Electrooptical "Amplitude Inversion" of Short DNA Fragments Observed by Brownian Dynamics Simulation

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Short DNA fragments are oriented by electric fields of a given duration, and this induces a birefringence or dichroism in the solution. When the field is turned off, the birefringence or dichroism decays to zero as the fragments return to an isotropic distribution. At low electric fields, the off-field relaxation has a large amplitude component which decays exponentially with lifetime $1/6D_R$ where D_R is the end-over-end rotational diffusion constant.[1-3] A smaller amplitude, faster decay process is also present which can be reproduced by Brownian dynamics simulations of wormlike chains modeled as semiflexible strings of beads.[4,5] In these simulations, the fragments were oriented by the electric field, but there was no field induced stretching/deformation of the chain. From experiment, it has been observed that the relative amplitude of the faster decay process increases with increasing field strength.[6] Under certain conditions, electrooptical decay of DNA fragments in the size range of 200 bp (base pair) can exhibit what is called "amplitude inversion".[7] In this case, the electric birefringence or dichroism decay starts negative, quickly decays to zero, then becomes positive, and finally decays to zero. This effect is favored by high salt concentrations (above 5 mM monovalent), where the electric polarizability is low, and by high electric fields (>16 kV/cm for 256 bp and >47 kV/cm for 179 bp DNA at 20 °C and 12.5 mM salt). Antosiewicz and Porschke were able to interpret this effect reasonably well in terms of a rigid bent rod model.[7]

Recently, Elvingson characterized the induced orientation and deformation of flexible wormlike chains placed in strong electric fields.[8] This study has gone far in helping us understand the electrooptical behavior of DNA fragments in strong electric fields. Our own simulations have corroborated those of Elvingson. When the field is turned on, DNA translates through the solution because it is a polyanion. Hydrodynamic interaction induces the chain to adopt a bowed conformation, and the bow tends to face into the flow direction as shown in Figure 1. It should be emphasized that this "bowing" is a consequence of the *translation* of the polyanion in an external field. The extent of deformation depends strongly on applied field strength, E, effective charge per base pair, q_{bp} (in units of the protonic charge), and the electric polarizability of the DNA, α. Deformation increases with increasing E and q_{bp} and decreasing α. Since α also decreases with increasing salt concentration,[7] we can suspect strongly that amplitude inversion is associated with field induced deformation of DNA.

The purpose of this note is to demonstrate that the phenomenon of amplitude inversion can be reproduced in Brownian dynamics simulations of DNA fragments using reasonable input parameters for the model chains. We shall focus on a 194 bp fragment since this is in the size range where amplitude inversion has been reported[7] and has also been studied in previous simulations of birefringence/dichroism decay but at low field strengths.[5] Briefly, the DNA is modeled as a string of 10 beads with bead

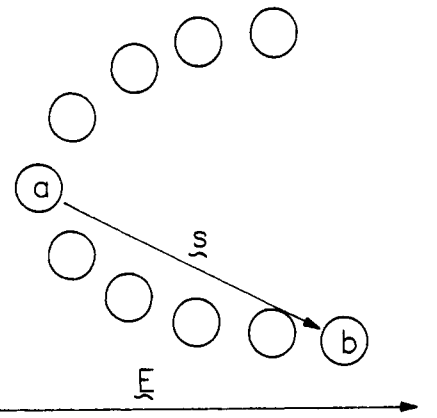

Figure 1. Schematic of a DNA fragment in an electric field. The molecule migrates to the left. Intersubunit vector, **s**, joins subunits on which negative (a) and positive (b) polarization charges are placed. Each bead also carries an effective subunit charge.

radius, virtual bond length, and bending force constant chosen to reproduce the ensemble-averaged eigenvalues of the rotational diffusion tensor of a rigid wormlike chain.[3-5] In the present study, we need to simulate the motion of the polyion when an external field is applied, which requires that we account for the subunit charge ($q_{subunit} = 19.4q_{bp}$) as well as the polarization charges, q_{pol}. The following simple model is used to account for q_{pol}. The induced dipole moment, **p**, due to polarization is taken to be

$$\mathbf{p} = \alpha\,(\mathbf{s}\cdot E)\mathbf{s}/s^2 = q_{pol}e\mathbf{s} \qquad (1)$$

where α is the polarizability of the DNA, **s** is the position vector from $-q_{pol}$ (on subuit a) to $+q_{pol}$ (on subunit b), E is the applied field strength, and $e = 4.8 \times 10^{-10}$ esu. Taking the field to be in the $+z$ direction, subunit a/b is chosen to be that subunit with the lowest/highest z coordinate. The subunits on which polarization charges are placed will, of course, change during a trajectory. In the monovalent salt concentration range 0.2–12.5 mM, the polarizability of 194 bp DNA ranges from 4.1×10^{-16} to about 0.35×10^{-16} cm^3, respectively.[2,7,8] A value for q_{bp} can be inferred from the work of Smith and Bendich, who studied the distortion of high molecular weight plasmid DNA's in electric fields which are immobilized by agarose fibers.[9] They determined an effective charge of -15 e per persistence length which yields $q_{bp} = -0.1$ if a persistence length of 500 Å is assumed. However, a value this low does not lead to amplitude inversion in the simulations unless field strengths in excess of 80 kV/cm are used. Around 25 kV/cm, a q_{bp} of this magnitude results in a fairly low electrophoretic mobility and there is little flow induced deformation of the fragment. A value of $q_{bp} = -0.24$ does yield amplitude inversion in the 25 kV/cm range and is used in the rest of this work. Although this is larger than expected on the basis of the Smith and Bendich value, it should be pointed out that those experiments were done at 0.05 M salt, which is substantially higher than that used in the electrooptical experiments we are trying to mimic here. In the model used by Smith and Bendich, the charge of $-15e$ represents not only the bare charge on a DNA persistence length but also an effective charge that represents the bare polyion charge and the associated ion atmosphere. Hence, it is expected to depend on salt concentration.

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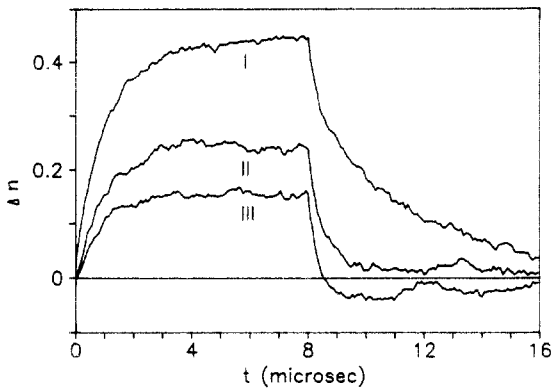

Figure 2. Birefringence decay from 194 bp DNA, E = 25 kV/cm, q_{bp} = −0.24. The field is turned on at t = 0 and off at t = 8 μs. The three curves from top to bottom correspond to α = 1.00 (I), 0.50 (II), and 0.25 × 10^{-16} cm^3 (III), respectively.

Brownian dynamics simulations of electric birefringence and dichroism are described in detail elsewhere.[5] In the present work, full hydrodynamic interaction is used and each simulation consists of between 800 and 1000 independent trajectories of 16-μs duration each. Chains are selected from an isotropic equilibrium ensemble to begin with and the field (along +z) is turned on at t = 0 and off at t = 8 μs ($1/6D_R \sim 3.5$ μs for 194 bp DNA[5]). A reduced birefringence/dichroism is computed from

$$\Delta n(t) = (N-1)^{-1}\sum_{j=1}^{N-1}\langle z_j^2(t) - x_j^2(t)\rangle \qquad (2)$$

where $z_j(t)/x_j(t)$ is the projection of the jth virtual bond along the z/x direction at time t, $N-1$ is the number of virtual bonds in the chain, and brackets denote an average over all trajectories. The average rms end-to-end distance is also computed. The actual dichroism, $\Delta A(t)$, is related to $\Delta N(t)$ by[5]

$$\Delta A(t) = (N-1)cl(a_\parallel - a_\perp)\,\Delta n(t) \qquad (3)$$

where c is the concentration of macromolecule, l is the path length, and $a_\parallel - a_\perp$ is the difference in absorption cross section of a virtual bond parallel and perpendicular to the bond axis. A similar expression is obtained for birefringence. Since $a_\parallel - a_\perp$ is negative for DNA, the actual dichroism starts off negative. Nonetheless, $\Delta n(t)$ will be independent of the relative magnitude of $a_\parallel$ and $a_\perp$ (or the corresponding indices of refraction in the case of birefringence).

Shown in Figure 2 is the rise and decay of $\Delta n(t)$ for several simulations in which the polarizability is reduced from 1 × 10^{-16} cm^3 (I) to 0.5 × 10^{-16} cm^3 (II) to 0.25 × 10^{-16} cm^3 (III). The field strength during the first 8 μs is 25 kV/cm. Consider case I first. Once the field is turned off, the time required for Δn to decay to $1/e$ of its saturation value is τ_1^{off} = 2.5 μs and the time required to decay to $1/e^2$ of its saturation value is τ_2^{off} = 6.3 μs. This is similar to the decay seen at low fields[5] which consists of a large amplitude component with a lifetime characteristic of overall tumbling along with a small amplitude component with a significantly shorter lifetime. In case II, the decay is much more rapid with τ_1^{off} = 0.5 and τ_2^{off} = 1.25 μs. However, $\Delta n(t)$ never drops below zero. If we view, probably incorrectly, case II as a superposition of two decays of the same lifetime as case I but different amplitudes, then clearly the amplitude of the fast decay process has grown at the expense of the slower. Amplitude inversion is clearly evident when the polarizability is reduced to 0.25 × 10^{-16} cm^3 (case III). In this case, the decay cannot be fit by a distribution of relaxation times unless we allow the amplitudes to be negative. Once the field is turned off, the initial decay is very rapid with τ_1^{off} and τ_2^{off} = 0.24 and 0.38 μs, respectively. Amplitude inversion is also seen at α = 0.10 and 0.00 × 10^{-16} cm^3. Consider the effect of increasing α holding q_{bp} and E constant. However, q_{bp} and E are assumed to be sufficiently large to result in amplitude inversion when α = 0. At low α the chain adopts a steady state bowed conformation and amplitude inversion results. Viscous drag on a translating polyion is reduced if it adopts a bowed conformation.[8] The chain is compressed relative to its equilibrium (no E) conformation. However, as α increases, the chain will extend since the induced polarization charges are pulled in opposite directions by the applied field. This extension will tend to offset the bowing of the translation polyion and thereby reduce the amplitude inversion. For sufficiently large α, chain extension will dominate and no amplitude inversion is observed. Under conditions where amplitude inversion is observed when α = 0, there will be a threshold α above which it will not be observed.

In summary, model simulations on a 194 bp DNA fragment at an applied field strength of 25 kV/cm and effective charge per base pair of −0.24 exhibit amplitude inversion at $\alpha \sim 0.25 \times 10^{-16}$ cm^3 or below. Since the molecular polarizability decreases with increasing salt, we can interpret this as corresponding to an onset of amplitude inversion above a monovalent salt concentration of about 12.5 mM, which is qualitatively consistent with experiment.[7] Because the model of the interaction of the polyion and its ion atmosphere with an external electric field is rather primitive, quantitative agreement with experiment cannot be taken seriously.

References and Notes

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