Electrohydrodynamic Stretching of DNA in Confined Environments

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(Received 25 August 1997)

The effect of confinement on the dynamics of polymers was studied by observing the transient extension and relaxation of single DNA molecules as they interacted with obstacles in a specially designed thin slit. Viscous drag was found to increase with the degree of confinement, which we interpret in terms of hydrodynamic screening by the planar surfaces of the slit. Since the DNA was driven by an electrophoretic force, the experimental data support the notion that an electric field acts on a tethered polyelectrolyte equivalently to a hydrodynamic flow. [S0031-9007(98)05566-5]

PACS numbers: 87.15.He, 82.45.+z, 83.10.Nn, 83.20.Di

The dynamics of polymers in confined environments is a fundamental problem of considerable technological importance. A sound understanding of their behavior would be advantageous in numerous practical situations, including the extraction of oil from porous media and the separation of biological macromolecules by electrophoresis. One needs to comprehend both the Brownian motion of the polymer molecules and their response to forces applied either locally or globally via a hydrodynamic flow. Recent direct observations of single DNA molecules elongated in a moving fluid [1–4] have provided us with a deeper understanding of the static and dynamic properties of single polymer chains. They have also promoted the development of new theoretical models that describe how tethered polymers get stretched by a hydrodynamic flow [5–7]. These previous experiments were concerned with molecules in free solution, and have not addressed the effect of confinement. In this Letter we present direct evidence that the dynamics is altered when a polymer is confined in a thin slit between planar surfaces, and show that this is a consequence of the screening of hydrodynamic interactions between distant parts of the molecule by the walls of the slit. By using electrophoresis to force the molecules to move, rather than a laminar flow, we also test the proposition of Long *et al.* [8] that an electric force acts on a tethered polyelectrolyte in the same way as a hydrodynamic flow.

In order to conduct a carefully controlled study, we used specially constructed microfabricated chambers (Fig. 1). Widely spaced rows of cylindrical posts were etched from a silicon wafer by optical lithography. The posts had uniform height, which could be varied over the range 0.1–10 μm, and the resulting structure was sealed with a glass cover slip to make a thin slit. The posts had two functions. First, they acted as spacers between the walls, ensuring that the slit depth was known with precision. Second, they provided obstacles to the motion of DNA molecules introduced into the chamber. Fluorescently stained DNA was propelled through the chamber by an electric field, directed orthogonally to the rows of posts, and observed by optical video microscopy. Individual molecules which impinged on a post would get hooked on it and stretch out into a U-shaped conformation, like a ribbon caught on a lamp post in the wind. In a matter of seconds, these molecules would slither around the obstacle and free themselves. Once disengaged, they would immediately start to contract, gradually relaxing towards their equilibrium, randomly coiled state. This transient extension and relaxation of individual molecules was the subject of our study. We examined how the degree of elongation and the time scale of relaxation were affected by the depth of the slit.

Considering that hydrodynamics at low Reynolds numbers is governed by long-range velocity profiles, it is expected that a thin slit geometry has a significant effect on the dynamics of a long polymer molecule [9]. Nonslip boundary conditions at the walls cause the screening of hydrodynamic interactions at scales longer than the slit depth. Thus the polymer, which is opaque to the flow in an unbounded fluid, becomes completely free draining

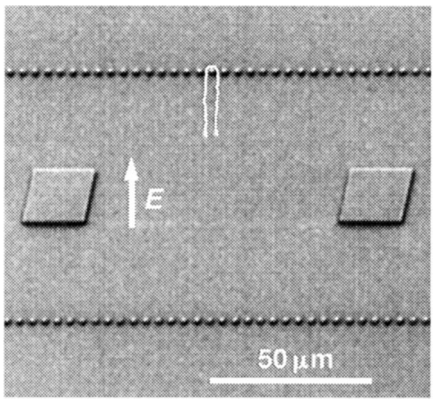

FIG. 1. Scanning electron micrograph of the microfabricated obstacle array used in the experiments. A cartoon DNA molecule is shown hooked over a post in a symmetric U shape—the configuration in which extension was measured.

(i.e., the flow penetrates the coil) when the slit depth approaches the persistence length. The primary motivation for our study was to quantify this effect. A second was to test theoretical concepts about the simultaneous application of electric and nonelectric forces to polyelectrolytes [8], which we summarize here. When a charged DNA molecule migrates freely under the influence of an electric field E, the electrostatic forces acting on the molecule and on the surrounding cloud of counterions combine in such a way that hydrodynamic interactions are screened on a scale longer than the Debye length [10]. As a consequence, DNA molecules of different lengths migrate at a unique velocity, $v = \mu E$, where μ is the electrophoretic mobility. However, if a contact force is applied locally to the DNA alone, without acting on the counterions, an effect is transmitted to the rest of the molecule via a hydrodynamic interaction. This has some surprising consequences. For instance, a uniformly charged polyelectrolyte held immobile by one end in an electric field extends as though it were in a hydrodynamic flow of uniform velocity $v = \mu E$, and not, as one might naively suppose [11], as if it experiences a constant electrostatic force per unit length. Our experiment in a thin slit permits a clear distinction between these two situations. If, at a given field strength, the extension of a hooked DNA molecule depends on the slit depth, then the hydrodynamic interaction must be present and the proposition of Long *et al.* is corroborated [12].

The thin slits were fabricated using standard optical lithography techniques. Cylindrical posts, with diameter of 2 μm and a gap of 2 μm between them, were disposed in rows at 90 μm intervals. Large square support columns, of side 20 μm, were included in the design of the chamber in order to prevent the structure from collapsing. Three different arrays were etched, with depths 0.09, 0.3, and 5.0 μm, and sealed with glass coverslips using a spun-on optical adhesive (Norland 81). T4 DNA molecules (167 000 base pairs) were chosen for our experiments, since they are commercially available molecules of a uniform size that is suitable for imaging [13]. The DNA was stained with 5 μM TOTO-1 dye in a 1/2 Tris-borate-EDTA (TBE) buffer containing 2% mercaptoethanol. At the concentration of DNA used, the DNA molecules were saturated with dye molecules. After the solution penetrated into an array, the DNA was imaged using epifluorescence techniques. An electric field, which varied in magnitude from 1 to 50 V/cm, was applied in the direction perpendicular to the rows. Elongation and relaxation of single DNA molecules interacting with the obstacles were observed with a 60× oil-immersion objective ($NA = 1.4$) and recorded using a silicon intensified target camera (SIT, Hamamatsu 2400).

In order to obtain reproducible measurements, elongation data were collected using only those molecules which happened to form symmetric U-shaped configurations when they got hooked on a post (see Fig. 1). The end-to-end length was accurately measured by following the polymer contour, using an ARGUS-10 image processor. The electrophoretic flow velocity was determined by tracking undeformed molecules in the obstacle-free region. Figure 2 shows the dependence of the molecular extension on the migration velocity in the three different slits. A clear effect of confinement is apparent: At the same velocity (i.e., at the same electric field strength), the extension is greater the shallower the slit.

A molecule which is hooked symmetrically over a post is momentarily stationary and thus at equilibrium in the electrohydrodynamic flow. Accordingly, it should be stretched as though it were held immobile at its midpoint in a hydrodynamic flow $v = \mu E$ [8]. The degree of extension is determined by a balance of the elastic restoring force and the hydrodynamic drag at all points along the chain. The former is now well characterized: the wormlike chain model has been shown to describe the entropic elasticity of DNA with precision [6]. When a molecule of length L and persistence length A is uniformly extended, so that its end-to-end separation is z, the tension F in the chain is

$$\frac{FA}{kT} = \begin{cases} \frac{3}{2}\frac{z}{L}, & z \ll L, \\ \frac{1}{4}(1 - \frac{z}{L})^{-2}, & L - z \ll L. \end{cases} \quad (1)$$

The viscous drag is more complicated to evaluate, since the long-range hydrodynamic interactions between different parts of the molecule mean that the overall hydrodynamic drag force depends on the detailed molecular conformation. Moreover, in a narrow slit the drag is modified by the walls, which screen these hydrodynamic

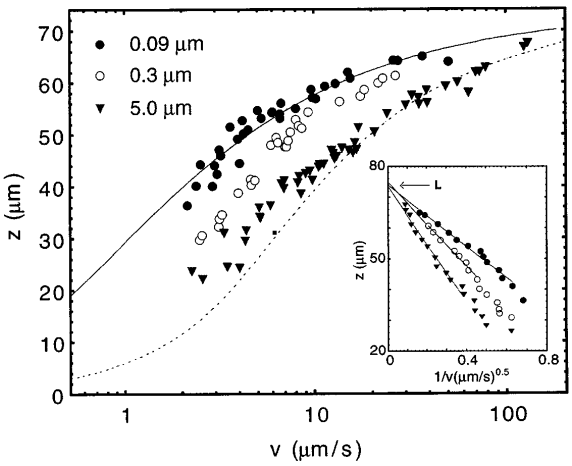

FIG. 2. Extension as a function of flow velocity in three slits of different depth. The error in the data is approximately 10% in v and ± 1 μm in z (due to limited screen resolution and the short depth of field of the objective, which affected focusing in the deeper arrays). The curves are the theoretical prediction of the extension in an unbounded fluid (dotted line), and the scaling function for the case where hydrodynamic interactions are completely screened (solid line). The inset shows how the extended length scales as $(1/v)^{1/2}$ as predicted by Eqs. (1) and (2).

interactions. To understand these effects, we shall start with the crudest approximation and then proceed to more sophisticated models.

Consider first the case of a DNA molecule, held stationary by its end, in a moving fluid *with no boundaries.* The viscous drag can readily be calculated in the two limiting cases of weak and strong extension. In the former situation, the molecule is hardly deformed from a random coil, and its friction coefficient is given by the Zimm model [14]. In the latter case, the DNA is stretched almost to its full length L and makes lateral fluctuations of size $D \sim (\frac{kT}{\eta v})^{1/2}$, and so may be modeled as a cylinder of length L and diameter D. Hence the overall friction coefficient ζ is [14]

$$\zeta = \begin{cases} \frac{(3\pi)^{3/2}}{4}\eta(LA)^{1/2}, & z \ll L, \\ \frac{2\pi\eta L}{\ln(L/D)}, & L - z \ll L, \end{cases} \qquad (2)$$

where η is the viscosity of the solvent. The difference in scaling of ζ with L at high and low flow rates means that there is no universal scaling of the extension as a function of the molecular size. Equations (1) and (2), however, indicate that in the strong stretching limit, $L - z \propto v^{-1/2}$. This dependence is borne out by the three sets of data (inset of Fig. 2), with a constant of proportionality that depends on the etch depth (to be discussed later). Extrapolation of each curve to $z = 0$ permits an evaluation of the molecular contour length L. The value $L = 74 \pm 1$ μm obtained is 30% longer than an unstained T4 DNA molecule. Similar figures for the elongation of the DNA backbone by intercalating fluorescent dyes have been reported previously [1,3].

To understand the behavior at intermediate extensions, a more detailed model is required, in which the tension and drag are balanced locally at all points on the chain. Brochard-Wyart suggested that a tethered polymer is best pictured as a "stem and flower" [5]. The tension has a maximum value at the tethered end and decreases to zero at the free end; as a result, the polymer appears as a highly stretched "stem," surmounted by a "flower" of increasingly disordered random coils. The flower may be modeled as a sequence of Pincus blobs [15]—each of which has a Zimm-like drag—which increase in size until the terminal blob of diameter $D \sim (\frac{kT}{\eta v})^{1/2}$. We have developed a more accurate version of the stem and flower model which incorporates wormlike chain elasticity and hydrodynamic interactions between blobs [16]. Numerical evaluation of the model, which contains no adjustable parameters, gives results that are in good quantitative agreement with both the static and dynamic data of Perkins *et al.* [2,3]. The extension curve predicted for T4 DNA ($L = 74$ μm), held by its midpoint in an unbounded fluid, is plotted in Fig. 2 [17]. Note that all of the data lie above this curve: the DNA extends more readily in a thin slit than in free solution.

Why does confinement produce this effect? In a narrow slit, the hydrodynamic interactions are diminished at distances r greater than the slit depth h (the Oseen tensor is proportional to h/r^2 rather than $1/r$). As confinement increases, three classes of modified hydrodynamics may be distinguished. (1) First, when $h < L$, the long-range interactions between the flower and the stem, and those between blobs in the flower, are screened. Effectively, the stretched polymer has the drag of a cylinder confined between parallel walls. This modifies the term in the denominator of Eq. (2), changing it from $\ln(L/D)$ to $\ln(4h/\pi D)$, and the friction is augmented. (2) When $h < D$, interactions within individual blobs in the flower are also reduced, the structure of the flower is modified, and the drag increases further. (3) Finally, when $h < A$, the motion of each statistical segment of the polymer is hydrodynamically decoupled from that of the others. In this case, the DNA is effectively free draining, and the molecule has a uniform friction per unit length $\xi = c\eta$, where c is a numerical constant that depends on the slit depth. This means that the total drag force is independent of the molecular conformation, and the extension, in this case, *does* obey a scaling relation of the form $z/L \sim g(\eta v LA/kT)$. The total screening of hydrodynamic interactions means that each blob has a Rouse-like friction [14], which leads to a more evenly stretched chain with a smaller flower than occurs in an unbounded fluid: the terminal blob has size $D \sim (\frac{kTA}{\eta v})^{1/3}$.

The three data sets at decreasing etch depth fall roughly into each of these three classes. In the thinnest slit, hydrodynamic screening is almost complete, and the free-draining scaling function fits the data when the numerical constant c is treated as an adjustable parameter. A theoretical value of this constant can be estimated by assuming that, when the slit depth is of order the persistence length, the DNA is confined in the two-dimensional plane midway between the walls. The friction coefficient per unit length is then $\xi = 2\pi\eta/\ln(4h/\pi d)$, where $d = 2.5$ nm is the diameter of the DNA molecule. This yields the value $c = 1.6$ when $h = 0.09$ μm, in fair agreement with the value $c = 1.4$ used to fit the data [18].

Confirmation that the varying extension in the three different slits is due to differing viscous drag was obtained by examining the dynamics of the DNA molecules. Visually, it was obvious that the thermal motion was least agitated in the shallowest slit, but to obtain quantitative data we followed the entropic relaxation of stretched molecules (Fig. 3). Different field strengths were applied in the three arrays to ensure the same initial degree of extension of a hooked molecule. Then the contraction of individual DNA molecules, immediately subsequent to their detachment from an obstacle, was observed. Figure 4 clearly shows that the relaxation rate decreases as the arrays get shallower: There is a factor of 2 difference between the 5 μm and the 0.3 μm slit, and another factor of 2 between the 0.3 μm and the 0.09 μm

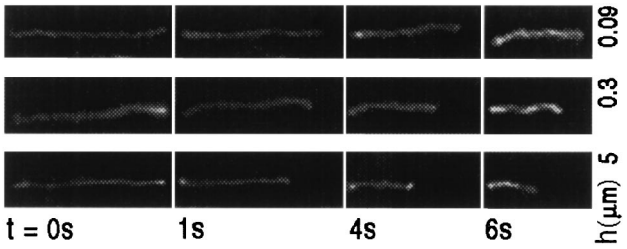

FIG. 3. Video captures of a T4 DNA molecule relaxing in three slits of different depth (the molecule is migrating from left to right). The Brownian dynamics is clearly slower in thinner slits. Higher monomer density in the "flower" is apparent as a luminous blob at the chain end, but note that this is less visible in the shallow array (see text).

slit. This is consistent with the ratios of the viscous drags, as evidenced by the relative magnitudes of electrophoretic drift velocity required to produce a given extension in the three arrays (see Fig. 2).

The ability to vary the dynamical time scales of a polymer by altering the degree of confinement could prove to be useful. Various strategies have been proposed for the electrophoretic separation of DNA molecules in microfabricated structures [19]; some work better when relaxation is rapid, others require the DNA molecules to remain stretched for long periods. For example, in methods based on collisions with obstacles [20,21], fast relaxation ensures that molecules quickly regain randomly coiled configurations, thereby providing a maximal collision cross section which improves separation. By contrast, pulsed-field electrophoresis techniques rely on the molecules being maintained in an almost fully stretched state [22], and so slow relaxation is favorable for the process of fractionation. We have demonstrated that the etch depth of the microfabricated array is a useful control parameter by which the Brownian relaxation rate may be adjusted, without altering the electrophoretic migration velocity, to enhance the separation.

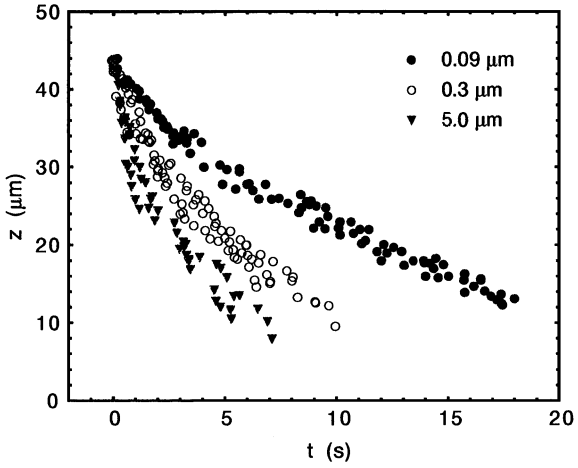

FIG. 4. Relaxation of the extension of a molecule, subsequent to disengagement from a post (at time $t = 0$) in the three slits of different depth. Data were collected from three different molecules in each array. All molecules were initially stretched by the same amount ($z = 44$ μm).

We thank P. Cluzel for useful discussions, A. Ajdari for critical comments, S. Turner for help in microfabrication, and T. T. Perkins for providing us with the experimental data of Refs. [2] and [3]. We are especially grateful for the initial contribution of T. Strick who began this work as his senior thesis at Princeton University. This work was funded by NIH Grant No. GM55453-01, the Royal Society, and BBSRC Grant No. E08580.

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