bound MeAO remain the same as those of MeAO itself (an assumption yet to be verified), the reduced dichroism of the visible band is

mostly influenced by the angle θ_1 (the ^1L_b band is denoted by 1) which is about 70° relative to the orientation axis (Figure 8c). The relative intensity of ^1L_b to ^1L_a is about 50 near the absorption maximum of 505 nm for MeAO;[38,39] therefore, the angle θ_2 for the ^1L_a transition may vary widely between 90° and about 50°, without affecting the dichroic spectrum appreciably (Table II).

We believe that the exact knowledge of the directions of the transition moments of each of the four paired DNA bases and those of individual bound dyes is a prerequisite for any further agrument on the conformation of DNA and DNA–dye complexes. Simulation of both linear dichroic and isotropic spectra such as those shown in Figures 4 and 5 should then be pursued. Finally, it may be worth noting that, although the high molecular weight calf thymus DNA is flexible, it shows a reduced dichroism spectrum which resembles the one shown in Figure 4.[1] A very close similarity between sDNA– and hDNA–MeAO complexes is also observed.[1] The coiled-coil or the extended wormlike structure of hDNA may assume an overall conformation close to that of rodlike sDNA. We hope that the conformation of hDNA and hDNA–dye complexes can be resolved eventually from the accumulated electric dichroism data of sDNA.

Conclusion

The field dependence of dichroism signals of sDNA and sDNA–MeAO could be described by the classical orientation function $\Phi(\beta,\gamma)$. The signals obeyed the Kerr law in low fields and showed a saturating trend in high fields. The angles between the orientation axis of rodlike sDNA and the transition moments of DNA bases near 260 nm and bound MeAO near 505 nm were both in the range 69–70°. The wavelength dependence of reduced dichroism, however, indicates that the UV band of sDNA is composed of both in-plane and out-of-plane transitions while the visible band of bound MeAO contains only in-plane transitions.

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