Internal Dynamics of DNA Probed by Transient Electric Birefringence

Mi K. Hong,(1) Onuttom Narayan,(2) Raymond E. Goldstein,(1) E. Shyamsunder,(1) Robert H. Austin,(1)
Daniel S. Fisher,(2) and Mike Hogan(3)
(1)*Department of Physics, Princeton University, Princeton, New Jersey 08544*
(2)*Department of Physics, Harvard University, Cambridge, Massachusetts 02138*
(3)*Center for Biotechnology, Baylor College of Medicine, The Woodlands, Texas 77381*

(Received 31 October 1991)

Experiments on the field-free transient electric birefringence decay of dilute solutions of monodisperse DNA fragments whose length L is much larger than the persistence length L_p are reported. They reveal that the highly nonexponential decay is independent of L. We present a simple theoretical model which includes the internal rigidity of the polymer, explains the qualitative results of the measurements, and describes quantitatively the dynamics of long semiflexible polymers such as DNA, providing an accurate determination of the persistence length.

PACS numbers: 87.15.He, 36.20.Ey, 78.20.Jq

The static and dynamic properties of a particular semiflexible polymer, DNA, are of fundamental importance in biology. The stiffness of DNA determines both its persistence length and its dynamic properties, as well as the strain energy stored in highly distorted DNA. The latter is of direct biological interest in areas ranging from the highly condensed nature of the DNA in eukaroyotic cells [1] and the enthalpic strain energy in DNA-protein complexes [2], to the conformation of supercoiled circular DNA [3]. Modern restriction enzyme techniques [4], which have revolutionized the study of nucleic acids, can also be used to produce absolutely monodisperse polymer solutions of DNA. These yield a beautiful system for studying the properties of semiflexible polymers. We report here experimental and theoretical work which demonstrates how the response of a polymer to transient electric fields can be used to determine the elastic constants of the chain and to reveal information about its dynamics.

The important parameters that characterize the static properties of a polymer are its total length L, proportional to the number of monomers [here, DNA base pairs (bp)], the persistence length L_p, which measures the decay of correlations of the local tangents along the chain, and its effective hard-core radius which gives rise to effects of self-avoidance. While extensive previous experimental [5-7] and theoretical [8-10] work has focused on the dynamics of short chains, our analysis focuses on the internal dynamics of DNA fragments for which $L/L_p \gg 1$. We have measured the transient electric birefringence (TEB) decay of monodisperse DNA of varying lengths L in dilute solutions (i.e., with concentration less than that for chain overlap). The experimental quantity of interest is the field-free decay $S(t;T)$ of induced birefringence following application of an electric pulse of duration T and amplitude E_m which partially aligns the DNA molecules. All the data [11] were obtained in the Kerr regime, where the signal amplitude is proportional to E_m^2.

Figure 1 shows the field-free TEB decay from dilute solutions [12] (0.01 mM bp) of *E. coli* DNA fragments of 22000 bp, for varying pulse widths in the range $2 \leq T \leq 60$ μs. The time resolution of the experimental system was 50 ns, as determined by the rise and fall times of the Cober pulse generator. The data reveal that the decay of the birefringence for long fragments is strongly nonexponential (as seen before in *polydisperse* solutions of polystyrene sulfonate [13] and in "living polymers" [14]). A remarkable saturation effect is also seen (Fig.

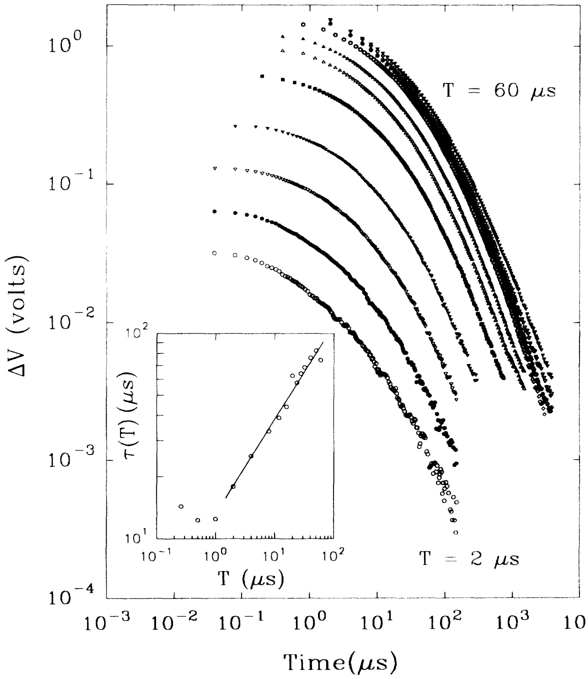

FIG. 1. Transient electric birefringence decay $S(t;T)$ from a sample containing restriction enzyme fragments of length 22000 base pairs. Decay curves were obtained with an aligning field of magnitude 1500 V/cm of duration $2 \leq T \leq 60$ μs, increasing to the right. Inset: The characteristic time of the various decays determined directly from integration of the data. Power-law fit shown in the inset yields an exponent of $\psi = 0.46 \pm 0.06$.

2) in which the form of the decay (in the range of t accessible) becomes *independent* of L for L sufficiently large. The nonexponential form of the decay implies the existence of a distribution of relaxation times, which in turn yields strong pulse-width dependence to the decay. Only those components of the distribution with relaxation time $\tau < T$ respond appreciably to the aligning pulse and thus contribute to the observed field-free decay. The relaxation associated with the shortest fragments (with $L \le L_p$) is nearly exponential, indicative of an extremely narrow distribution of relaxation times.

In the Kerr regime, if the electric field polarizes the molecule sufficiently fast, the measured signal will depend only on $E_m^2(t)$. For a square pulse, linear response then implies (for $t > 0$)

$$S(t;T)=\int_0^\infty d\tau\,\tilde{G}(\tau)\tau(1-e^{-T/\tau})e^{-t/\tau}E_m^2\,, \qquad (1)$$

where $\tilde{G}(\tau=1/s)$ is the Laplace transform (in s) of the linear response $G(t)$ to a δ-function electric field pulse. We have explicitly verified that the data are in this linear regime by comparing the signals from independent pairs of pulses to those from a single longer pulse.

Since inverse Laplace transformation of data tends to be fraught with numerical instabilities, it is useful to extract from the measured signal a quantity which directly yields information on the large-τ behavior of $\tilde{G}(\tau)$ which, as we shall see, is determined by the long-wavelength dynamics of the polymer. We thus define a characteristic time, $\tau(T)=\int_0^\infty dt\,S(t;T)/S(0;T)$, which is plotted in the inset of Fig. 1. For large T, $\tau(T)$ appears to exhibit a power-law form $\tau(T)\sim T^{\psi}$ with an exponent $\psi=0.46\pm0.06$. In this paper we propose and analyze a simple model for the polymer dynamics which results in $\tilde{G}(\tau)\sim\tau^{-\phi}$ with $\phi=\frac{8}{3}$ for a wide range of τ in long chains. This is reasonably consistent with $\phi=3-\psi=2.54\pm0.06$ extracted from the data, especially considering the rather limited range of T (1 to 60 μs) used for the fit.

In order to formulate a reasonable model for the polymer dynamics, we must first clarify the role of self-avoidance. In the *absence* of self-avoidance, the rms end to end distance $\bar{R}_L$ of a long DNA segment of contour length L is given in terms of the persistence length L_p by $\bar{R}_L^2=2LL_p$. From a variety of physical techniques [15], L_p has been estimated to be ~650 Å under the present solution conditions. To estimate roughly the effects of self-avoidance on the equilibrium configurations of the DNA, we consider all configurations that would be permitted in its absence. If the typical number of self-intersections $\langle n_{\mathrm{int}}\rangle$ in such configurations is much less than 1, self-avoidance will not change the allowed configurations significantly. Simple considerations yield that $\langle n_{\mathrm{int}}\rangle$ scales with the polymer chain length as $\sim L^2/\bar{R}_L^3\sim L^{1/2}$. If the DNA cross-sectional radius a is small, $\langle n\rangle_{\mathrm{int}}$ then has the form $\sim a(L/L_p^3)^{1/2}$. (An estimate of the numerical prefactor yields a result considerably less than unity.) Using the measured values of $L_p\sim650$ Å and $a\sim15$ Å [16], we can verify that even for the longest DNA segments we have used here (~20000 base pairs), this quantity is much less than unity. Thus the effects of self-avoidance on the *statics* will be negligible. Nevertheless, even for small chain radius a, the *dynamics* will be affected by self-avoidance at least at very long times [17].

For a semistiff thin polymer like DNA we thus have a separation of length scales between the microscopic length a, the persistence length L_p, and the length above which self-avoidance will be important, L_{SA}: $a\ll L_p\ll L_{\mathrm{SA}}$. The region probed by the present data is $L_p\ll L\ll L_{\mathrm{SA}}$. Since the fluctuations in the shape of the chains are strongly overdamped, the dynamics can be described by a Langevin equation of the form [18]

$$\frac{\partial\mathbf{R}(q)}{\partial t}=-\Gamma(q)\frac{\delta\mathcal{F}[\mathbf{R}]}{\delta\mathbf{R}(q)}+\eta(q,t)\,, \qquad (2)$$

where $\mathbf{R}(q)=L^{-1/2}\int\mathbf{R}(x)\exp(iqx)dx$ is the Fourier transform of the position $\mathbf{R}(x)$ of a point on the chain, and the thermal noise η has correlations

$$\langle\eta_i(q,t)\eta_j(q',t')\rangle\sim\delta_{ij}\Gamma(q)\delta(t-t')\delta(q-q')\,.$$

(We choose units of energy so that k_B times the temperature is unity.) Long-range hydrodynamic coupling between different sections of the chain caused by the response of the solvent water molecules to motion of the chain results in an effective nonlocal viscous drag, so that $\Gamma(q)$ depends on q [19]. Since the hydrodynamic radius and hence the drag on a section of polymer of length l scales as $l^{1/2}$ rather than l, we expect that modes with wavelengths $q\sim1/l$ will have their drag reduced by a factor of $l^{1/2}$, yielding an enhanced $\Gamma(q)=\gamma q^{-1/2}$ at long wavelength. The diffusion coefficient D_L of a polymer of length L is similarly proportional to $L^{-1/2}$ rather than

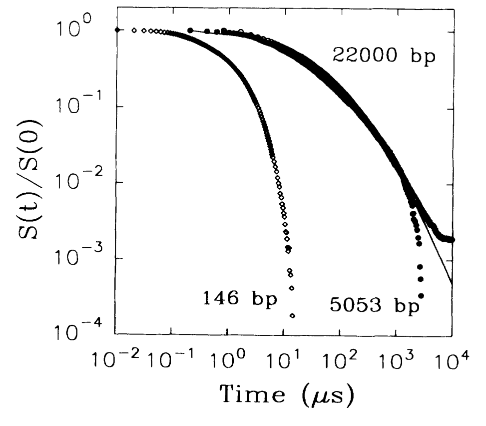

FIG. 2. Transient electric birefringence decay for 146, 5053, and 22000 bp DNA fragments. The solid line is a fit to the data for the 22000 bp fragment using the model presented in Eq. (5). Note that the decay for the shortest chains is essentially exponential, in contrast to that of the longer fragments.

$1/L$, and can be related to γ within the "preaveraging approximation" [18], $D_L = 8\gamma(9\pi L/2)^{-1/2}$, allowing for an experimental determination of γ. This scaling of D_L with L is borne out experimentally for DNA fragments in the range of lengths used here [20].

On scales shorter than L_p, the free energy of the polymer is given by $\frac{1}{2}L_p\int dx(\partial^2\mathbf{R}/\partial x^2)^2$, with the constraint $|\partial\mathbf{R}/\partial x| = 1$. At scales larger than the persistence length, however, it is more convenient to work with an effective free energy in terms of a coarse-grained position $\mathbf{R}(x)$, which includes the effects of the entropy of short-wavelength deformations. The dominant terms are

$$\mathcal{F} = \int_0^L dx\left[\frac{\sigma}{2}\left(\frac{\partial\mathbf{R}(x)}{\partial x}\right)^2 + \frac{\kappa}{2}\left(\frac{\partial^2\mathbf{R}(x)}{\partial x^2}\right)^2 - \alpha\left(\mathbf{E}(t)\cdot\frac{\partial\mathbf{R}(x)}{\partial x}\right)^2\right], \tag{3}$$

in which the first term is the conventional entropic term [21], and the second is a residual rigidity term [22]. The last term is a contribution from the electric field, which tries to align the polymer parallel to $\mathbf{E}$, and has been assumed to be local in time (as justified earlier).

The relationship between the two material parameters σ and κ and the persistence length L_p is found by requiring that correlations between the local tangents to the chain should decay in exactly the same way as for a continuous elastic rod, for which [23] $\langle q^2\mathbf{R}(q)\cdot\mathbf{R}(-q)\rangle \sim (1+q^2L_p^2)^{-1}$. For this to be true, we must choose $\kappa/\sigma = L_p^2$. For very large L, the leading term in the mean square extent is $3L/\sigma$. Accordingly, we find $\sigma = 3/2L_p$, and thus $\kappa = L_p^2\sigma = 3L_p/2$.

Since the electric contribution to $\mathcal{F}$ is of the same form as the entropic term, it can be absorbed into it by making the coefficient anisotropically time dependent. The resulting Langevin dynamics of Eq. (2) constitutes a first-order (time-dependent) linear equation for $\mathbf{R}(q)$: We write Eq. (3) in terms of the Fourier components of $\mathbf{R}(x)$, $\mathbf{R}(q)$, and substitute in Eq. (2). For a square pulse, this can be easily integrated to find $R_\parallel(q,t)$ and $R_\perp(q,t)$, the components of $\mathbf{R}(q)$ parallel and perpendicular to the aligning field, the two differing because of the field-induced anisotropy.

The birefringence signal is proportional to the difference in indices of refraction parallel and perpendicular to the field, and apart from material properties such as polarizabilities is given by [18]

$$n_\parallel - n_\perp \sim \int dx\left[\left(\frac{\partial\mathbf{R}_\parallel(x)}{\partial x}\right)^2 - \left(\frac{\partial\mathbf{R}_\perp(x)}{\partial x}\right)^2\right]. \tag{4}$$

Substituting $\mathbf{R}(q,t)$ obtained as in the previous paragraph in Eq. (4), in the Kerr regime the birefringence signal can be found to be of the form anticipated in Eq. (1),

$$S(t;T) = \alpha E_m^2\sum_q \frac{q^4}{K(q)^2}[1-e^{-T/\tau(q)}]e^{-t/\tau(q)}, \tag{5}$$

where $K(q) = \sigma q^2 + \kappa q^4 = 3[q^2+L_p^2q^4]/2L_p$ and the decay time of the mode of wave vector q is $1/\tau(q) = 2\Gamma(q)K(q)$. From the boundary conditions $\partial\mathbf{R}(x)/\partial x = 0$ at the two ends, we have $q = n\pi/L$, where n ranges over all nonzero integers in the summation in Eq. (5).

In order to understand the form of the decay function, it is instructive to examine the behavior of our model in the continuum limit, in which the summation over q in Eq. (5) is replaced by an integral. There is a characteristic "turn-on" time at which, for the modes that dominate the integral, both parts of $K(q)$ are of the same order; this occurs at a time $t_p \sim L_p^{5/2}/\gamma$. Second, when t is large, for the modes that are important we can approximate $K(q)$ by its first term, σq^2. Equation (5) is then a Gaussian integral, which yields

$$S \sim t^{-5/3}, \text{ for } t \gg t_p, T. \tag{6}$$

Finally, there is a long-time cutoff to the signal, which arises from the fact that Eq. (5) is really a summation over q rather than an integral. This time is set by the condition $2\Gamma(\pi/L)K(\pi/L)t \sim 1$. This results in an exponential cutoff at $t_L \sim L^{3/2}L_p/\gamma$. Since $\Gamma(q)$ and $K(q)$ are both independent of L, when we approximate the signal by an integral over q, for a given value of the pulse width T the observed decay for $t \ll t_L$ is also *independent* of L, up to a normalization. The long-time cutoff does, however, depend on L.

The chain-length independence of the decay, for times less than t_L for large L, reflects the fact that the birefringence signal is a nonsingular function of wave vector q as $q \to 0$. As a result, for large L, the sum in Eq. (5) is well approximated by an integral over q from 0 to some upper cutoff. (This is in contrast to the behavior for any probe that is singular for small q, which would not show the kind of length independence that we have found.)

If we recast the result, Eq. (5), in terms of an integral over relaxation times as in Eq. (1), and consider the large-τ behavior, we find from the limiting forms for $q \to 0$ of $\Gamma(q)$ and $K(q)$ that $\tau(q) \sim q^{-3/2}$ and $q^4/K(q)^2 \sim q^0$. Thus $\tilde{G}(\tau) = \tau^{-1}dq/d\tau \sim \tau^{-8/3}$.

The decay curve $S(t;T)$ is completely specified (up to a normalization) by the two characteristic times t_p and t_L, which in turn depend on two physically important quantities which can be determined by other experimental techniques: the persistence length L_p and the translational diffusion constant D_L. The signal decay should thus be fitted *without any adjustable parameters.* However, our data are not particularly sensitive to the long-time cutoff. The best fit estimates from an analysis of several hundred decay curves at varying pulse widths and varying contour lengths yield a value of $L_p = 200 \pm 30$ base pairs (or 680 ± 100 Å at 3.4 Å/bp) when we use measured values of the diffusion constant D_L for different values of L as obtained from sedimentation studies [20]. Figure 2 shows the best fit obtained for the DNA fragment 22000 base pairs long with a pulse width $T=20$ μs. The value obtained for L_p is consistent with values reported in the literature [15], again given the errors involved in both the experiment and the assumptions of our model.

In conclusion, we have demonstrated that a simple model for the internal dynamics of relatively long DNA molecules yields a good fit to the transient electric birefringence decay, and provides a means to determine the elastic constants and persistence length of such polymers. Extensions to include the effects of self-avoidance should be interesting. During preparation of this manuscript, we received preprints on related work by Wu *et al.* [14] and by B. Zimm.

We thank E. Frey, A. Georges, T. Hwa, D. Nelson, and C. Yeung for useful discussions. This work was supported in part by NSF Grants No. DMB88-16340, No. DMR 9106237, and No. CHE9106240, ONR Grant No. N00014-91-J-1543, the Harvard University Materials Research Laboratory, and the A. P. Sloan Foundation.

[1] V. C. Gordon, C. M. Knobler, D. E. Olins, and V. N. Schumaker, Proc. Natl. Acad. Sci. U.S.A. **75**, 660 (1987).

[2] M. E. Hogan and R. H. Austin, Nature (London) **329**, 263 (1987).

[3] M. Wadati and H. Tsuru, Physica (Amsterdam) **21D**, 213 (1986).

[4] M. Singer and P. Berg, *Genes and Genome* (University Science Books, Mill Valley, CA, 1991).

[5] R. J. Lewis, R. Pecora, and D. Eden, Macromolecules **19**, 134 (1986).

[6] R. J. Lewis, R. Pecora, and D. Eden, Macromolecules **20**, 2579 (1987).

[7] Z. Wang, R. Xu, and B. Chu, Macromolecules **23**, 790 (1990).

[8] P. Hagerman and B. Zimm, Biopolymers **20**, 1481 (1981).

[9] M. D. Barkely and B. H. Zimm, J. Chem. Phys. **70**, 2991 (1979).

[10] J. M. Schurr, Chem. Phys. **84**, 71 (1984).

[11] For a description of the experimental apparatus, see S. S. Chan, K. J. Breslauer, M. E. Hogan, D. J. Kessler, R. H. Austin, J. Ojemann, J. M. Passner, and N. C. Wiles, Biochemistry **29**, 6161 (1990).

[12] Solutions were prepared in 10 mM TRIS buffer, at pH 7.5, with 10 μM EDTA.

[13] V. Degiorgio, T. Bellini, R. Piazza, F. Mantegazza, and R. E. Goldstein, Phys. Rev. Lett. **64**, 1043 (1990).

[14] X-l. Wu, C. Yeung, M. W. Kim, J. S. Huang, and D. Ou-Yang, preceding Letter, Phys. Rev. Lett. **68**, 1426 (1991).

[15] P. J. Hagermann, Ann. Rev. Biophys. Biochem. **17**, 265 (1988).

[16] The radius used here is the effective hydrodynamic radius, which in fact is larger than the geometrical radius. The water used as a solvent in the experiment was buffered with 10-mM salt; this screens Coulomb interactions sufficiently strongly so that the charged DNA is effectively neutral. (For low salt concentrations, a and L_p would increase.)

[17] Estimates of such dynamic effects are rather difficult, and will depend on the process considered, but one would expect that as long as typical sections of the polymer do not move far enough to run into other sections in experimental times, the dynamics will not be affected strongly by self-avoidance. This is supported by the linearity of the measured response: If dynamic self-avoidance were important, one might expect nonlinear behavior due to the constraints.

[18] M. Doi and S. F. Edwards, *The Theory of Polymer Dynamics* (Oxford Univ. Press, Oxford, 1989).

[19] B. H. Zimm, J. Chem. Phys. **24**, 269 (1956).

[20] J. E. Hearst and D. A. Reese, J. Chem. Phys. **73**, 3007 (1980).

[21] P. E. Rouse, J. Chem. Phys. **21**, 1272 (1953).

[22] K. Freed, J. Chem. Phys. **54**, 1453 (1971). (Freed's expression differs by a factor of $\frac{3}{2}$ from ours due to a use of the short-length-scale coefficient in a long-length-scale effective free energy.)

[23] L. D. Landau and E. M. Lifshitz, *Theory of Elasticity* (Pergamon, Oxford, 1986), Vol. 7.