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Dependence of Electric Polarizability of Rodlike DNA in Aqueous Solutions on Ionic Strength as Studied by Electric Dichroism and Birefringence. A Comparison between Experimental and Theoretical Results

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Synopsis. Orientation of a sonicated DNA fragment in aqueous solutions containing sodium chloride (0.2—4 mmol/dm^3) by an external electric field was discussed in terms of counterion-induced electric moments by using the electric dichroism and birefringence data experimentally determined at limiting low field strengths.

Ample evidence indicates that highly charged DNA in aqueous solutions can be oriented by an external electric field.[1—3] In previous studies of electric dichroism (ED) and electric birefringence (EB), we have shown that the electric field orientation of sonicated DNA fragments (sDNA) obeys the Kerr law in the low field strength region under diverse conditions.[4,5] This result is in good agreement with recent reports by other workers.[6—10] The orientation of sDNA has been attributed to the interaction between the ionized site and counterion and the applied field. Detailed analyses have shown, however, that the polyion behaves as if it possesses both a permanent dipole moment and a covalent polarizability anisotropy.[4—7] Thus, this electric dipole has been termed either the pseudo-[4,5,9] or quasi-permanent dipole[11] or the saturated induced dipole.[12] The electric moment was recently shown to result from the fast polarization of the "bound" counterion.[4,7] For the understanding of the polyelectrolyte property of DNA, its field-orientation mechanism should be fully clarified. Since the counterion-induced dipole is affected by the added simple ions, we examined the dependence of the steady-state ED and EB at limiting low electric field on the ionic strength (thus, saturation of the counterion-induced dipole moment could be avoided). We also present a critical comparison of observed results with recent theoretical developments.[13—15]

Experimental

Materials and Measurements. The sDNA sample is the same as before.[5] In order to adjust the counterion (Na$^+$) concentration, the sDNA solution was dialyzed at 4 °C for at least 48 hours against distilled water containing sodium chloride at the desired level. Both ED and EB were measured at 7 °C.[5] The observed data were expressed with the parallel (specific) dichroism, $\Delta A_{\parallel}/A$ at 260 nm, and the specific birefringence, $\Delta n/C$ at 535 nm.[5] At limiting low field strengths, E, the following expression generally holds for charged polyions:

$$\frac{(\Delta A_{\parallel}/AE^2)_{E^2\to 0}}{(\Delta A_{\parallel}/A)_s}=\frac{(\Delta n/CE^2)_{E^2\to 0}}{(\Delta n/C)_s}=\frac{1}{15}\left[\left(\frac{\mu_3}{kT}\right)^2+\frac{\Delta\alpha^t}{kT}\right], \tag{1}$$

where $(\Delta A_{\parallel}/A)_s$ and $(\Delta n/C)_s$ are the corresponding quantities at limiting high fields, μ_3 is the permanent dipole moment along the symmetry axis, and $\Delta\alpha^t$ is the polarizability anisotropy, which is generally the sum of the covalent (*i.e.*, electronic and atomic) and ionic polarizabilities ($\Delta\alpha^t=\Delta\alpha^c+\Delta\alpha^i$).

Results and Discussion

Dependence of ED and EB on Added Sodium Ions. In the added-salt concentration range of 0.2—4 mM (1 M=1 mol/dm^3), values of $\Delta A_{\parallel}/A$ and $\Delta n/C$ for the rodlike sDNA sample are proportional to the second power of electric field in the limiting low field region. The results are given in Table 1. The linear portions in the plots of these values against E^2 become narrower and the magnitudes are larger at lower concentrations of added salt (*cf.* Figs. 1 and 2 of Ref. 5). Values of $(\Delta A_{\parallel}/A)_s$ and $(\Delta n/C)_s$ are nearly independent of the added-salt concentration,[5] *i.e.*, the optical part in Eq. 1 is constant. Therefore, the electric parameter should be responsible for the variation of the observed limiting-low-field quantities with salt concentration. Since the double-helical DNA possesses no permanent dipole moment under ordinary conditions ($\mu_3=0$ in Eq. 1), the variation of $(\Delta A_{\parallel}/AE^2)_{E^2\to 0}$ and $(\Delta n/CE^2)_{E^2\to 0}$ should result from the change in the electric polarizability $\Delta\alpha^t$, in particular the ionic polarizability $\Delta\alpha^i$. Values of $\Delta\alpha^t$ decrease with the increase in added-salt concentration, and are given in Table 1. Their magnitude is of the order of 10^{-16} cm^3, in good agreement with reported values.[5—7,9,12]

In Fig. 1, values of $(\Delta A_{\parallel}/AE^2)_{E^2\to 0}$ and $(\Delta n/CE^2)_{E^2\to 0}$ are plotted against the total concentration of sodium ions, $[\mathrm{Na}^+]_t$, *i.e.*, the sum of those ions from the added sodium chloride and from the counterions of DNA-phosphates. In Fig. 1, values of $(\Delta A_{\parallel}/AE^2)_{E^2\to 0}$ at

TABLE 1. VALUES OF $(\Delta A_{\parallel}/AE^2)_{E^2\to 0}$ AT 260 nm AND $(\Delta n/CE^2)_{E^2\to 0}$ AT 535 nm OF A SONICATED DNA SAMPLE AT VARIOUS CONCENTRATIONS OF ADDED SODIUM CHLORIDE [NaCl] AT 7 °C[a)]

[NaCl] mM	$\left(\frac{\Delta A_{\parallel}}{AE^2}\right)_{E^2\to 0}\times 10^2$ (kV/cm)$^{-2}$	$\left(\frac{\Delta n}{CE^2}\right)_{E^2\to 0}\times 10^3$ M^{-1}(kV/cm)$^{-2}$	$\Delta\alpha^t\times 10^{16}$ cm^3 [b)]
0.2	−1.33	−0.36	10.4(9.37)[c)]
1.0	−0.75	−0.22	6.68(6.99)[c)]
2.0	−0.39	—	3.43[d)]
4.0	−0.23	—	2.02[d)]

a) The concentrations of DNA, C, in nucleotide units are 0.17—0.18 mM. b) 1 cm^3 = 1.113×10^{-16} F m^2. c) Values in the parentheses are obtained with the limiting low-field strength birefringence. d) Value of $(\Delta A_{\parallel}/A)_s$ is −0.593.

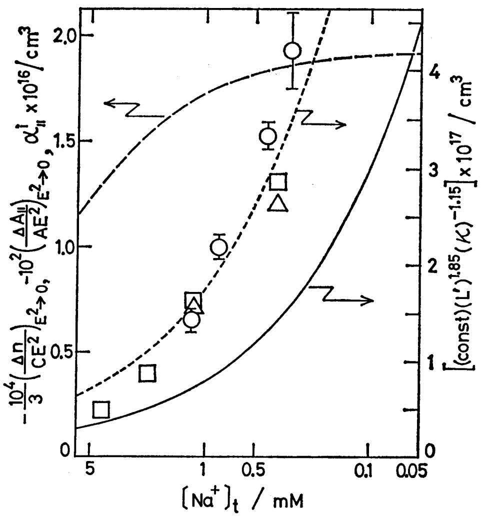

Fig. 1. Dependence of the measured limiting low-field dichroism, birefringence, and the theoretical electric polarizabilities of rodlike sDNA solutions on the total concentration of sodium ions, $[\text{Na}^+]_t$. Experimental values: $(\Delta A_\parallel/AE^2)_{E^2\to 0}$ (□) in this work and (○) cited in Ref. 9, and $(\Delta n/CE^2)_{E^2\to 0}$ (△) in $(\text{kV/cm})^{-2}$. Theoretical curves: Fixman's polarizability (— — —) was calculated from Eq. 2 with the parameters: $q_1 = -q_2 = 1$ and $\gamma^2 = 28.52 \times 10^{12}\ C_1$, where C_1 (mol/dm^3) is the bulk concentration of Na$^+$. Rau and Charney's polarizabilities (———), for which the (const) is unity, and (- - - -), for which the (const) is 2.2, were calculated from Eq. 3. Dielectric constant is 80 and temperature is 280 K. The $[\text{Na}^+]_t$ includes the concentration of DNA in nucleotide units.

270 nm for a nearly identical sDNA sample are also plotted. They were calculated from published values of $(\Delta A_\parallel/A)$ (*cf.* Fig. 1 of Ref. 9) but they agree well with the present data, which were obtained with an entirely different apparatus.

Comparison between Experimental and Theoretical Results. Recently, Manning derived an expression for the parallel component ionic polarizability $\alpha^1_\parallel$ on the basis of the ion condensation model.[16] The slight dependence of this theoretical $\alpha^1_\parallel$ on ionic strength is, however, in opposition to experimental results.[7,9,13] More recently, Fixman proposed an expression for electric polarizability (with his own notations):[14]

$$\alpha^1_\parallel \equiv \frac{\mu}{E_0} = \frac{2q_1KDL}{(q_1-q_2)\gamma^2}\left[1-\frac{\tanh(\gamma L)}{\gamma L}\right], \tag{2}$$

where $K = [2\ln(4L/a) - (14/3)]^{-1}$. Approximating the B-DNA form for the present sDNA sample regardless of salt concentrations ($a = 13$ Å, $L = 319.6$ Å),[5] we have calculated the polarizability in Eq. 2 as a function of concentrations of added sodium chloride. The result is shown by a dashed curve in Fig. 1. This curve *decreases* monotonously but in a *convex* fashion with increasing salt concentrations in the range $<$ 6 mM.

Another theoretical advance has been made lately by Rau and Charney.[15] By calculating the electric moment for an electric field-induced polarization of a Debye-Hückel ion atmosphere, they show that the polarizability along a charged rod, $\alpha^1_\parallel$, is approximately proportional to the half length of the rod, $L'(=319.6$ Å), and to the Debye-Hückel shielding parameter κ (with their own symbolism):[15]

$$\alpha^1_\parallel = (\text{const})(L')^{1.85}(\kappa)^{-1.15}. \tag{3}$$

As a function of the ionic strength of NaCl, we have calculated the quantity $(L')^{1.85}(\kappa)^{-1.15}$ and plotted it in Fig. 1 (solid curve). This curve, too, *decreases* monotonously and in a *concave* fashion with the increase in the total sodium concentration. The curve is nearly parallel to experimental values of the limiting low-field dichroism and birefringence. To visualize this trend quantitatively, the solid curve was shifted upward by multiplying a factor of 2.2 (shown by the dotted curve); the agreement with the experimental points is good.

In summary, the effect of ionic strength on observed limiting low-field dichroism or birefringence now appears to be explicable reasonably well by the Debye-Hückel ion atmosphere polarization concept. Another test for the Fixman *vs.* the Rau and Charney model may reside on measurements of ED, EB, or dielectric constants as a function of temperature, since the polarizability in Eq. 2 is temperature-independent but that in Eq. 3 is inversely proportional to temperature. A remaining question on the counterion-induced dipole moment is to assess the contribution from the condensed or "bound" counterions.[8,13] This contribution would be most prominent, if it exists, in the DNA solution in which no excessive salt ions are present. Values of, *e.g.*, $(\Delta A_\parallel/AE^2)_{E^2\to 0}$ may show an asymptotic trend in salt-free solutions. Measurements of ED or EB of rodlike sDNA under such conditions are highly desirable.

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