Entropic Trapping and Escape of Long DNA Molecules at Submicron Size Constriction

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We studied the passage of DNA molecules, driven by an electric field, through a microfabricated channel with 90 nm size constrictions. DNA molecules were entropically trapped at the constriction and escaped with a characteristic lifetime. Counterintuitively, longer DNA were found to escape entropic traps faster than shorter ones. DNA molecules overcome the entropic barrier by stretching their monomers into the constriction, which results in the fact that the energy barrier for DNA escape is independent of the chain length.

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Recently, the concept of entropic trapping was introduced as a new dynamic regime in gel electrophoresis [1]. A long polymer molecule can be trapped entropically in a random restrictive environment such as a gel, and this effect becomes important when the dimension of pores in a retarding matrix is comparable to the radius of gyration (R_0) of the polymer. So far, entropic trapping has been demonstrated by computer simulation [2] and experiment in gel [3]. However, the lack of information on the structure of gel has hindered researchers from obtaining detailed microscopic understanding of the effect. Volkmuth and Austin [4] suggested the use of an artificial structure as a substitute for gel in electrophoresis. Aside from the application to polymer separation [5,6], microfabricated structures provide an ideal environment for studying these polymer dynamics problems, because one can easily control the dimension of obstacles or retarding matrix.

The object of this Letter is to characterize the motion of long DNA polymer in an artificial channel with entropic traps. As a model pore-constriction system, we designed a channel with regions of two different depths. Figure 1(a) shows a schematic diagram of the device. The thick regions of the channel are as deep as 1 μm, comparable to the R_0 of the double stranded DNA molecules we used in this experiment. However the depth of the thin region is 90 nm, which is much smaller than R_0. These thin and thick regions alternate along the channel, and DNA molecules in thick regions are entropically hindered from entering thin regions. Therefore at low electric fields DNA molecules are trapped at the entrance of the thin region and are unable to overcome the trapping barrier. We designed four different channels with different spatial periods of structure (4, 10, 20, and 40 μm). One period is divided by equal lengths of a thick and a thin region, which ensures that the fluid (electrical) resistance of the channel, as well as E_s and E_l (the electric field in the thin and thick region, respectively), are the same in all four channels. DNA molecules are free to relax while they are traveling in thick regions. By changing the period we can vary the time for DNA to relax before it meets another constriction.

The channel was fabricated by standard photolithography techniques on Si substrate, and the front surface was anodically bonded to a Pyrex coverslip. The bonded channels were filled with a buffer solution containing DNA molecules. To visualize DNA motion, we labeled the DNA with a fluorescent dye (YOYO-1 from Molecular Probes). As a buffer solution we used tris-borate-EDTA (TBE) with 2% to 4% of β-mercaptoethanol [7]. DNA molecules were observed on an optical microscope with a 100× oil immersion objective lens (N.A. = 1.3) and standard filter sets for fluorescence detection. DNA motion was recorded by an intensified charge-coupled device camera into video format.

The motion of DNA through the channel is affected by the existence of entropic traps. Whenever a DNA molecule reaches an entropic trap, it is trapped for a certain

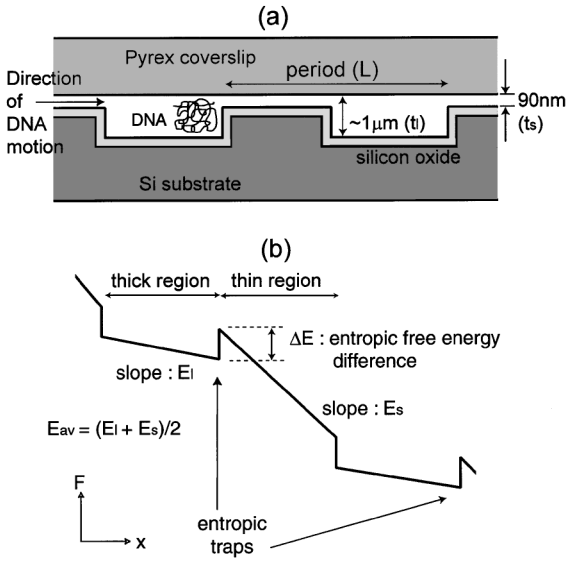

FIG. 1. (a) Schematic diagram of the device. (b) Free energy landscape for DNA in the channel. ΔE is the entropic free energy difference between a DNA molecule in a thick region and a thin region. E_s and E_l are the electric field at thin and thick regions, respectively. E_{av} is the average electric field over the channel.

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trapping lifetime τ before it escapes the trap. Including the entropic trapping effect, the mobility (μ) of DNA could be written as

$$\frac{\mu}{\mu_0} = \frac{t_{\text{travel}}}{t_{\text{travel}} + \tau}, \tag{1}$$

where τ is the trapping lifetime, $t_{\text{travel}} = L/\mu_0 E_{\text{av}}$ (see Fig. 1 for a legend) is the transit time between two adjacent entropic traps, and μ_0 is the conformation-independent (so-called "free draining") mobility of DNA. We obtained the mobility of DNA (μ) by measuring the average velocity over many periods of the channel at various electric fields. The value of μ_0 was extracted from the high electric field plateau of the mobility curve, where entropic trapping is negligible ($\tau = 0$) and $\mu = \mu_0$. From measured μ and μ_0, averaged values of τ at each electric field were calculated. A more detailed description of the experimental conditions is available elsewhere [8].

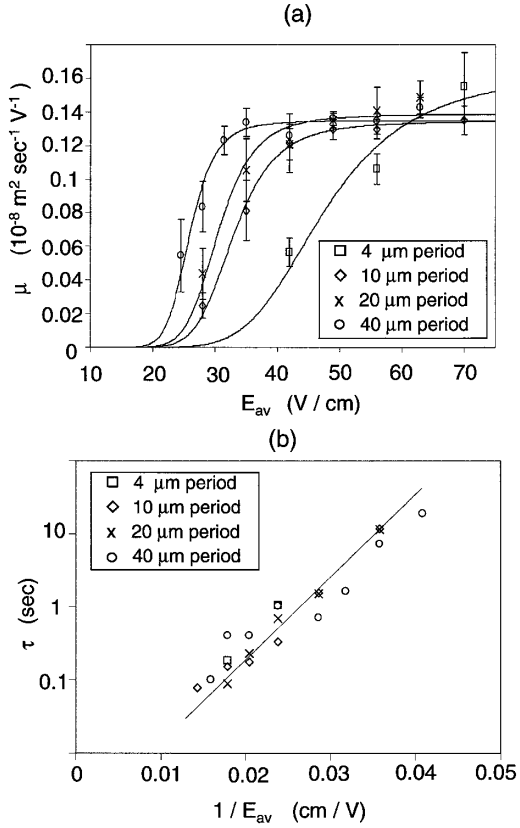

FIG. 2. (a) Mobility of λ DNA in the channel. $t_l = 1.4$ μm and $t_s = 90$ nm. To fit the mobility data to $\mu = \mu_0/[1 + \alpha_1 \exp(\alpha_2/E_{\text{av}})]$ [from Eqs. (1) and (2)], the α_2 value (same for all four curves) was extracted from the slope of (b). The fit for a 4 μm period channel seems to be a little different from others, probably because of a limited number of data points and the poor accuracy of measurement for these data. Other curves are showing nearly the same μ_0 value (μ at high field). (b) $\log(\tau)$ vs $1/E_{\text{av}}$ (τ in seconds), calculated from the mobility data. τ is a function of E_{av} because $E_s = \kappa E_{\text{av}}$, where κ is a constant.

The motion of a polymer chain through a narrow constriction by hydrodynamic flow was studied theoretically by Daoudi and Brochard [9]. They showed that there should be a critical fluid current density (J_c) to force a polymer into the constriction. They calculated J_c for several different geometries. For the case of a thin slit present on a flat surface, they showed that $J_c \sim N^{-3/5}$. Our channel is similar to this case (a slit on a flat wall), because the depth of the channel changes abruptly at the interface of the thin and thick region. Figure 2(a) is the measured mobility of λ phage DNA (48.5 kbp) in our channels. DNA mobility shows the transition behavior between trapping and flowing regimes, as described in their work. However, the transition between these two regimes is not abrupt. At intermediate field strength, DNA molecules are trapped with a characteristic lifetime before they escape to the next thick region [8]. This escaping motion was not considered in Daoudi and Brochard's work. Figure 3 is a sequence of video frame images, separated by 0.1 sec, showing the escape of a λ phage DNA molecule. DNA molecules formed nearly spherical blobs when they moved in the thick region or when they are trapped at entropic traps. Escape of a DNA molecule from an entropic trap is initiated by stretching of a small part of DNA into the thin region. Once this "beachhead" is formed the rest of the molecule becomes stretched and enters the thin region sequentially.

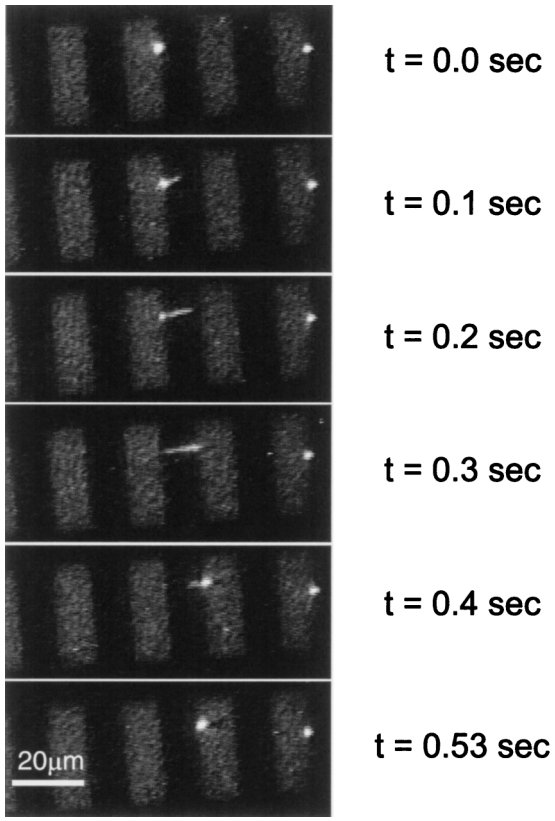

FIG. 3. Video images showing the escape of a λ phage DNA. $t_l = 1.4$ μm and $t_s = 90$ nm.

A simple kinetic model can be constructed by considering the free energy difference after a portion of DNA has been introduced into the thin region [see Fig. 4(a) for a legend]. The amount of DNA already in the thin region should be proportional to the length x, either fully stretched or forming a series of blobs [10]. The decrease of electrical potential energy due to the deformation should depend on x^2E_s, whereas the increase of entropic free energy is proportional to xT. Therefore, the total free energy difference due to the introduction of monomers by the length x is $\Delta F \sim xT - x^2E_s$, as shown in Fig. 4(b). The positive energy barrier $\Delta F_{\max}$ (for the transition state $x = x_c$) is the activation barrier for the escape of DNA molecules from the trap and its height is proportional to $1/E_s$ [11]. If $\Delta F_{\max} \ll k_BT$ (high E_s) DNA can readily escape the trap without being retarded. When $\Delta F_{\max} \gg k_BT$ (low E_s) DNA molecules are trapped completely and cannot escape the trap. For intermediate fields ($\Delta F_{\max} \sim k_BT$) DNA molecules have a finite probability per unit time to overcome the barrier. This probability should be proportional to the Boltzmann factor $\exp(\Delta F_{\max}/k_BT)$. Thus, the trapping lifetime can be written

$$\tau = \tau_0 \exp(\Delta F_{\max}/k_BT) = \tau_0 \exp(\alpha/E_s k_BT), \quad (2)$$

where α is a constant. It is important to note that we calculated the free energy difference between the trapped state and the transition state, not between the trapped state and the state when the entire DNA coil is in the thin region [ΔE in Fig. 1(b)]. The energy barrier for escape of DNA is not ΔE because DNA molecules overcome (and lower) the barrier by stretching themselves.

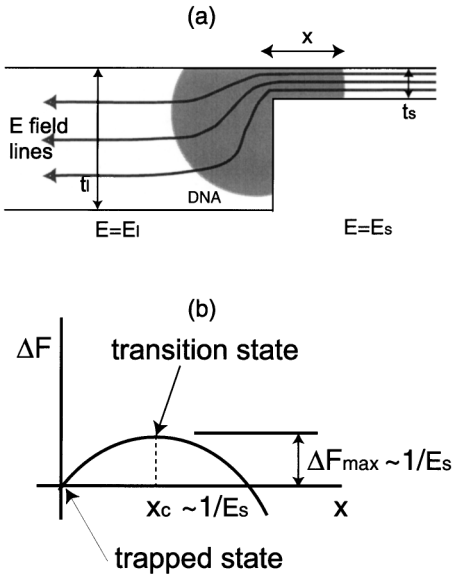

FIG. 4. (a) Escape of a DNA molecule from the trap. x is the length of DNA section in the thin region. (b) Plot of ΔF vs x. The transition state is when $x = x_c$.

Figure 2(b) is a logarithmic plot of the trapping lifetime of λ DNA against $1/E_{\rm av}$. A clear linear relationship between $\log(\tau)$ and $1/E_{\rm av}$ can be seen, which demonstrates the validity of our simple model. However, there is a slight difference between data from different channels. Data from a 40 μm period channel deviate more from the trend line than others. This suggests that the relaxation of DNA molecules also has an effect on the entropic trapping lifetime. However, a detailed model for this effect is not yet available.

To test the chain length dependence, we measured the mobility of two different DNA species (T7 and T2 DNA) in the channel. T2 DNA (164 kbp) is longer than T7 DNA (37.9 kbp) by 4.3 times. Two DNA species were mixed and dyed at the same dye-to-base pair (bp) ratio. Mobility of both molecules showed the transition behavior at nearly the same threshold electric field [see Fig. 5(a)]. At high electric field when almost no trapping is observed, T7 DNA mobility is slightly higher than that of T2, probably because of the hooking of longer molecules at defects in the channel, or other interactions with the walls. However, in the intermediate regime, mobility of the T2 DNA turned out to be higher than that of T7 DNA [8]. In other words, T2 DNA is trapped with a shorter trapping lifetime than T7 DNA. This can be seen in Fig. 5(b),

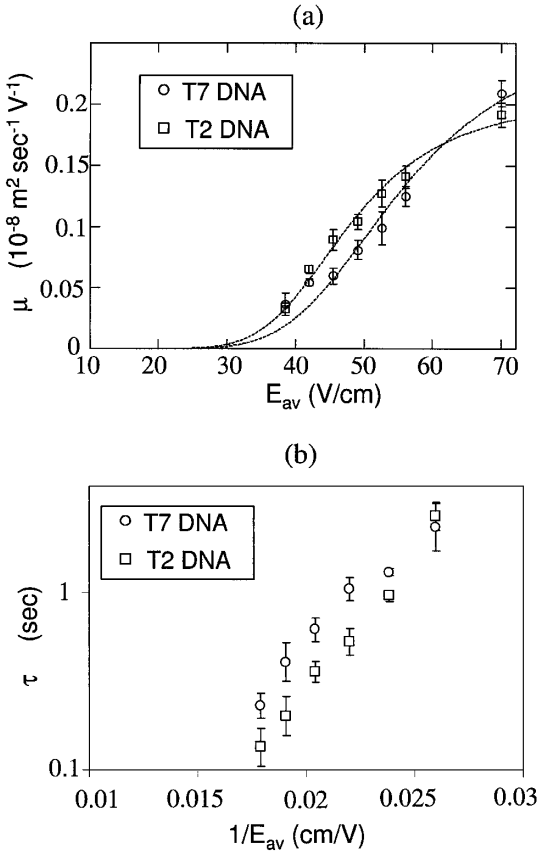

FIG. 5. (a) Mobility of T2 and T7 DNA in a 4 μm period channel with fitting curves. $t_l = 0.65$ μm and $t_s = 90$ nm. (b) Plot of $\log(\tau)$ vs $1/E_{\rm av}$, calculated from (a).

where we plotted $\log(\tau)$ as a function of $1/E_{\mathrm{av}}$. Another important point in Fig. 5(b) is that the slope in this graph for T2 and T7 DNA is essentially the same. The slope of this graph [$\sim\alpha/k_BT$ in Eq. (2)] is related to the energy barrier height for escape. This means that ΔF_{max} for both T2 and T7 DNA is the same, given the same electric field. Escape from a trap is initiated by the introduction of a small portion of DNA into the thin region—just enough to overcome the escaping activation barrier [x_c in Fig. 4(b)]. Only the monomers directly facing the thin slit can go into the thin region by thermal fluctuations. Therefore this initiation process is local in nature, and the energy barrier does not depend on the total length of the trapped DNA molecule. Once a DNA molecule is in the transition state (once a proper length of beachhead is formed), it readily escapes the entropic trap, regardless of the length of the remaining molecule in the trap.

This result is important for designing future "artificial gel" structures for polymer separation. In their theoretical paper, Ajdari and Prost [12] calculated the motion of DNA through a series of "dielectric traps" given by an ac electric field. The free energy landscape of their system is quite similar to ours [Fig. 1(b)], where the depth of the energy trap is proportional to $N^{3/2}$. However our result suggests that, contrary to their prediction, this does not necessarily mean that longer DNA is more strongly trapped. Just as in our channels DNA would not escape the trap as a point particle, and deformation of DNA would change the energy barrier.

The difference of mobility between T2 and T7 DNA comes not from the escaping activation energy barrier, but from the fact that the surface area of a DNA molecule facing the thin slit is different because of the difference in their size. Bigger molecules escape faster simply because more monomers are facing the thin slit, and are able to form a beachhead for escape. This difference affects the prefactor τ_0, not the exponential factor of Eq. (2). Then, the escaping probability of DNA would be proportional to the size of the DNA blob in the trap, which is roughly proportional to R_0. Therefore one can expect $\tau \sim 1/R_0$ dependence. From our data, the ratio between the trapping lifetime of T2 and T7 DNA is about $\sim$1.9 for a 4 μm period channel, and $\sim$1.7 for a 10 μm period channel. These ratios are rather small compared to predicted values assuming ideal chain ($\sim$2.08) or Flory chain ($\sim$2.41). Considering that R_0 is just a rough estimate for the size of DNA in the trap, this is not an unreasonable discrepancy. Also the monomer distribution for trapped DNA molecules is different from the equilibrium distribution because of the converging electric field and the wall of the channel which deforms the overall shape of DNA molecules.

Data for T7 DNA, after being trapped more than $\sim$1 sec, deviate from the linear behavior in Fig. 5(b). This is possibly because trapped DNA molecules have a transition to a quasiequilibrium state after being trapped for a time, in response to the existence of a wall and a converging electric field. This argument is backed by the fact that data for T2 DNA still conform to the linear behavior while T7 DNA data do not, probably because of the longer relaxation time of the larger molecule. However, further work is required to include monomer distribution and relaxation in our model.

In conclusion, we demonstrated entropic trapping of long DNA in a microfabricated structure, and established a model to describe the observed escape of DNA molecules from an entropic trap. It is important to recognize that DNA molecules can deform to reduce the energy barrier for escape. The activation barrier for the escape is independent of the chain length N, because deformation of DNA into the constriction is local and does not involve the whole molecule. This result could be applied directly to separate long polymer molecules, which is currently done only by pulsed field gel electrophoresis.

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