Transient electric dichroism of rod-like DNA molecules

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ABSTRACT We report transient electric dichroism studies on monodisperse rod-like DNA molecules. By using restriction fragments and DNAs of known length, it is shown that the orientation time is accurately predicted by the theoretical calculation of rotational diffusion coefficient. The field dependence of the steady-state dichroism values is not consistent with the induced electric dipole orientation mechanism, and the time dependence is not consistent with the presence of a permanent dipole moment. In order to explain the dependence of the dichroism on the electric field, the ionic strength of the medium, and the length of the macromolecule, we propose a new model in which anisotropic ion flow produces an asymmetric ion atmosphere around the polyelectrolyte, resulting in an orienting torque. From the limiting dichroism at high field, we estimate that the DNA bases are inclined at an angle of 73° or less relative to the helix axis, in good agreement with the revised model of B-form DNA suggested by Levitt, in which the base pairs have a propeller-like twist. Our results establish transient electric dichroism measurements as a technique well suited for study of alterations in the length and base pair inclination of rod-like DNA molecules.

Electric dichroism, a form of linear dichroism induced in a solution of optically anisotropic macromolecules that have permanent or induced electrical anisotropy, is potentially of great value for study of structural alterations in DNA (1-5). Theory indicates that the orientation time should depend on the third power of the length of rod-like double helical fragments, whereas the dichroism amplitude, extrapolated to perfect molecular orientation, depends on the angle between the electronic transition moment and the molecular orientation axis. These features make electric dichroism well suited to problems of changes in DNA length and base pair orientation when drug or protein molecules are bound. However, for several reasons, this potential has not been fully realized. Monodisperse DNA fragments are required, and the restriction enzymes necessary for their production have only recently become widely available. Furthermore, electric dichroism equipment capable of resolving orientation times of a few microseconds and operating at salt conditions of at least 1 mM to avoid irreversible degradation (6) is not generally available. Perhaps most important, the physical origin and general properties of the orienting force have not been clear, because the field-dependence of the dichroism implies a constant or permanent dipole moment (3), whereas the 2-fold symmetry of the DNA phosphodiester chains in a double helix rules out a permanent molecular dipole moment directed along the helix axis.

The purpose of this paper is to show that electric dichroism can be used to obtain accurate values for the length of DNA molecules in the size range 100-250 base pairs, to characterize the variables that control the dichroism, and to suggest a new model for the origin of the torque in the electric field-induced orientation of polyelectrolytes. As have others (6, 7), we have adapted our temperature-jump instrument to perform the electric dichroism measurements.

MATERIALS AND METHODS

Materials. Calf thymus DNA (Sigma V) was dissolved in 6 mM Na2HPO4/2 mM NaH2PO4/1 mM EDTA/1 mM Na cacodylate, sonicated for 30 min at ice bath temperature with a Branson sonifier, deproteinized by repeated phenol extractions, dialyzed against a large volume of 8 mM Na2HPO4/2 mM NaH2PO4/180 mM NaCl/1 mM EDTA. The purified DNA in the dialysis buffer was fractionated on a Sepharose 4B column. Staphylococcal nuclease digestion fragments of calf thymus chromatin, kindly provided by L. Klevan and H. Eshaghpour, were sized by electrophoresis on polyacrylamide gels, with dye standards (8) and sequenced fragments from φX174 as references. Hae III digestion fragments of ColE1 DNA (160 and 230 base pairs) were a generous gift from D. Bastia. These fragments were further purified on a DEAE-Sephadex column to remove UV-absorbing impurities. DNA solutions used for dichroism measurements were prepared by overnight dialysis against the experimental buffers listed, and the DNA concentration was determined by absorbance measurements on a Cary 14 spectrophotometer.

Dichroism. Electric dichroism was measured on a device originally designed for temperature-jump kinetic studies and modified by placing a prism polarizer (Claus-Thomson, kindly made available by S. Colson) in the incident beam just in front of the temperature-jump cell. Calibration by using a second polarizer in the output beam showed that only about 2% depolarization of the beam occurs on passage through the sapphire cell windows, which must be selected so that the beam is aligned parallel to the crystal axis. No rotation of the beam could be detected and, at the concentrations studied (<0.1 mM nucleotides), no field-induced change in transmission of polarized light was detected outside the absorbance bands, indicating no measurable optical artifacts due to birefringence effects. Moreover, the absence of any dichroism amplitude when the polarizer was set at an angle of 54° to the field direction indicates that only dichroism, not chemical changes, was measured (7). The temperature-jump cell had an optical path length of 0.7 cm and an electrode separation of 1.22 cm. The maximum voltage applied to the cell usually did not exceed 40 kV. The rise time of the field was rapid compared to the 100-nsec response time of the electronics. Because of electronic disturbances accompanying the initiation of the discharge, we did not attempt to resolve relaxation effects faster than 1 μsec.

Dichroism results are expressed in terms of the reduced dichroism, ρ = (A∥ - A⊥)/A, or its equivalent (9),

\[ ρ = \frac{3}{2} \left( \frac{A∥ - A⊥}{A} \right) \]

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in which \(A_p\) and \(A_\perp\) are the absorbances of light polarized parallel and perpendicular to the field, respectively, and \(A\) is the isotropic absorbance without the field. The magnitude of \(\rho\) depends on the angle \(\alpha\) between the chromosome transition moment and the molecular orientation, expressed by the orientation function \(\Phi\) defined by O’Konski et al. (10) (0 \(\leq\) \(\Phi\) \(\leq\) 1). Specifically (11),

\[
\rho = \frac{3}{2} (3 \cos^2 \alpha - 1) \Phi \tag{2}
\]

in which \(\Phi\) is a complicated function of the field, whose form depends on the angular dependence of the potential energy (10).

MODELS FOR THE ORIENTATIONAL FORCE

Permanent and Induced Dipole Moments. These are the two standard mechanisms for electric field-induced orientation of macromolecules, for which the orientation function \(\Phi\) has been calculated by O’Konski et al. (10). The potential energy \(U\) depends on the electric field \(E\), according to the equations

\[
U = -\mu E \cos \theta \quad \text{(permanent moment)} \tag{3a}
\]

\[
U = -\frac{\Delta \alpha}{2} E^2 \cos^2 \theta \quad \text{(induced moment)} \tag{3b}
\]

in which \(\mu\) is the molecular dipole moment, \(\Delta \alpha\) is the polarizability anisotropy of the molecule, and \(\theta\) is the angle between the field and the molecular orientation axis. The different dependence of these two expressions on the field allows them to be readily distinguished by experiment. The fundamental dilemma in electric dichroism studies of DNA is that the permanent moment expression fits the results at all tested values of the field (3), yet it does not make sense that DNA should have a permanent dipole moment; the induced moment expression does not fit the observed field dependence.

Saturation of an Induced Dipole Moment. Neumann and Katchalsky (6), developing the Mandel (12) model, suggested that saturation of the ionic polarizability, as a result of filling the ion binding sites at one end of the macro-ion, could be responsible for limiting the dipole moment to a maximum value. Consequently, a “permanent” moment would appear in the field dependence (13). However, our results indicate that saturation of the polarizability would have to occur at surprisingly small values of the field and charge displacement. In addition, the model for an induced dipole moment considers the macro-ion with its ion atmosphere to be isolated from other ions, thus neglecting the counter-ion flux which tends to restore positive charges to the more negative end of the macro-ion and remove them from the more positive end. For example, in 1 mM NaCl the Debye radius is about 100 Å, and the mean distance between counter-ions is roughly 120 Å. A field of 10^4 V/cm^-1 causes a Na^+, with mobility 5 \times 10^{-4} cm^2/sec-V^-1, to move a distance of 2500 Å during the average 5-μsec interval required for DNA orientation after application of the field. Therefore, many counter-ions cross the polyelectrolyte ion atmosphere during the orientation process, and one must consider the possible influence of their steady-state flow on DNA orientation. A principal feature of counter-ion flow is that the ions move more readily along the helix axis than perpendicular to it, because the latter motion requires escape from the local electrostatic energy minimum.

Orientation Due to Anisotropic Ion Flow. We propose, as a working hypothesis, that the primary cause of DNA orien-

![FIG. 1. Phenomenological description of an anisotropic ion flow model. An isotropic distribution of counter-ions is transported past a DNA rod. Flow of ions down the rod axis leads to a deficiency of counter-ions below the rod on the right and to an excess on the left. The force \(F_s\) arises from the induced asymmetry of the counter-ion distribution and produces a torque that tends to orient the molecule parallel to the field.](image)
RESULTS AND DISCUSSION

The Broersma Equation Fits the Observed Orientation Times. Rod-like molecules without a permanent dipole moment should orient at low fields, with an exponential time course given by (15)

$$\rho_t = \rho_0 (1 - e^{-t/\tau_e})$$

where the rotational correlation time is $\tau_e = (\mathcal{H}L)^{-1}$, and $\mathcal{H}$ is the rotational diffusion coefficient for recollection of the long axis of the rod. Eq. 7 assumes that the field is constant during orientation, a condition satisfied in our experiments by using salt concentrations low enough (<10 mM) so that the field decay time, the product of the high-voltage capacitance (0.05 $\mu$F) times the cell resistance, is long compared to $\tau_e$. We observed single exponential orientation curves for DNA samples that were not appreciably polydisperse; Fig. 2 shows a comparison of the measurements with the values predicted by Broersma’s equation which gives $\mathcal{H}$ for rods (16):

$$\mathcal{H} = \frac{3kT}{\pi \eta_0 L^3} \left( \ln \left( \frac{L}{b} \right) - 1.57 + 7 \left( \frac{1}{\ln(L/b)} - 0.28 \right)^2 \right).$$

Calculated results are insensitive to the rod radius $b$, which was taken to be 13 Å; $\eta_0$ is the solvent viscosity, and $kT$ is the Boltzmann constant times temperature. The DNA length was taken as 3.4 Å per base pair. As shown in Fig. 2, Perrin’s equation (17) for an ellipsoid of revolution does not fit the data.

These results are not consistent with a permanent dipole orientation mechanism, which would require a lag in the time course of orientation and a correlation time related to $\tau_e$ by $\tau_e = (\mathcal{H}L)^{-1}$ in the presence of the field (15). Ding et al. (3) reported a lag in the orientation kinetics, which we believe must arise from electronic delays in their instrument.

Polydispersity Causes $\tau_e$ to Depend on the Field Strength. We found that monodisperse fragments (as judged by narrow bands on gel electrophoresis) showed no detectable field dependence of $\tau_e$, whereas polydisperse samples exhibited longer orientation times at low values of the field. This result, expected from the $L^2$ dependence of the apparent dipole moment demonstrated below, is illustrated in Fig. 3. The high-field value of $\tau_e$ reflects approximately the weight average molecular weight, but the low field values yield a higher average.

$\tau_e$ Increases Linearly with the Weight Concentration of Neutral Polymer Added to Increase the Viscosity. Our dichroism device has a time resolution of only about 1 µsec, so measurement of $\tau_e$ for small objects (short pieces of DNA, protein–nucleic acid complexes such as nucleosomes, etc.) requires increase of the solvent viscosity, perhaps by as much as a factor of 10. We tested the influence of dextran (molecular weight, $5 \times 10^5$) at concentrations up to 8 g/100 ml and found that $\tau_e$ for a 140-base-pair DNA fragment varies linearly with dextran weight concentration, extrapolating to the value measured without dextran. However, the relationship of $\tau_e$ to bulk solution viscosity is not linear, presumably because $\tau_e$ depends on the microscopic viscosity in the region of the DNA molecule.

$\tau_e$ Increases Dramatically at 1 mM Na+. It has been observed that the field-free relaxation time of DNA in low-salt solutions increases considerably when the ionic strength is <1 mM, an effect interpreted (18) as arising from DNA stiffening due to phosphate repulsion. We found (Fig. 4) that, when the ionic strength of the medium is >2 mM for both mono- and divalent salts, $\tau_e$ is not dependent on salt concentration (nor on DNA concentration; data not shown). However, at 1 mM Na+, $\tau_e$ increases substantially; this effect is reversed by increasing the Na+ concentration. Divalent ions prevent the increase in $\tau_e$. A plausible possibility is that melting from the ends increases the hydrodynamic resistance. In any case, the results suggest caution in interpreting electric dichroism measurements when the Na+ concentration is below 2 mM.

The Permanent Dipole Model and the Anisotropic Ion
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Flow Model: Fit the Observed Field Dependence of the Dichroism Amplitude. Dichroism amplitudes provide useful information about molecular structure only if the influence of the orientation function $\Phi$ can be removed—for example, by extrapolation to perfect orientation at infinite field. Fig. 5 shows a test of our data against the three relevant models, showing that the permanent dipole and ion flow models fit within experimental error, whereas the induced moment model does not. As shown in the inset, a plot of dichroism versus reciprocal field is linear at high field and requires only a short extrapolation to infinite field (perfect orientation).

The Apparent Dipole Moment Varies with the Square of Molecular Length. This result is illustrated for the ion flow model in Fig. 6. The apparent dipole moment $A$ at constant ionic strength increases linearly with $L^2$ and extrapolates to zero when $L = 0$. This behavior is predicted by Eq. 8b. The theory for a pure induced dipole moment predicts that the dipole moment should vary with $L^3$ (19-21), which clearly is inconsistent with our data.

The data in Figs. 5 and 6 allow one to set limits on the field at which saturation of an induced dipole moment would have to occur to satisfy the observations. For example, for a 230-base-pair fragment, saturation would have to be essentially complete at only 1 kV/cm, yielding a limiting dipole moment of 8500 Debye, equivalent to a charge displacement from one end of the DNA to the other of 11 counter-ions, out of a total of 460 positive charges. We see no simple basis for expecting polarization saturation to occur at such low degrees of charge displacement. On the other hand, the ion flow model recognizes the reality of extensive ion movement through the solution and correctly predicts the field and length dependence of the orientation.

The Apparent Dipole Moment Is Linear in the Debye Radius. The Debye radius in an electrolyte solution depends on the reciprocal of the square root of the ionic strength. Fig. 6 shows that $A$ varies linearly with the Debye radius for several electrolytes, arguing strongly that the observed phenomenon involves the ion atmosphere, not the bound or condensed (22) ions, because $A$ (or $\mu$ for the permanent dipole model) does not depend on the nature of the ion. This result is qualitatively consistent with the ion flow model because, when the Debye radius is smaller, diffusion within it is more effective in countering the asymmetry established by the electrophoretic ion current. Furthermore, at high salt concentrations the ion atmosphere is better able to screen the effect of an asymmetric ion distribution at a given distance. Other plots that we found to be linear within experimental error include $A$ versus the surface or $\xi$ potential, evaluated (23) at a macro-ion radius of

FIG. 4. Variation of relaxation time with ionic strength, $\tau_0/\tau_1$, is the ratio of relaxation times at the salt concentration specified to $\tau_1$ at 2.5 mM Na+, $\bullet$ Na+, $\bigcirc$ Mg2+, $\bigcirc$ Ca2+, with calf thymus DNA; $\Delta$ Na+, with Escherichia coli DNA; $\triangle$ Na+, with Micrococcus luteus DNA; $\diamond$ Na+, with Clostridium perfringens DNA. The data show that the DNA sample is altered in Na+ solutions below 2 mM concentration.

FIG. 5. Dependence of reduced dichroism on field strength $E$: calculated variation of $\rho$ with field strength using the flow (---), permanent moment (•••••), and the induced moment (- - -) models. The adjustable parameters giving best fit to the flow model were $A = 13,370$ Debye and $\rho = 1.2$; for the permanent moment model, $\mu = 8860$ Debye and $\rho = 1.24$. The shapes of the field-dependence curves for the flow and permanent moment models differ only slightly, primarily in the very high field region, which is not experimentally accessible. $\bullet$, Experimentally measured points for a 250-base-pair nucleosome digestion fragment of calf thymus DNA; [Na+] = 2.5 mM; temperature 12°. Only the induced moment model cannot be accommodated to the observed field dependence. Inset, Extrapolation to infinite field by using a plot of $\rho$ versus $1/E$.

FIG. 6. Dependence of $A$ on ionic strength and length. Curve a, linear variation of $A$ with $L^2$. The samples, in order of decreasing lengths, are: nuclease digestion fragment, 250 base pairs; nuclease digestion fragment, 172 base pairs; nuclease digestion fragment, 140 base pairs; and calf thymus DNA fraction from Sepharose 4B. $I = 2.5$ mM Na+; temperature, 12°. Curve b, plot of $1/\sqrt{I}$, proportional to the Debye radius, versus $A$. $\bigcirc$, $\bigcirc$, $\bigcirc$, $\bigcirc$, Na+; Calf thymus DNA fraction from Sepharose 4B; 140 base pairs; temperature, 12°. $I$ is the ionic strength.
Table 1. Transition moment angles (at 265 nm) of DNA bases relative to helix axis

<table>
<thead>
<tr>
<th>DNA source</th>
<th>DNA base pairs</th>
<th>Counter-ion</th>
<th>Counter-ion concentration, mM</th>
<th>( \phi^* )</th>
<th>( \alpha ) degrees</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclease digest, calf thymus chromatin</td>
<td>230</td>
<td>Na(^+)</td>
<td>2.50</td>
<td>1.20</td>
<td>75.0</td>
</tr>
<tr>
<td>Calf thymus, fractionated on Sepharose 4B</td>
<td>154</td>
<td>Na(^+)</td>
<td>7.50</td>
<td>1.08</td>
<td>72.2</td>
</tr>
<tr>
<td>C. perfringens</td>
<td>143</td>
<td>Na(^+)</td>
<td>2.5</td>
<td>1.05</td>
<td>71.6</td>
</tr>
<tr>
<td>E. coli</td>
<td>151</td>
<td>Na(^+)</td>
<td>2.5</td>
<td>1.03</td>
<td>71.2</td>
</tr>
<tr>
<td>M. luteus</td>
<td>132</td>
<td>Na(^+)</td>
<td>2.5</td>
<td>0.93</td>
<td>69.0</td>
</tr>
<tr>
<td>Poly(dG)-poly(dC)</td>
<td>159</td>
<td>Na(^+)</td>
<td>2.5</td>
<td>1.08</td>
<td>72.2</td>
</tr>
</tbody>
</table>

* \( \phi^* \) is the reduced dichroism at infinite orientation obtained by extrapolation of the measured values, following the anisotropic ion flow model.

13 Å and extrapolating to \( A = 0 \) at the approximate ionic strength at which the Debye radius is equal to the assumed macro-ion radius. Also apparently linear was a plot of \( A \) versus the logarithm of ionic strength. Quantitative interpretation of the ion strength effect is beyond the scope of our dimensional analysis of the ion flow model.

The Limiting Dichroism Indicates that the Bases are Inclined at an Angle of 73° or Less with Respect to the Helix Axis. We extrapolated the dichroism of various DNA samples to infinite field and calculated \( \alpha \) from Eq. 2, with the orientation parameter \( \Phi \) set equal to 1. The results (Table 1) show angles of 73° ± 3°, with no apparent systematic dependence on DNA length or source, salt concentration, or nature of the counter-ion. Data are included only for conditions that give values of the correlation time in agreement with Fig. 2. This excludes, for example, monovalent cation concentrations below 2 mM.

The angle \( \alpha \) measures the average orientation of the base transition moments relative to the helix axis and cannot strictly be equated to the orientation angle of the bases for two reasons. First, it is possible that transition moments polarized out of the base plane contribute appreciably to the absorbance. We tested the wavelength variation of the reduced dichroism from 250 to 285 nm and found no dependence. We conclude that it is unlikely that out-of-plane transitions, such as \( n \rightarrow \pi^* \), contribute appreciably to the measured dichroism.

The second difficulty in equating \( \alpha \) to the base inclination angle is that \( \alpha \) measures the orientation of a vector (the transition moment) that lies in the plane of the base whose angle need not equal the angle between the base plane and the helix axis. It is possible for \( \alpha \) to be larger than the base plane inclination; for example, \( \alpha \) could be 90° even though the base is tilted away from the perpendicular to the helix axis. However, \( \alpha \) cannot be smaller than the base inclination angle. Therefore, we can conclude that the bases in B DNA seem to be inclined at an angle of 73° or less relative to the helix axis; the accepted value for this angle for B DNA is about 84° (24). In work to be published we will show that our apparatus is capable of measuring chromophore inclination angles of nearly 90°, supporting our view that our results are not due to unknown instrumental artifacts that reduce our measured dichroism amplitudes.

The Measured Base Inclination Angle Is Consistent with Levitt's Revised B-Form DNA Model. During the course of this work it came to our attention that Levitt has proposed, on the basis of energy minimization calculations, a revised model for B-form DNA in which the base pairs have a propeller-like twist, each base being inclined at an angle of about 73° relative to the helix axis (25), smaller than the accepted value. Given such a model, and the general orientation of the 260-nm band base transition moments perpendicular to the axis of twist between the bases in a pair (26), \( \alpha \) should be nearly equal to the base inclination angle. Hence, Levitt's calculation and our results are in good agreement, strongly suggesting further consideration of his modified model for the structure of DNA in solution.

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