# Electric Birefringence and Electric Dichroism of Sonicated DNA in Aqueous Solutions with Various Additives. Electro-optical and Hydrodynamic Properties

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Electric birefringence (EB) and electric linear dichroism (ED) of a sonicated DNA ($M_w = 1.24 \times 10^5$) sample were studied in solutions containing various additives at 7 °C. Signals of EB at 535 nm and of ED at 260 nm were measured in the electric field range 0—*ca.* 19 kV/cm. Both signals obeyed the Kerr law at low field strengths ($E <$ *ca.* 5 kV/cm) and showed a saturating trend at high fields. The mean relaxation time, $\langle\tau\rangle_{EB}$, obtained from the decay of EB, was field-strength dependent, indicating the effect of the polydispersity of sDNA. The field strength dependence of steady-state EB signals was nearly identical with that of ED signals. These results were analyzed with the "classical" orientation functions derived for mono- and polydisperse systems. The optical anisotropy factor ($g_3 - g_1$) of sDNA was *ca.* $-2.0 \times 10^{-2}$, while the average angle between the orientation axis of sDNA and the transition moment of the base pair was *ca.* 70° at 260 nm. The electric moment of sDNA was influenced by ionic strength, counterions, and other additives. The weight-average length $l_w$ of sDNA decreased in this order: 0.2 mM NaCl**>9-aminoacridinium chloride ($P/D = 10$)>1.0 mM NaCl>0.33 mM $MgCl_2$> 20 vol% glycerol. Values of $l_w$/base pair were 2.8—2.7 Å in the glycerol and 3.6—3.4 Å in the 1.0 mM NaCl.

Electric dipole moments of DNA in solutions have been investigated by various methods, *e.g.*, electric birefringence (EB),[1-16] electric dichroism (ED),[3-5,17-27] and dielectric relaxation.[28-33] Both EB and ED are powerful techniques for studying the optical, electric, and hydrodynamic properties of DNA in aqueous solutions. With reversing-pulse electric birefringence, Greve and Heij[7] and Yamaoka and Matsuda[15] recently demonstrated that DNA fragments have no permanent dipole moment. Hogan *et al.*[21] found that an apparent dipole moment of DNA is inversely proportional to the square-root of ionic strength. There have been numerous observations that the electro-optical property of DNA is very sensitive to its molecular weight and to external conditions such as ionic strength and small molecular weight additives in solutions.[5,11,13-16,19,21,23] Stellwagen[16] recently studied the electro-optical and hydrodynamic properties of restriction enzyme fragments of DNA whose lengths vary widely, exposing a number of interesting points to be clarified. The decay process of birefringence, for example, could not be explained by a monodisperse system when the DNA fragments are larger than 217 base pairs (bp). The previous studies[1-33] are unanimous in pointing out that native DNA in solution can be oriented by an external electric field, but they also indicate that the nature of the electric moments responsible for field orientation remains to be clarified. Sustained efforts are needed for resolving this problem. For this purpose, the intrinsic effects of the polydispersity of DNA length and the flexibility of DNA chain, and the external effects of the various ionic additives must be considered on a systematic basis.

The present study is the first of a series of work on the electro-optical properties of DNA and related biopolymers. In this paper, the electric field strength dependence of steady-state signals of EB and ED was examined with a sonicated rodlike DNA (sDNA) sample under various ionic conditions. The decay curve of the EB signal was also measured an a function of field strength. This is a result that cannot be explained if the sample is monodisperse. Hence, tan analytical method for the polydisperse system was utilized to compare the observed EB and ED data with theoretical orientation functions. The length of sDNA in solutions with various additives was not constant, but was influenced by external factors. The applicability of the "classical" mixed orientation function $\Phi(\beta, \gamma)$[34] was tested for the sDNA system.

** 1 mM = $10^{-3}$ mol dm$^{-3}$.

## Experimental

*Materials.* A calf thymus DNA sample was purchased from Worthington Biochemical Corp (USA). The sonication procedure for sDNA was described elsewhere.[26] The protein content was determined to be less than 0.01 mg/1 mg DNA by the microbiuret method. The weight-average molecular weight was determined to be *ca.* $1.24 \times 10^5$ by ultracentrifugation.[26] (The ratio of the weight-average to the number-average molecular weight for sonicated DNA samples is known to be in the range between 1.02 and 1.11.[35]) The cationic dye, 9-aminoacridinium chloride (AA), was described in previous papers.[36-38] Other chemicals used were of reagent grade.

*Preparation of Sample Solutions.* The stock sDNA solution (*ca.* 1 mg cm$^{-3}$) containing *ca.* 1 mM NaCl was prepared in an ice bath. The solutions were unbuffered to avoid any effect of buffer cations.[37] Each stock DNA solution was diluted ten-fold and dialyzed against NaCl or $MgCl_2$ solutions of desired ionic strength at 4 °C for *ca.* 48 h. The concentration of AA was determined by using a molar absorption coefficient, $\varepsilon$, of $1.03 \times 10^4$ M$^{-1}$ cm$^{-1}$ at 401 nm.[37] The sDNA–AA solution was prepared by adding the dye solution to the sDNA solution at a molar mixing ratio of DNA-phosphate residue-to-dye ($P/D$) of 10, the final concentration of AA being 10 μM. The concentration of sDNA solution was determined photometrically with a molar absorption coefficient of 6400 M$^{-1}$ cm$^{-1}$ at 258.5 nm.[39] The sDNA in a 20 vol% glycerol–0.8 mM NaCl solution was prepared in an ice bath by adding glycerol to the aqueous sDNA solution. The hyperchromicity of the denatured sDNA solution containing either 1.0 mM NaCl or 0.2 mM NaCl was higher than 30%.[39,40] Absorption spectra were measured at 7 °C on a

Hitachi EPS-3T double-beam recording spectrophotometer equipped with a thermostated cell holder.

*Measurements of Electric Birefringence and Electric Linear Dichroism.* An EB apparatus constructed in our laboratory was used.[41] The voltage and the duration of a single square-wave electric pulse field were continuously varied between 0.248 and 3.24 kV and between *ca.* 40 and 150 μs, respectively. The instrumental response time for a transient birefringence signal was usually faster than 0.5 μs; this was checked with nitrobenzene.[42] The linear response between the transmitted light intensity and the output voltage generated across a load resistor was verified by testing the Malus law.[42] In order to determine the sign of the birefringence and to increase the sensitivity of the signal, a quarter-wave plate was placed before the analyzer. The phase retardation was measured at 535 nm and at 7 °C with the procedure described previously.[4,5,42] An electric linear dichroism apparatus was also built in our laboratory and has been described in detail.[24,38] Both the parallel dichroism ($\Delta A_{\parallel}/A$) and the perpendicular dichroism ($\Delta A_{\perp}/A$)[24,25,38] were measured at 7 °C and at 260 nm. The voltage and the duration of a single square-wave electric pulse field were varied between 0.455 and 3.91 kV and between 50 and 200 μs. The linear response between the photocurrent and the incident light intensity was checked with a metal mesh of a known absorbance (=1.20),[24] the deviation being always kept less than 1%. For both EB and ED, optical path lengths of the "Kerr" cell were 2 cm (electrode gap 0.33 cm) and 1 cm (electrode gap 0.207 cm).[38] Two electric pulses were usually applied to each fresh solution, but the sample solution was discarded after a single high-field pulse to avoid denaturation of sDNA.

## Method of Data Analysis

*Analysis of Steady-state.* For a monodisperse system, the steady-state birefringence ($\Delta n$) of a rodlike polymer of axial symmetry (the 3-axis) may be given as[34]

$$\Delta n = \frac{\lambda}{d}\cdot\frac{\delta}{2\pi} = \frac{2\pi C_v}{n}(g_3-g_1)\Phi, \tag{1}$$

where $\lambda$ is the wavelength of light *in vacuo*, $d$ is the path length, $\delta$ is the phase retardation of solution, $n$ is the refractive index of the solution, $C_v$ is the volume fraction of solute, $(g_3-g_1)$ is the optical anisotropy factor, and $\Phi$ is the orientation function. Correspondingly, the steady-state reduced dichroism ($\Delta A/A$) is given as[4,38,43]

$$\frac{\Delta A}{A} = \frac{A_{\parallel}-A_{\perp}}{A} = \frac{\Delta A_{\parallel}}{A} - \frac{\Delta A_{\perp}}{A} = \frac{3}{2}(3\cos^2\theta-1)\Phi, \tag{2}$$

where $\Delta A_{\parallel}/A$ and $\Delta A_{\perp}/A$ are the specific parallel and perpendicular dichroisms, respectively. $A$ is the isotropic absorbance of the solution in the absence of an electric field. $\theta$ is the angle between the transition moment of a chromophoric group and the orientation axis of sDNA.

For a polydisperse system, the molecular weight (or the length for rodlike molecules) distribution of a polymer sample must be considered. For this purpose, the Schulz-Zimm function was assumed;[44,45] then, the number-average length distribution density function $f_n(l)$ is given as[38]

$$f_n(l) = \frac{(ym_0)^k}{\Gamma(k)}\, l^{k-1}e^{-ym_0 l}, \tag{3}$$

where $y$ and $k$ are related to the number-average and the weight-average molecular weights ($M_n$ and $M_w$) as $M_n=k/y$ and $M_w=(k+1)/y$. The conformation of sDNA is a rigid double-stranded helix and is approximated by a cylindrical model. The molecular weight $M$ of an sDNA molecule may be equated to $m_0 l$, where $l$ is the contour length and $m_0$ is the molecular weight per unit length. The ratio $M_w/M_n$ may be equated to the ratio of weight-average length to number-average length, *i.e.*, $l_w/l_n$. According to the analytical method devised for the polydisperse system,[27] Eqs. 1 and 2 may be expressed in terms of the sum of the contributions from component species of heterogeneous lengths, for which the length distribution density function is defined by Eq. 3:[38]

$$\Delta n = \frac{2\pi(g_3-g_1)\bar{C}_v}{n}\cdot\frac{\int_{L_1}^{L_2} l f_n(l)\Phi\,dl}{\int_{L_1}^{L_2} l f_n(l)\,dl} \equiv \frac{2\pi(g_3-g_1)\bar{C}_v}{n}\langle\Phi\rangle_w, \tag{4}$$

and similarly,

$$\frac{\Delta A}{A} \equiv \frac{3}{2}(3\cos^2\theta-1)\langle\Phi\rangle_w, \tag{5}$$

where $\bar{C}_v$ is the total volume of all solute molecules with various chain lengths divided by the volume of solution and $\langle\Phi\rangle_w$ is the weight-average orientation function. Both $L_1$ and $L_2$ are the lower and upper limits of $l$.

The field strength dependence of the steady-state EB or ED for many DNA systems[5,13,14,16,18,19,21,25,27] has been described by the "classical" orientation function, $\Phi(\beta,\gamma)$, where $\beta=\mu E/kT$ and $\gamma=\Delta\alpha E^2/2kT$ ($\geq 0$).[34] Notations are the same as those in Refs. 34 and 38.

*Analysis of Decay Process.* The electric birefringence-average relaxation time $\langle\tau\rangle_{EB}$ and initial slope $\langle S\rangle_{EB}$ of the decay process of EB for the polydisperse system are given as[27,46,47]

$$\langle\tau\rangle_{EB} = \int_0^{\infty}\frac{\Delta n(t)}{\Delta n(0)}\,dt = \frac{\int_{L_1}^{L_2}\tau(l) l f_n(l)\Phi\,dl}{\int_{L_1}^{L_2} l f_n(l)\Phi\,dl}, \tag{6}$$

$$\langle S\rangle_{EB} = -\int_{L_1}^{L_2}\tau(l)^{-1} l f_n(l)\Phi\,dl \Big/ \int_{L_1}^{L_2} l f_n(l)\Phi\,dl, \tag{7}$$

where $\Delta n(t)$ is the birefringence at time $t$ after removal of an electric field, $\Delta n(0)$ is the birefringence of the steady-state. $\tau(l)$ is the relaxation time for a cylindrical molecule of length $l$ and may be calculated from the Broersma equation:[48]

$$\tau(l) = \frac{\pi\eta_0 l^3}{18kT}\left[\ln\frac{l}{b} - 1.57 + 7\left(\frac{1}{\ln(l/b)} - 0.28\right)^2\right]^{-1}, \tag{8}$$

where $\eta_0$ is the viscosity of the solvent and $b$ is the radius of the cylinder.

At infinitely high field strength, $\Phi$ approaches unity.[34,43] Then, Eqs. 6 and 7 become the weight-average relaxation time,

$$\langle\tau\rangle_w = \int_{L_1}^{L_2}\tau(l) l f_n(l)\,dl \Big/ \int_{L_1}^{L_2} l f_n(l)\,dl, \tag{9}$$

and the weight-average initial slope,

$$\langle S\rangle_w = -\left\langle\frac{1}{\tau}\right\rangle_w = -\int_{L_1}^{L_2}\tau(l)^{-1} l f_n(l)\,dl \Big/ \int_{L_1}^{L_2} l f_n(l)\,dl, \tag{10}$$

respectively. These quantities are independent of the electric parameters, $\beta$ and $\gamma$, since the orientation function is not involved. Once $\langle\tau\rangle_w$ and $\langle S\rangle_w$ are obtained from the experimental data, the ratio $l_w/l_n$ and the length $l_w$ can be evaluated;[27] with these values, the most likely Schulz-Zimm function may be determined.

## Results and Discussion

*Electric Field Strength Dependence of the Steady-state Birefringence and Linear Dichroism.* Figure 1 shows the field strength dependence of the birefringence ($\Delta n/C$) of sDNA in solutions with various additives. Values of $\Delta n/C$ tend to saturate at high field strengths. The sign of $\Delta n$ is negative in all cases. No positive or peculiar signal of $\Delta n$ could be observed at low fields, contrary to the report by Colson *et al.*[6] At a given field strength, *e.g.*, 10 kV/cm, values of $-\Delta n/C$ of sDNA decrease with solvent compositions in this order: 0.2 mM NaCl>AA($P/D$=10)–1.0 mM NaCl>1.0 mM NaCl> 0.33 mM $MgCl_2$>20 vol% glycerol–0.8 mM NaCl. This result does not necessarily indicate a difference in the degree of orientation of sDNA in solutions with various additives. The birefringence at an electric field strength and at a wavelength is given by the product of the orientation factor and the optical factor (*cf.* Eq. 1). Hence, the different values of $\Delta n$ arise from the differences in these factors. In order to obtain values of $(g_3-g_1)$, $\mu$, and $\Delta\alpha$ of sDNA, the experimental points will be compared with the theoretical orientation functions (see Discussion, together with Fig. 5).

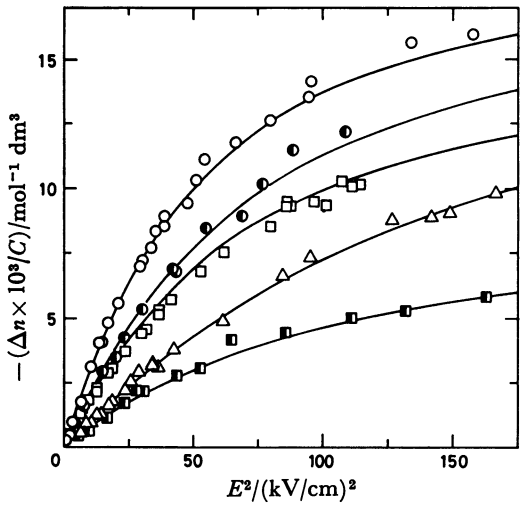


Fig. 1. The field strength dependence of the birefringence ($\Delta n/C$) of sDNA at 535 nm and at 7 °C in solutions with various additives. The residue concentration of sDNA ($C$) and the concentration of additives are: $C$=0.165 mM and [NaCl]=0.2 mM (○); $C$= 0.10 mM, [AA]=0.01 mM, and [NaCl]=1.0 mM (◐); $C$=0.171 mM and [NaCl]=1.0 mM (□); $C$= 0.22 mM and [$MgCl_2$]=0.33 mM (△); $C$=0.10 mM, [NaCl]=0.8 mM, and 20 vol% glycerol (◧). Symbols indicate experimentally determined points, while solid curves are the theoretical ones calculated by using the orientation function $\langle\Phi(\beta,\gamma)\rangle_w$ for the polydisperse system.

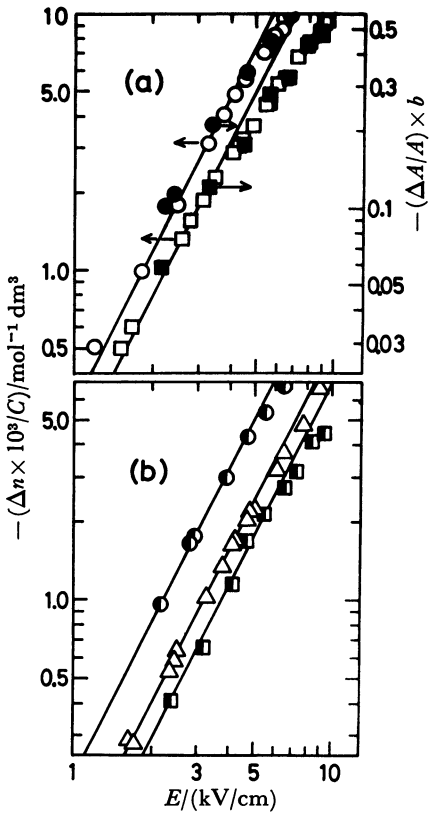


Fig. 2. The double-logarithmic plots of the field-strength dependence of both $\Delta n/C$ and $\Delta A/A$ of sDNA in the low field strength region. The slope of the straight lines is 2. The right ordinate refers to values of $\Delta A/A$ of sDNA at 260 nm and at 7 °C in 0.2 mM NaCl (●), for which $b$=1.1, and in 1.0 mM NaCl (■) for which $b$=1.0. Other symbols and conditions are the same as in Fig. 1.

Figure 2 shows the double logarithmic plots of $\Delta n/C$ against $E$. In the low field strength region, the experimental points follow a straight line with a slope of 2; thus, the Kerr law holds for sDNA in various solvents ($E$<5 kV/cm). The field strength dependence of reduced dichroism ($\Delta A/A$) of sDNA in 0.2 and 1.0 mM NaCl solutions is also shown in Fig. 2(a) (closed symbols). The sign of $\Delta A/A$ is negative for both cases. In the same low field strength region ($E$<5 kV/cm), the Kerr law holds for sDNA. This quadratic field strength dependence of EB and ED has been predicted by the electro-optical theories for molecules with cylindrical symmetry.[34,43,49] Hence, the dependence of EB and ED on the first power of electric field in the low field region, as was observed by Sokerov and Weill for the aqueous sDNA solution,[12] must be considered rather anomalous. From our result in Fig. 2, it is now verified that the Kerr law holds for the low molecular weight, rodlike sDNA in diverse cases. The fact that the straight lines of $\Delta n/C$ and $\Delta A/A$ in Fig. 2(a) are superimposed upon each other is reasonable, considering that the orientation function $\Phi$ in Eqs. 1 and 2 must be the same for a given sDNA solution provided that the backbone conformation of the sDNA molecule is rigid.

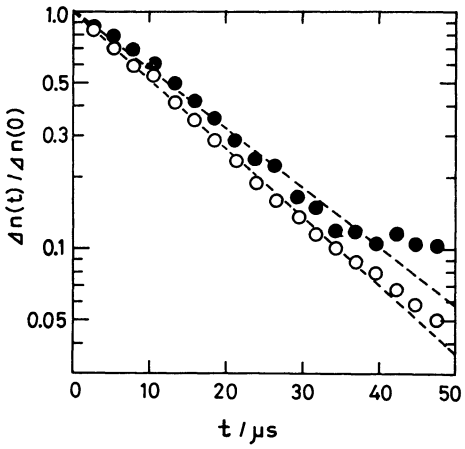


Fig. 3. The semi-logarithmic plots of the field-free decay curves of EB for sDNA in a 20 vol% glycerol–0.8 mM NaCl solution at a low field strength ($E$=6.45 kV/cm) (●) and at a high field strength ($E$=10.5 kV/cm) (○). Dashed lines (------) are straight lines.

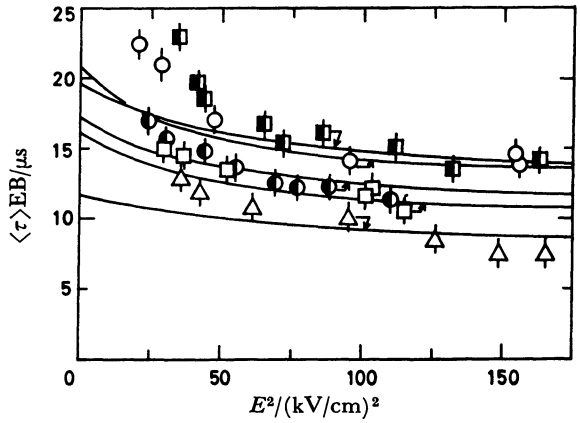


Fig. 4. The dependence of birefringence-average relaxation times, $\langle\tau\rangle_{EB}$, of sDNA solutions obtained from the field-free decay curves on the applied electric field strength. Symbols and conditions are the same as in Fig. 1. Solid lines are theoretical curves calculated by the use of Eq. 6 with the parameters ($l_w/l_n$, $l_w$, $\beta_w^2/2\gamma_w$). These values for each curve are: $l_w/l_n$=1.16, $l_w$=742 Å, and $\beta_w^2/2\gamma_w$=2 (—○—); $l_w/l_n$=1.16, $l_w$=674 Å, and $\beta_w^2/2\gamma_w$=2 (—□—); $l_w/l_n$=1.16, $l_w$=533 Å, and $\beta_w^2/2\gamma_w$=4 (—■—); $l_w/l_n$=1.16, $l_w$=682 Å, and $\beta_w^2/2\gamma_w$=4 (—◐—); $l_w/l_n$=1.16, $l_w$=596 Å, and $\beta_w^2/2\gamma_w$=2 (—△—).

*Electric Field Strength Dependence of the Relaxation Time of sDNA.* As a precautionary example, two normalized decay curves of birefringence, $(\Delta n(t)/\Delta n(0))$, are shown in Fig. 3 for sDNA in a 20 vol% glycerol–0.8 mM NaCl solution. The field-free decay curve (open circles) of EB after removal of a high electric field yields a nearly straight line (dashed line) on a semilogarithmic plot, whereas the curve displayed by a solution subjected to a low electric field (filled circles) deviates slightly from the straight line, being larger than the former. This example clearly indicates that a single decay curve of EB at a relatively high electric field may often result in an ambiguity over whether or not a polymer system is monodisperse. In order to determine whether the polymer system is polydisperse, the electric field strength dependence of relaxation times must be examined. The field-free relaxation time of sDNA from a low electric field should be larger than that of sDNA from a high electric field, if the sample is polydisperse.

Figure 4 shows the electric field dependence of birefringence-average relaxation time $\langle\tau\rangle_{EB}$. The persistence length of DNA is generally $1.5\times10^3$ Å in 5 mM NaCl[50] or $1.3\times10^3$ Å in 30 mM NaCl.[51] These values are much larger than the contour length of an sDNA molecule of $1.24\times10^5$ daltons. Therefore, the present sDNA sample is probably a rigid rod with little flexibility at low ionic strengths. The results in Fig. 4 may be best explained by the effect of the length polydispersity of sDNA. An analytical method for treating the decay process of the polydisperse rigid rodlike system was recently developed.[27] According to this method, the values of $l_w/l_n$ and $l_w$ for a given sDNA sample can be determined from the weight-average relaxation time $\langle\tau\rangle_w$ and the weight-average initial slope $\langle S\rangle_w$ at infinitely high field, provided that an appropriate length distribution is assumed. The best values of $l_w/l_n$ and $l_w$ can be determined by comparing experimental values of $\langle\tau\rangle_w$ and $\langle S\rangle_w$ with the theoretical values of $\langle\tau\rangle_w$ and $\langle S\rangle_w$ which were calculated from Eqs. 9 and 10. In this work, the Schulz-Zimm function (Eq. 3) was used for the length distribution density function, together with two values of 10 Å[52] and 13 Å[16,21] for the radius $b$ of the cylinder assumed for sDNA. Two sets of parameters ($l_w/l_n$, $l_w$) thus determined are given in Table 1. The theoretical relaxation times calculated from Eq. 6 with the experi-

Table 1. The weight-average relaxation time, $\langle\tau\rangle_w$, the weight-average length, $l_w$, and the weight-average length per base pair for sDNA in solutions containing different counterions

| sDNA in | $I\times10^3$ [a] | $\langle\tau\rangle_w$ [b] | $b$[c]=10 Å ($l_w/l_n$=1.16)[d] | | $b$[c]=13 Å ($l_w/l_n$=1.17)[d] | |
|---|---|---|---|---|---|---|
| | | μs | $l_w$[d]/Å | ($l_w$/bp)[e]/Å | $l_w$[d]/Å | ($l_w$/bp)[e]/Å |
| 0.2 mM NaCl | 0.2 | 12.5 | 742 | 3.9 | 712 | 3.8 |
| 1.0 mM NaCl | 1.0 | 9.7 | 674 | 3.6 | 645 | 3.4 |
| 20 vol% glycerol, 0.8 mM NaCl | 0.8 | 11.4 | 533 | 2.8 | 509 | 2.7 |
| AA($P/D$=10), 1.0 mM NaCl | 1.0 | 10.0 | 682 | 3.6 | 635 | 3.4 |
| 0.33 mM $MgCl_2$ | 1.0 | 7.0 | 596 | 3.2 | 569 | 3.0 |

a) $I$ is the ionic strength. b) Values obtained from the relaxation time $\langle\tau\rangle_{EB}$ which is extrapolated to infinitely high field strength. c) $b$ is the radius of the cylinder for sDNA in Å (1 Å=0.1 nm). d) Values of $l_w$ were evaluated from Eqs. 9 and 10 with experimental values of $\langle\tau\rangle_w$ and $-\langle S\rangle_w^{-1}$(=4.68μs) by using a constant value of $l_w/l_n$. e) bp is the number of base pairs, which is 188 for sDNA.

mentally obtained parameters ($l_w/l_n$, $l_w$, and $\beta_w^2/2\gamma_w$) for each sDNA solution are also shown by solid curves in Fig. 4. The "classical" mixed orientation function $\langle\Phi(\beta, \gamma)\rangle_w$ was utilized for the calculation of the theoretical relaxation times (the reason is given later). Except for the low field strength region, the experimental points are in fairly good agreement with the theoretical curve in each case.

The weight-average length ($l_w$) of sDNA given in Table 1 decreases with the increase of the ionic strength or the valence of counterion. The value of $\langle\tau\rangle_{EB}$ or $\langle\tau_w\rangle$ for the sDNA in the 20 vol% glycerol–0.8 mM NaCl solution is almost equal to that for the sDNA in the 0.2 mM NaCl solution. However, the values of $l_w$ are very different. This is because the solvent viscosity of the former solution is about 2.2 times larger than that of the latter. The weight-average length per base pair ($l_w$/bp) is also given in Table 1; it decreases in the order: 0.2 mM NaCl>AA ($P/D$=10)–1.0 mM NaCl> 1.0 mM NaCl>0.33 mM $MgCl_2$>20 vol% glycerol–0.8 mM NaCl. The value of $l_w$/bp in 1.0 mM NaCl is 3.4—3.6 Å, which is close to that for B-form DNA, whereas it is 2.7—2.8 Å in 20 vol% glycerol–0.8 mM NaCl, which is close to that of A-form DNA. The latter value appears to support the proposals by Nelson and Johnson[53] and Ivanov *et al.*[54,55] that the A-form DNA exists in water–alcohol solutions.

The decay curve of ED was not analyzed in the present work, because the time constant for the signal detector of our ED apparatus was *ca.* 2.7 μs (with a load resister of 12 kΩ) for good signal-to-noise ratios. The good transient signals of EB could be measured with the instrumental decay time less than 0.5 μs (at a load resister of 2 kΩ) for the same sDNA solutions under the identical experimental conditions; therefore, the analysis of decay curves was possible in this case. Since the field strength dependence of the steady-state EB and ED is superimposable upon each other (*vide post*), the mean relaxation times of EB and ED may be the same for a given sDNA solution.

*Electro-optical Properties of sDNA.* Figure 5 shows the field strength dependence of the steady-state EB and ED signals of sDNA in 0.2 mM (circles) and 1.0 mM (squares) NaCl solutions. In order to obtain both the electric dipole moments responsible for field orientation and the intrinsic birefringence and dichroism ($\Delta n_s$ and $(\Delta A/A)_s$), the experimental data may be compared with the theoretical orientation function. The orientation function $\Phi(\kappa)$ proposed by Kikuchi and Yoshioka,[49]

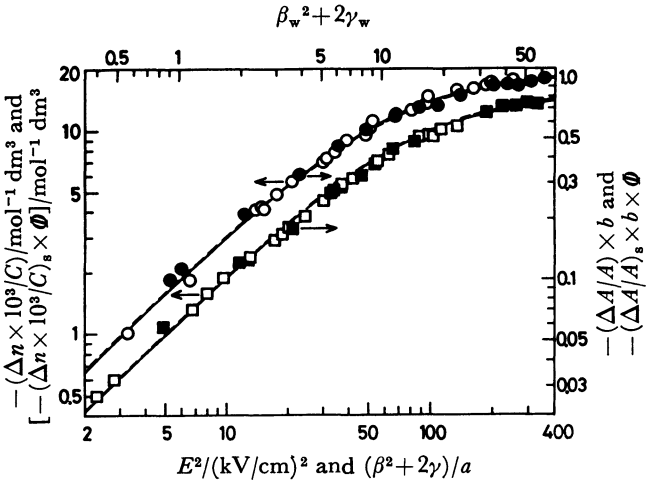


Fig. 5. Comparison of experimental values of birefringence and reduced dichroism for sDNA in 0.2 mM NaCl (○ for EB and ● for ED) and 1.0 mM NaCl (□ for EB and ■ for ED) solutions with the theoretical orientation functions, $\Phi$, for the monodisperse and polydisperse systems. Two sets of the double-logarithmic plots are shown: $\Delta n/C$ against $E^2$ (○, □); $(\Delta n/C)_s$ $\Phi(\beta, \gamma)$ against $(\beta^2+2\gamma)/a$ (——); $(\Delta n/C)_s$ $\langle\Phi(\beta, \gamma)\rangle_w$ against $(\beta_w^2+2\gamma_w)$ (----); $\Delta A/A$ against $E^2$ (●, ■); $(\Delta A/A)_s$ $\Phi(\beta, \gamma)$ against $(\beta^2+2\gamma)/a$ (——); $(\Delta A/A)_s$ $\langle\Phi(\beta, \gamma)\rangle_w$ against $\beta_w^2+2\gamma_w$ (----). The intrinsic values of $(\Delta n/C)_s$ and $(\Delta A/A)_s$ are given in Table 2. The factor $a$ is 0.192, while the factor $b$ is 1.0 for (■) and 1.1 for (●). Symbols and experimental conditions are the same as in Fig. 2 (a).

in which ion-atmosphere polarization is considered, seems to be the most reasonable one for the polyelectrolyte with axial symmetry. The applicability of $\Phi(\kappa)$ has been already tested for sodium poly(*p*-styrenesulfonate) (NaPSS) and NaPSS–AA systems.[38] It was, however, found that the fitting of $\Phi(\kappa)$ to the experimental data is good only in the low field strength range. In view of this fact, the function $\Phi(\beta, \gamma)$ has been employed for many DNA systems.[5,13,14,16,18,19,21,25,27] It will be also used in this work, although it was originally derived for nonionized polymers.

The experimental points in Fig. 5 (open symbols for EB and closed symbols for ED) fit not only the theoretical curves for monodisperse system, $\Phi(\beta, \gamma)$.[34] but also those for polydisperse system, $\langle\Phi(\beta, \gamma)\rangle_w$, fairly well over a wide range of field strength, provided that values of the interaction terms, $\beta$ and $\gamma$, or, $\beta_w$ and $\gamma_w$, are

Table 2. Electro-optical properties of sDNA in solutions of different counterions

| sDNA in | $I\times10^3$ | $\mu_w\times10^{-3}$ [a] / D [b] | $\mu_w$/bp / D | $\langle\Delta\alpha\rangle_w\times10^{16}$ [a] / $\mathrm{cm^3}$ [b] | $-(\Delta n/C)_s$ [c] / $\mathrm{mol^{-1}\,dm^3}$ | $-(g_3-g_1)$ | $-(\Delta A/A)_s$ |
|---|---|---|---|---|---|---|---|
| 0.2 mM NaCl | 0.2 | 4.5 | 24 | 2.6 | 0.023 | 0.027 | 1.0(70)[d] |
| 1.0 mM NaCl | 1.0 | 4.0 | 21 | 2.0 | 0.016 | 0.018 | 0.88(68) |
| 20 vol% glycerol, 0.8 mM NaCl | 0.8 | 3.3 | 18 | 0.7 | 0.009 | 0.011 | |
| AA($P/D$=10), 1.0 mM NaCl | 1.0 | 3.8 | 20 | 0.9 | 0.019 | 0.022 | |
| 0.33 mM $MgCl_2$ | 1.0 | 2.7 | 14 | 0.9 | 0.018 | 0.021 | |

a) The number-average distribution density function $f_n(l)$ with the parameters ($l_w/l_n$=1.16, $l_w$ in Table 1) was used. b) 1 D=3.336×10$^{-30}$ C m, and 1 cm$^3$=1.113×10$^{-16}$ F m$^2$. c) $C$ stands for the residue concentration of sDNA. d) Values in parentheses are the angles $\theta$ in degrees.

adjusted. This is because the curve of $\Phi(\beta, \gamma)$ for the monodisperse system differs only slightly from that of $\langle\Phi(\beta, \gamma)\rangle_w$, if $l_w/l_n<1.3$. Hence, it is difficult to determine uniquely the exact electric property of sDNA in solutions from the field strength dependence of $\Delta n$ (or $\Delta A/A$). Nevertheless, $\langle\Phi(\beta, \gamma)\rangle_w$ should be employed because, as noted in the preceding section, it was verified that the sDNA sample is a polydisperse system. The best values of $\beta_w$ ($=\mu_o l_w E/kT$) and $\gamma_w$ ($=\Delta\alpha_o l_w E^2/2kT$), where $\mu_w=\mu_o l_w$ and $\Delta\alpha_w=\Delta\alpha_o l_w$, were read by the curve fitting method[25,34,38,42] and are given in Table 2. It has been shown by reversing-pulse electric birefringence that sDNA has no appreciable permanent dipole moment, but has only a pseudo-permanent dipole moment.[15] It is clear that this moment depends strongly on the ionic strength and the species of counterions and decreases in the order (*cf.* Table 2): 0.2 mM NaCl>1.0 mM NaCl>AA ($P/D=10$)–1.0 mM NaCl>20 vol% glycerol–0.8 mM NaCl>0.33 mM $MgCl_2$. In conclusion, the electric dipole moment of sDNA may be accounted for by the counterion-induced dipole moment which originates from the interaction between the counterions of sDNA and the applied external field.

From values of $\Delta n_s$ at infinitely high field strength, together with the partial specific volume of 0.55 $cm^3/g$ in 1 mM NaCl (Emonds-Alt *et al.*),[13] the optical anisotropy factor ($g_3-g_1$) was estimated to be $-(2.7-1.1)\times10^{-2}$ for sDNA at 535 nm. This result is in good agreement with the values of $-(2.0-0.5)\times10^{-2}$ at 550 nm (Houssier *et al.*)[5,13] and $-(2.8-2.3)\times10^{-2}$ at 633 nm (Stellwagen).[16] These values are much larger than $-(7.1-6.4)\times10^{-4}$ for high molecular weight DNA at 633 nm (Marion *et al.*).[14]

The apparent angle $\theta$ between the plane of base pair and the orientation axis of sDNA helix was estimated from Eq. 5 and is given in Table 2. It is in good agreement with the $\theta$ value of $73°\pm3°$ at 265 nm obtained for digested fragment DNA by Hogan *et al.*[22] It is very close to the value for A-form DNA or the revised B-form DNA,[56] but differs from that for the original B-form DNA. The apparent tilt angle remains unchanged by a 5-fold increase in NaCl concentration, while the weight-average length per base pair, $l_w$/bp, decreases about 10% (*cf.* Table 1). In 1.0 mM NaCl, however, the value of $l_w$/bp is 3.6 Å, which is close to that of B-form DNA ($l_w$/bp=3.4 Å). The value of $(\Delta A/A)_s$ should represent the true tilt angle of *ca.* 70° between the plane of the bases and the orientation axis of the sDNA helix, only if no out-of-plane transition (such as the n-$\pi^*$ transition) is involved in the 260 nm band of the bases. In order to determine the exact conformation of rigid rodlike DNA in solutions with various additives, we should study the wavelength dependence of reduced dichroism spectra of DNA over the entire UV region.

## Conclusion

The field strength dependence of both the steady-state EB and the field-free relaxation time of sDNA in solutions with various additives can be described over a wide range of field strength by the "classical" mixed-dipole orientation function $\langle\Phi(\beta, \gamma)\rangle_w$ for the polydisperse system. Similarly, the dependence of steady-state ED of sDNA is fitted by this orientation function with the same electric and distribution parameters ($\beta_w$, $\gamma_w$, $l_w/l_n$, and $l_w$). The weight-average length and the electric dipole moment for sDNA are strongly influenced by the ionic strength, the species of counterions, and additives, while the optical properties such as the apparent tilt angle (*ca.* 70°) and the optical anisotropy factor (*ca.* −0.02) are constant. The value of $l_w$/bp for sDNA in 20 vol% glycerol is close to 2.5 Å for A-form DNA, but that of $l_w$/bp for sDNA in 1 mM NaCl is close to 3.4 Å for B-form DNA.

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