The Hydrodynamics of DNA Electrophoretic Stretch and Relaxation in a Polymer Solution

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ABSTRACT Theories of DNA electrophoretic separations generally treat the DNA as a free draining polymer moving in an electric field at a rate that depends on the effective charge density of the molecule. Separations can occur in sieving media ranging from ultradilute polymer solutions to tightly cross-linked gels. It has recently been shown that DNA is not free-draining when both electric and nonelectric forces simultaneously act on the molecule, as occurs when DNA collides with a polymer during electrophoretic separations. Here we show that a semidilute polymer solution screens the hydrodynamic interaction that results from the application of these forces. Fluorescently labeled DNA tethered at one end in a semidilute solution of hydroxylethyl cellulose stretch more in an electric field than they stretch in free solution, and approach free-draining behavior. The steady stretching behavior is predicted without adjustable parameters by a theory developed by Stigter using a hydrodynamic screening length found from effective medium theory. Data on the relaxation of stretched molecules after the electric field is removed agree with the Rouse model prediction, which neglects hydrodynamic interactions. The slowest relaxation time constant, τ_R, scales with chain length as $\tau_R \sim L^{1.9\pm0.17}$ when analyzed by the data collapse method, and as $\tau_R \sim L^{2.17\pm0.17}$ when analyzed by multiexponential fit.

INTRODUCTION

Electrophoresis in a sieving medium is commonly used to separate DNA molecules by size. Separations of DNA molecules varying in size from oligonucleotides to chromosomes are conducted in a range of media, from ultradilute polymer solutions to tightly cross-linked gels (Viovy, 2000). Because of this wide variety of solute sizes and media employed in their separation, many of the physical processes affecting resolution and efficiency remain poorly understood. The models most commonly employed to describe gel electrophoresis are the Ogston sieving model and the biased reptation with fluctuations model (Sartori et al., 2003). The Ogston model is applicable only for very small molecules in a covalent gel, since it treats DNA as a rigid sphere and the gel as a static network of randomly distributed pores. The biased reptation with fluctuations model is useful for crosslinked or highly entangled gels, but does not provide a quantitative prediction of the dependence of DNA mobility on size. To develop a predictive model for electrophoretic separations, the electrophoretic behavior of DNA in gels and polymer solutions must be better understood.

Epifluorescence microscopy has shown that the conformation of long DNA molecules during electrophoretic separations is highly dynamic, fluctuating between collapsed and highly extended states (Schwartz and Michael, 1989; Smith et al., 1989; Sunada and Blanch, 1998a). Because DNA itself is a polymer, the description of these dynamics has often been pursued using the classical theories of polymer dynamics. Rouse (1953) developed a bead-spring model of a polymer fluctuating around its equilibrium conformation, a model that has been used extensively since. The model assumes that each bead has the same friction coefficient (i.e., the polymer is free-draining), the restoring force of the springs is linear, and the solvent is athermal. The position of each bead with time is found from the differential equation

$$\frac{\partial \mathbf{R}(n,t)}{\partial t} = \frac{3k_BT}{b^2\zeta}\frac{\partial^2 \mathbf{R}(n,t)}{\partial n^2} + \mathbf{g}(n,t), \tag{1}$$

where $\mathbf{R}$ is the position of bead n at time t, k_B is Boltzmann's constant, T the temperature, b the Rouse segment length, ζ the friction factor of the bead, and $\mathbf{g}$ is a noise term that accounts for Brownian motion. From the solution to Eq. 1, the motion of a polymer, ranging from the diffusion of the whole molecule to rearrangements of single segments, can be described. For example, the time correlation function of the end-to-end vector, $\langle \mathbf{P}(t) \cdot \mathbf{P}(0) \rangle$ for a polymer with N segments, is described by a sum of independent, normal modes, each with its own relaxation time, τ_R:

$$\langle \mathbf{P}(t) \cdot \mathbf{P}(0) \rangle = Nb^2 \sum_{p_{Odd}} \frac{8}{\pi^2 p^2} \exp\left(-\frac{tp^2}{\tau_R}\right). \tag{2}$$

The characteristic relaxation time is

$$\tau_R = \frac{\zeta N^2 b^2}{3\pi^2 k_B T}. \tag{3}$$

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Equation 3 predicts that for a free-draining polymer in a θ-solvent, the characteristic relaxation time should scale with the square of the polymer length. However, the Rouse model neglects both hydrodynamic interactions between segments and the solvent-mediated excluded volume interaction. Zimm (1956) extended the bead-spring model to account for hydrodynamic interactions using the Kirkwood approximation (Kirkwood and Riseman, 1948), resulting in a different form for the relaxation time that also depends on the solvent viscosity, η_s:

$$\tau_R = \frac{\eta_s(\sqrt{N}b)^3}{\sqrt{3\pi}k_BT}. \quad (4)$$

The Zimm model correctly predicts the scaling behavior of the diffusion coefficient in dilute solutions where hydrodynamic interactions are important (Doi and Edwards, 1986).

Solvent quality has been explicitly introduced into the Zimm model (Doi and Edwards, 1986), but a simple scaling argument first put forward by de Gennes (1976) gives the same functional form for the dynamical properties. De Gennes proposed that the dynamical properties of polymers will scale similarly to their static properties, depending on the solvent quality through the scaling parameter ν (which is equal to 1/2 in a θ-solvent and ~3/5 in a good solvent). For example, if the number of segments in a chain is changed from N to N/λ (where λ is a constant) then the physical properties can be held constant by simply changing the segment length from b to $b\lambda^{\nu}$ (since these properties do not depend on the local structure of the chain). By this transformation, the invariant static and dynamical properties should change from A to $A\lambda^{x}$, where x is a scaling parameter that depends on ν. It can be shown that for a Rouse chain and a Zimm chain, respectively, that

$$\tau_R \propto N^{1+2\nu}, \quad (5a)$$

$$\tau_R \propto N^{3\nu}. \quad (5b)$$

The dynamic behavior of DNA during electrophoretic separations should range from Zimm behavior in ultradilute polymer solutions, to Rouse behavior at higher concentrations of polymer where hydrodynamic interactions are screened, to reptation at high concentrations of long polymer (or in cross-linked gels) where the polymer matrix forms a long-lived constraining tube around the DNA. In the present work, we focus on the separations of DNA in dilute and semidilute solutions of uncrosslinked polymers where neither the Ogston model nor the biased reptation with fluctuations model applies. In this regime, because reptation tubes do not exist or are not long-lived, separation occurs by the transient entanglement coupling mechanism (Sunada and Blanch, 1998b). In transient entanglement coupling, DNA molecules electrophorescing through solution entangle with or are briefly associated with free polymer molecules, slowing their progress. Larger DNA will interact with polymers more often, and will therefore be slowed relative to smaller molecules. The model closely predicts the electrophoretic mobility of DNA for separations where the DNA does not entangle with the polymer; i.e., where the polymer molecules and/or DNA molecules are sufficiently small. However, the mechanism is more complex for larger DNA and polymer. In this case polymer-DNA entanglements will occur, causing the DNA to stretch from a globular form into a U-shaped configuration, releasing in a pulley-like motion, and relax back to a condensed state (Sunada and Blanch, 1998a). These long-range conformational fluctuations have not been included in any theory to date and require a much more detailed analysis. Recently, by visualizing single tethered DNA molecules stretching in an electric field by epifluorescence microscopy, we experimentally verified that in free solution DNA is not free-draining in the presence of both electric and nonelectric forces (Ferree and Blanch, 2003). Consequently, the hydrodynamic interactions that arise when a long DNA molecule entangles with a polymer molecule in solution will affect DNA stretching and relaxation. Quantitative data for the stretch and relaxation of single DNA molecules in free solution have shown Zimm-like behavior (Perkins et al., 1994) and are relevant to separations of DNA in ultradilute polymer solutions.

The hydrodynamic features of any electrophoretic separation are going to be system-dependent. The polymer identity, concentration, and possibly polydispersity could all affect the manner in which single molecules of DNA will stretch and relax. However, whether the presence of neutral polymer at a concentration relevant to electrophoretic separations will screen these hydrodynamic interactions, and whether this screening will significantly affect the electrophoretic stretch and relaxation, remain open questions. This article addresses the magnitude and result of these hydrodynamics on DNA stretch and relaxation in a semidilute polymer solution. We use epifluorescence light microscopy to measure the end-to-end distance of DNA molecules tethered in an electric field. Data on both the steady stretching as a function of electric field and dynamic relaxation after the field is removed are presented for DNA molecules of varying length. The data are interpreted using several theories of polymer physics.

MATERIALS AND METHODS

Polymer network characterization

Hydroxyethyl-cellulose (average M_W = 250,500 g/mol, Sigma, St. Louis, MO) (HEC 250) in 45 mM tris, 45 mM boric acid, 5 mM EDTA (TBE, all Sigma) was used as the polymer solution in all experiments. Polymer solution viscosities at concentrations ranging from 0.03 to 1.5 wt % were found using an automatic Ubbeholde capillary kinematic viscometer (Schott AVS-300, Mainz, Germany). Viscosity is reported from the average of four separate measurements at 21°C. The number average molecular weight (M_N)

was found from osmotic pressure measurements using a Wescor 4420 colloid osmometer with a 10,000 g/mol cutoff membrane (Wescor, Logan, UT). Dynamic rheological experiments were performed using a cone and plate rheometer (Rheometrics RMS-800, Piscataway, NJ).

Device fabrication

We wish to examine only the effects of the neutral polymer in solution on the electrophoretic stretch and relaxation of single DNA molecules. Consequently, we need to have the DNA molecules tethered far enough away from any solid surfaces so that the hydrodynamic screening is dominated by the neutral polymer and not by the surface. As will be described in detail later, the hydrodynamic screening length at the polymer concentration used here is ~20 nm. The DNA was therefore attached to a microstructure consisting of a 500-nm-high, 2-μm-wide gold wall deposited on a silicon wafer. The P-type silicon wafers were first cleaned by immersing in piranha (5:1, H_2SO_4:H_2O_2) solution for 10 min, rinsed in a tank of nanopure deionized water until resistivity reached 13 MΩ, and blown dry under a stream of nitrogen. A 1.5-μm-thick layer of silicon oxide was grown on the surface of the wafer by wet thermal oxidation at 1000°C. Positive photoresist was then patterned on the wafer by contact lithography to leave an array of 2-μm-wide lines of bare oxide exposed. A 7-nm layer of chromium was deposited by thermal evaporation at 5×10^{-6} torr, followed by a 500-nm layer of gold, without breaking the vacuum. After liftoff of the metals in a bath of acetone, the wafers were left patterned with a series of parallel 2-μm-wide lines, 500 nm in height.

The silicon oxide surfaces of the microfabricated device will generate electro-osmotic flow under our experimental conditions. Therefore, before immobilizing the DNA all surfaces were coated with a brush of polyacrylamide, which we have previously shown eliminates electroosmotic flow in microfluidic channels (Ferree and Blanch, 2003). Before use, the microcells and glass coverslips were cleaned with piranha solution then rinsed thoroughly in deionized water. All surfaces were then coated with a self-assembled monolayer of methacryloxypropyltrimethoxysilane (MPTS, Sigma, St. Louis, MO) by immersing in a 2% solution of MPTS in 95% ethanol (0.3% acetic acid) for 10 min, rinsing with ethanol, and curing at 110°C for 5 min. A polyacrylamide brush was then polymerized from the surface by soaking overnight in a 8% solution of acrylamide (Bio-Rad Laboratories, Hercules, CA) in 0.5× TBE buffer, pH 8.0 (45 mM tris, 45 mM borate, 1 mM EDTA, all Sigma) using ammonium persulfate as a free-radical generator and TEMED (n,n,n',n'-tetramethylenediamine, Sigma) as a stabilizer. The unbound polymer was then removed by rinsing with TBE.

DNA immobilization

The chromosome of the λ-phage was labeled at one end with a 12 basepair 3′-biotinylated oligonucleotide (Genemed Synthesis, South San Francisco, CA), and purified by filtering through a Microcon 50 microcentrifuge filter (Amicon, Beverly, MA). Concatamers of the molecule were made by incubating a solution of λ with T4 DNA ligase (New England Biolabs, Beverly, MA) for several hours at room temperature before labeling. The biotinylated DNA was then immobilized on the gold surfaces by a technique reported in detail previously (Zimmerman and Cox, 1994). Briefly, a self-assembled monolayer of thiolated biotin (Biotin-HPDP, Pierce-Endogen, Rockford, IL) was formed on the gold lines, to which was bound the protein neutravidin (Pierce-Endogen). Since neutravidin is a tetramer, the immobilized protein retains free biotin-binding sites that are used to bind free molecules of biotinylated DNA.

Fluorescence imaging

A 30-μm-deep channel was made by separating a glass coverslip and the silicon device with Kapton tape (3M, Minneapolis, MN) and sealing with nail polish. The channel was filled with a solution of λ-molecules, labeled with the fluorophore YOYO-1 at an 8:1 basepair/dye ratio (Molecular Probes, Eugene, OR) in HEC/TBE, by capillary action. Photobleaching and photoscission of the DNA were minimized by adding 3% β-mercaptoethanol, 15 μg/ml catalase, 50 μg/ml glucose oxidase, and 0.1% glucose (all Sigma). All experiments were performed on an upright Zeiss epifluorescence microscope (Carl Zeiss, Zurich, Switzerland) with a 100× oil immersion lens ($NA = 1.3$). The samples were illuminated with a xenon arc lamp using a standard FITC filter set ($450 < \text{ex} < 490$, $\text{em} > 510$), intensified and recorded to a computer hard drive with a low light vidicon camera. For steady-state measurements, images were captured at 10 fps and the extension averaged over 10 frames for each electric field. For the relaxation measurements of longer DNA (>25 μm in length) images were captured at 5 fps and 3–4 full relaxation curves averaged for each molecule. For the relaxation of shorter molecules of DNA (<25 μm) images were captured at 10 fps to ensure that the faster relaxation dynamics were acquired.

RESULTS

Entanglement properties of HEC 250

The entanglement threshold of a polymer is the concentration, c^*, in solution where single chains begin to interact with one another. Because transient concentration fluctuations are always present in polymer solutions, there will not be a discrete transition from a dilute solution of polymers to an entangled solution, even in monodisperse systems. However, as the polydispersity of a polymer sample increases, the concentration range over which a system evolves from dilute to entangled will become broader. HEC samples are, in general, quite polydisperse and therefore will have a broad entanglement threshold. The weight average molecular weight of the HEC employed here (as characterized by the manufacturer) was 250,000 g/mol. Using membrane osmometry, we found the number average molecular weight to be 63,000 ± 3000 g/mol, indicating a polydispersity of ~4.

The entanglement threshold can be found from solution viscosity measurements by a number of different techniques. Often, the log of the specific viscosity is plotted as a function of the log of the concentration and the entanglement threshold designated as the point where the data deviate from linearity (Grossman and Soane, 1991). From the data in Fig. 1 we designated $c^* = 0.4$ wt % as the polymer overlap concentration. As a second estimate, we also calculated the entanglement threshold from the intrinsic viscosity of the polymer in TBE. We found the intrinsic viscosity of HEC 250 from a dual Huggins-Kraemer plot to be $[\eta] = 315 \pm 20$ ml/g. From geometric arguments it can be shown that $c^* = 2.5[\eta]^{-1} = 0.79$ wt % (Allcock et al., 2003). This quantity is approximately double our estimate from Fig. 1; the difference is probably due to the sample polydispersity and the approximate nature of the geometric arguments. We therefore used a concentration of 0.7 wt % HEC 250 in TBE as the solution to study the stretch and relaxation of DNA, a concentration above the entanglement threshold, but still in the semidilute regime and a concentration relevant to electrophoretic separations (Barron and Blanch, 1995).

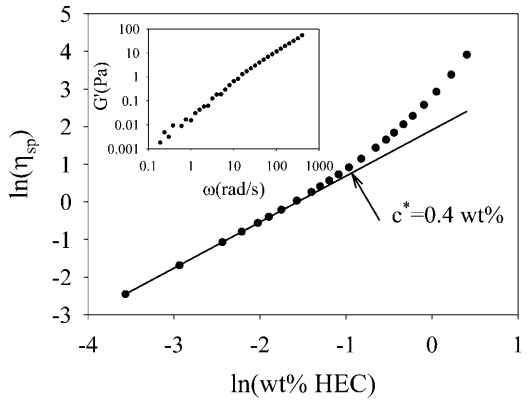

FIGURE 1 Log of the specific viscosity of HEC 250 versus log of the concentration in wt %. The point of deviation from linearity is the entanglement threshold concentration $c^* = 0.4$ wt %. (*Inset*) Dynamic rheology of a 4 wt % solution of HEC 250. The steady increase of the storage modulus with frequency indicates that no long-lived entanglements are present.

In the semidilute regime of polymer solutions, physical entanglements of polymer molecules are short-lived. During DNA electrophoretic separations in these matrices, topological constraints on the DNA are easily broken, so the degree of alignment of long DNA in the field is decreased compared to a covalent gel of the same pore size (Duke and Viovy, 1994). It has been postulated that constraint release will increase the separable size range of DNA and also eliminate DNA trapping—a phenomenon that can lead to decreased resolution. In semidilute solutions, the DNA molecule will not be tightly constrained to a reptation tube, but instead should have considerable conformational freedom. However, in the case of a solution comprised of short polymers, the pore size will be quite small at the entanglement threshold, so the hydrodynamic features should be comparable to what would be observed in a covalent gel (such as polyacrylamide or agarose) often used for DNA sequencing (Grossman and Soane, 1991).

To ensure that the HEC network did not form long-lived entanglements that would constrain DNA to a reptation tube, we performed dynamic rheology measurements on HEC 250 solutions at a concentration much higher than that used in our DNA stretching experiments. Linear viscoelastic theory predicts an entanglement plateau in a plot of the storage modulus versus frequency (Larson, 1999). Polymers that are too short or solutions that are too dilute will instead show a steadily increasing modulus with frequency. The inset of Fig. 1 indicates that even at a concentration of 4 wt. % (~6 times more concentrated than the solutions used in the DNA stretching experiments reported here), there are no long-lived HEC entanglements.

Steady stretching in a polymer solution

In free electrophoresis DNA behaves as a free-draining polymer because the long-range hydrodynamic perturbation caused by the molecule moving through the quiescent solution is cancelled out by the electro-osmosis of the electric double layer around the DNA. However, during gel electrophoresis when a DNA molecule interacts with a gel fiber, it is temporarily held stationary. In this scenario the hydrodynamic perturbation is removed and the electro-osmotic flow around the charged DNA backbone creates a long-ranged hydrodynamic interaction along the chain. We recently reported on the steady stretching of single tethered molecules of DNA in a gel-free electrophoresis buffer (Ferree and Blanch, 2003). It was observed that the hydrodynamic interaction results in tethered DNA stretching the same in an electric field, E, as it would stretch in a flow field with a velocity equal to μE (where μ is the free solution electrophoretic mobility). When the surrounding medium is replaced with a solution of uncharged polymers in electrophoresis buffer, much of this hydrodynamic interaction should be screened. The hydrodynamic interaction acts to lessen the force felt by a DNA molecule, so when it is screened by the polymers the molecule should stretch even further at a given electric field. We measured the extension of single DNA molecules tethered by one end to a chemically modified gold surface in a microfluidic device as a function of electric field from <1 V/cm up to 25 V/cm. Since DNA has been shown to behave as a worm-like chain (Bustamante et al., 1994; Marko and Siggia, 1995), which approaches its contour length with an inverse square-root dependence on force, the contour length of each molecule was found as the y intercept of a plot of extension versus $E^{-0.5}$. Fig. 2 is a plot of the extension of several molecules as a function of the electric field in both free solution and in 0.7% HEC. In the solution of HEC 250, the DNA extension is seen to increase above the free solution value as a result of hydrodynamic screening.

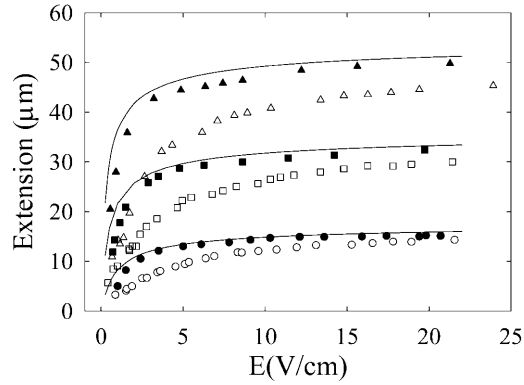

FIGURE 2 Extension of single molecules of fluorescent λ-DNA monomers (●,○), dimers (■,□), and trimers (▲,△) as a function of electric field. Solid symbols are in 0.7 wt % HEC and open symbols are in free solution. The solid lines are the theoretical prediction of the Stigter theory for stretching in a uniformly porous medium using a hydrodynamic screening length of 21 nm found from the effective medium theory.

For a tethered, completely free-draining polyelectrolyte (when no hydrodynamic interaction is present), the electrophoretic force on a segment of the chain will be independent of that segment's location in the chain. The tension at any segment will then be proportional to the length of chain downstream from the segment. From this argument it follows that $x/L \sim EL^1$, where x is the extension of a molecule length L in an electric field E. A plot of the fractional extension for five different chains as a function of the electric field multiplied by the contour length is shown in Fig. 3. The collapse of all of the data to one universal curve shows that the HEC solution used here does screen out nearly all of the hydrodynamic interaction along the DNA backbone.

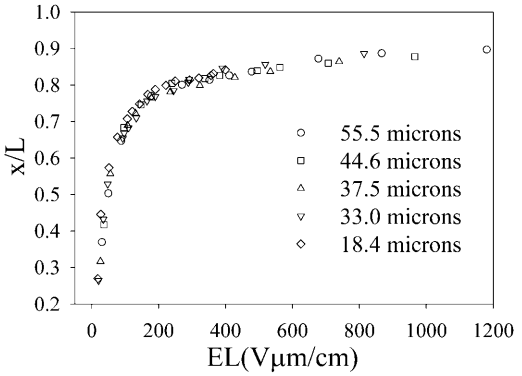

FIGURE 3 The fractional extension of five DNA molecules of different lengths in 0.7 wt % HEC plotted vs. *EL*. The collapse of the data onto one curve indicates that the hydrodynamic interaction has been screened by the polymer solution.

Relaxation in a polymer solution

To measure the relaxation dynamics of single molecules of DNA in a polymer solution, we first applied a strong electric field (~25 V/cm) to a sample of tethered DNA, then suddenly switched off the field and followed the relaxation of single molecules until they reached an equilibrium size. A time lapse of the relaxation of a 51.5-μm-long DNA molecule is shown in Fig. 4. The relaxation dynamics depend on the molecular conformation, so data from three or four separate relaxation experiments were averaged. Fig. 5 is a plot of extension versus time for several molecules of different lengths in a solution of 0.7% HEC 250. By fitting a universal curve to the data in Fig. 3, we were able to estimate the contour length of each molecule from the extension before turning off the electric field.

The inset of Fig. 5 shows the decay of the fractional extension with time for DNA molecules of different lengths. It is obvious from this plot that the relaxation normal modes decay much more slowly for longer molecules than for shorter molecules. The Rouse model predicts that the relaxation normal modes of a polymer chain near equilibrium in a θ-solvent should scale with the square of the chain

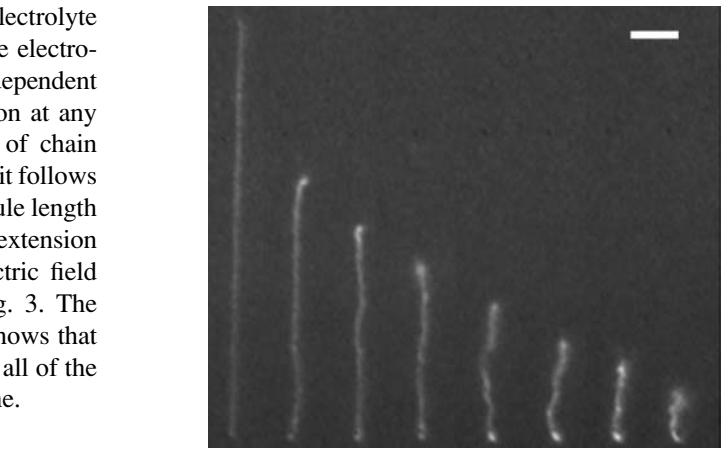

FIGURE 4 A time-lapse sequence of the relaxation of a single tethered 51.5-μm-long molecule of DNA at 1.5-s intervals in a 0.7% solution of HEC 250. The scale bar is 5 μm.

length. We analyzed the relaxation data by two model independent methods: data collapse and multiexponential fit. The data collapse method has proven effective in previous studies of relaxation of DNA in free solution and relies on the different data sets having similar shapes (i.e., same functional form with a similar number of relaxation modes; Perkins et al., 1994). With this technique, one data set (the template chain) is chosen and rescaled so that the data collapses on to the same curve as a test chain of different length. Both the time and extension data are multiplied by constant factors and a baseline offset is added to the extension. The optimum values for these three parameters are found by a least-squares minimization. The change in the time rescaling parameter, λ_t, with the length of the test chain

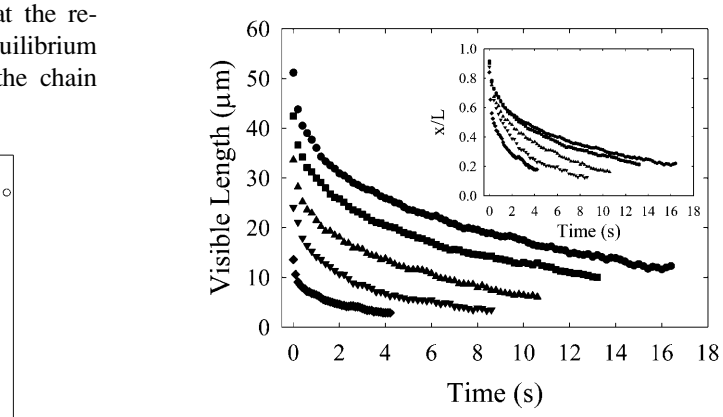

FIGURE 5 Relaxation data for 16.2- (♦), 27.5- (▼), 37.5- (▲), 46.8- (■), and 55.9- (●) μm-long DNA molecules in a 0.7% solution of HEC 250 where each plot is the average of three or four different experiments on the same molecule. For comparison, a 50-μm-long molecule in free solution relaxed from a fractional extension of 0.9–0.1 in 10 s (data not shown for clarity). (*Inset*) The relaxation of the fractional extension for the same molecules shows the strong dependence of the relaxation time constants on DNA length.

will reflect the scaling of the normal relaxation mode. In Fig. 6 *a*, the data for relaxation of a 37.5-μm template chain is rescaled to fit data for 16.2- and 46.8-μm test chains. A plot of log(λ_t) versus log(*test chain length*) should lie on a line with slope equal to the dynamical scaling constant as shown in the inset of Fig. 6. We repeated this technique using three template chains of different lengths: 16.2, 37.5, and 55.9 μm. We found the average of the dynamical scaling exponent for relaxation from these three calculations to be 1.93 $\pm$ 0.20.

To corroborate our findings by the data collapse method, we also analyzed the second moment of the relaxation data by fitting the data of length squared versus time to a sum of exponentials using the FORTRAN program DISCRETE (Provencher, 1976). Since the bead-spring models were developed to describe polymer dynamics around an equilibrium conformation, it is expected that the slowest relaxation times, τ_R, would scale most closely with the Rouse model. Two-thirds of our data were described best by a sum of just two exponentials (plus a baseline offset), whereas the other third were split evenly between one and three exponentials. By performing a least-squares linear regression on data of log(*length*) versus log(τ_R), we found the dynamical scaling constant to be 2.17 $\pm$ 0.17 (Fig. 7).

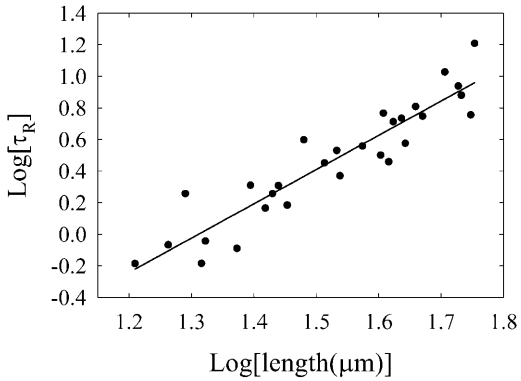

FIGURE 7 The log of the slowest mode time constant found from a multiexponential fit of the second moment of relaxation is plotted as a function of the log of the chain length giving a dynamical scaling constant of 2.17 $\pm$ 0.17.

DISCUSSION

Scaling theories have proven very useful in describing polymer systems, but for many applications where quantitative predictions are needed, more detailed theories have been developed. Here we apply the theory of Stigter (Stigter, 2000; Stigter and Bustamante, 1998) to describe the steady stretching of DNA in a polymer gel. We have shown previously that this theory correctly predicts the electrophoretic stretching of tethered DNA in free solution (Ferree and Blanch, 2003). The original articles provide a full description, so here we will only point out the most important aspects. The DNA is modeled as a chain of freely jointed prolate ellipsoids equal in size to the Kuhn length of DNA. The bare electrophoretic force on each segment is calculated by assuming that it will equal the friction a segment would feel if moving through the fluid at its electrophoretic velocity. The hydrodynamic interaction is explicitly included by calculating the flow field around each segment based on its orientation to the field using low Reynolds number transport theory. However, when a polymer network is present, the hydrodynamic interaction between segments will be screened. To account for screening, the network is modeled as a uniformly porous medium comprised of stationary beads at a concentration c, with each having a friction factor f_b. When fluid flows through this medium at a velocity $\mathbf{u}$, the beads exert a volume force on the fluid equal to $-cf_b\mathbf{u}$. This volume force changes the creeping flow form of the Navier-Stokes equation to

$$\nabla^2\mathbf{u} + \frac{cf_b}{\eta}\mathbf{u} + \frac{1}{\eta}\nabla p = 0, \tag{6}$$

where η is the fluid viscosity and p is the pressure. Equation 6 is solved along with the continuity equation for an incompressible fluid by assuming that the prefactor of the second term is equal to $1/S^2$, where S is the hydrodynamic screening length. This calculation results in new equations for the friction factors of each DNA segment, as well as for the hydrodynamic perturbation around each segment. With the fluid perturbation around each segment known, the total force is equal to the bare electrophoretic force plus the hydrodynamic interaction with all other segments in the chain. The tension at each segment is then used to find a new orientation with respect to the electric field from a worm-like chain entropic elasticity formula. The calculation then begins

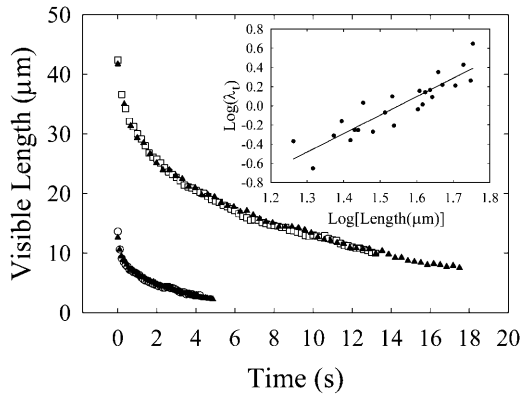

FIGURE 6 The collapse of the relaxation data in a 0.7% solution of HEC 250 for a 37.5-μm template molecule (▲) onto 16.2- (○) and 46.8- (□) μm test molecules. Both the length and time coordinates are multiplied by constant factors and a baseline offset is added. (*Inset*) The dynamical scaling of the relaxation with chain length is found by plotting the log of the rescaling parameter, λ_t, versus the log of the chain length. The dynamical scaling constant is found to be 1.93 $\pm$ 0.20.

again—finding the tension at each segment based on the new chain configuration—and continues until self-consistency is found. The only unknown parameter in the theory is the screening length.

The screening length could be used as an adjustable parameter, but we would prefer to use Stigter's model to predict the behavior of DNA in polymer solutions. We can derive a value for the hydrodynamic screening length of the polymer solution using the effective medium theory developed by Freed, Edwards, and Muthukumar (Edwards and Freed, 1974; Edwards and Muthukumar, 1984; Freed and Edwards, 1974). The fluid is assumed to obey the Navier-Stokes equation under the assumptions of incompressibility and creeping flow. The polymer is described by a Rouse-like bead-spring model and its motion is explicitly coupled with the motion of the fluid. At low frequencies it is found that

$$S = \frac{2}{\pi c_m b^2}, \tag{7}$$

where c_m is the concentration of monomers in solution and b is the segment length. For our sample of HEC 250 at a concentration of 0.7 wt % (monomer molecular weight = 250 g/mol and segment length 0.425 nm; Bandrup and Immergut, 1989), we calculate S to be 21 nm. In our calculations we set the persistence length of DNA equal to the generally accepted value of 50 nm (Zimm, 1998), the hydrodynamic radius equal to 2.28 nm (Fernandes et al., 2002), and the ζ-potential equal to 0.079 V (Ferree and Blanch, 2003). The result of the Stigter theory with these values is shown as the solid lines in Fig. 2. Although there is no direct relationship between the hydrodynamic screening lengths in the two models, the fit of theory to experiment is very good without the use of adjustable parameters.

As described in the Introduction, the Rouse model predicts that the normal modes for relaxation of a free-draining polymer will scale with $N^{1+2\nu}$ where ν is 1/2 in a θ-solvent and ~3/5 in a good solvent. Kantor et al. (1999) used fluorescence microscopy to follow the relaxation of single molecules of yeast chromosomal DNA stretched by an alternating electric field in the interface between an agarose gel and a glass coverslip, a technique they termed optical contour maximization. A log-log plot of τ_R versus length for five different molecules resulted in a scaling exponent of 1.45. There are several aspects of the work which are questionable. First, the DNA molecules studied were very long, and therefore extremely sensitive to shearing and breakage. However, the contour length of each DNA molecule was assumed to be equal to the length of the molecule as found from pulsed field gel electrophoresis. This method of determining the contour length is very inaccurate since many molecules will break during handling and this may be the main source of the large error in their data. Second, the environment in which the DNA molecules were stretched is inhomogeneous and uncharacterized. The authors state that the molecules are trapped in a region very near to the glass where the agarose concentration is low, explaining the Zimm-like scaling of the relaxation time. However, since the solvent in these experiments is good, the scaling exponent should actually be closer to 1.8, as was found by Perkins et al. (1994). Because the environment around the DNA molecules is not well characterized and the technique is highly irreproducible, optical contour maximization is not a reliable method for measuring the dynamics of single DNA molecules.

We have found the relaxation scaling time in a semidilute solution of HEC 250 to be 1.93 ± 0.20 using data collapse and 2.17 ± 0.17 using multiexponential fit—values significantly higher than found in free solution (1.7 ± 0.1; Perkins et al., 1994). Since the electrophoresis buffer used here is a good solvent for DNA, the expected value for the scaling exponent of the relaxation time is 2.2 for Rouse dynamics. This prediction is higher than our experimental measurements, although still within the experimental uncertainty for the multiexponential fit. Since the length scale for the hydrodynamic perturbation (the persistence length, ~50 nm) is of the same order of magnitude as that for the hydrodynamic screening length, hydrodynamic interactions between segments are not entirely screened and will lead to a decrease in the scaling exponent. This interaction could explain the difference between the scaling prediction and our results. It has also been proposed that the dynamical scaling constants in a good solvent may approach the theoretical value slowly as the polymer length increases (Weill and Des Cloizeaux, 1979). Our experiments involve DNA molecules ranging from ~300 to 1100 persistence lengths, which are short compared to many synthetic polymers, and may also lead to a decrease in the scaling exponent.

CONCLUSIONS

The data presented here provide the first quantitative description of the electrophoretic stretch and relaxation of single molecules of DNA in a semidilute polymer solution. Because the uncharged polymers in solution screen the hydrodynamic interaction along the DNA backbone, the force felt by the molecule is higher than in free solution, causing them to stretch more. The Stigter theory for electrophoretic stretch in a uniformly porous media provides a good description of the steady stretching without adjustable parameters when the hydrodynamic screening length from the effective medium theory is used.

The dynamic relaxation of ~30 separate molecules ranging in length from 300 to 1100 persistence lengths was measured. Because the HEC samples studied are too short to form long-lived reptation tubes, the DNA can be described by the bead-spring models of Rouse and Zimm. The relaxation time of the slowest mode, τ_R, was found to scale with length as $\tau_R \sim L^{1.93\pm0.20}$ using data collapse and

$\tau_R \sim L^{2.17\pm0.17}$ using multiexponential fit. These values agree well with the Rouse model prediction.

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