SINGLE MOLECULE DYNAMICS OF DNA DURING ELECTROKINETIC TRANSPORT THROUGH NANOFLUIDIC CHANNELS

L.D. Menard, J.S. Zhou, M.E. Woodson, C.E. Mair, J.P. Alarie and J.M. Ramsey*

University of North Carolina at Chapel Hill, USA

ABSTRACT

The electrokinetically-driven translocation of single DNA molecules through nanochannels in quartz devices was monitored using fluorescence microscopy. Observation of fast intramolecular relaxation dynamics was possible with the use of high intensity excitation and high-gain, high-speed detection. These dynamics are two orders of magnitude faster than observed in previous related studies. A study investigating the dependence of relaxation times on the driving voltage and DNA length suggested that the rapid relaxation was due to a highly strained initial conformation induced during the threading process. These findings are relevant to various high throughput applications in which measurements are made on translocating polynucleotides.

KEYWORDS: Nanochannels, DNA, Single-Molecule, Translocation

INTRODUCTION

Nanopores and nanochannels are being developed as components in devices for single-molecule analysis of biopolymers. Important examples include the sizing [1], restriction site mapping [2], and sequencing [3,4] of polynucleotides. These nanofluidic conduits serve several functions. First, insertion of a macromolecule into a channel or pore that is smaller than the molecule's hydrodynamic radius results in extension of the molecule, facilitating spatial mapping or characterizations. This region of extension may be smaller than the molecule's contour length in the case of a nanopore or it may confine the entire molecule in the case of a long nanochannel. Second, precise positioning of the biopolymer relative to other components integrated with or interfaced to the device (e.g., tunneling electrodes [4] or laser illumination [5]) can be achieved with the nanopore or nanochannel. Third, the nanopore or nanochannel acts as the locus at which the forces driving macromolecular translocation are applied. Thus, its geometry defines the force gradients and magnitudes and intrinsically affects translocation dynamics. This paper discusses these fundamental effects in the context of double-stranded DNA that is electrokinetically driven through nanochannels longer than the DNA's contour length.

THEORY

Much of the theoretical framework describing polymer confinement was well developed even before the fabrication of nanochannels became routine. These theories are important in understanding systems such as polymer melts and reptation through gels. Two relevant theories describing the extension of polymers confined in nanochannels are those of de Gennes [6] and Odijk [7]. These two theories are appropriate when the confining dimensions are greater than or less than the polymer's persistence length, respectively. The persistence length is a measure of the chain stiffness. In the de Gennes model, the polymer can fold over on itself and forms a string of impenetrable blobs. In the Odijk model, blob formation is unfavorable and the molecule adopts a deflecting chain geometry. It should be noted that these models are also only strictly applicable for systems near equilibrium.

In systems far from equilibrium, such as in the case of highly strained biopolymers, it is important to consider the force-extension relationship for polymers. Polymer extension is opposed by an entropic restoring force, resulting in behavior that can be described by considering the polymer as a spring. At small extensions, the spring is Hookean, but at extension lengths approaching the polymer's contour length, the spring stiffens and the force increases dramatically. For worm-like chains (the model that best describes double-stranded DNA) the force-extension relationship was approximated by Marko and Siggia using the following equation [8].

$$F = \frac{k_B T}{L_P}\left[\frac{1}{4(1 - x/L_0)^2} - \frac{1}{4} + \frac{x}{L_0}\right] \quad (1)$$

In the above equation F is force, k_B is Boltzmann's constant, T is the absolute temperature, L_p is the persistence length, x is the extension, and L_0 is the contour length. At sufficiently high forces (~ 65 pN), a reversible transition is observed in which the DNA assumes an extension that is ~1.7 times its B-form contour length [9].

EXPERIMENTAL

Devices in planar quartz substrates were fabricated using standard photolithography and wet etching techniques to pattern microfluidic components. Focused ion beam (FIB) milling was used to pattern the nanofluidic components. The network was then sealed by fusion bonding of a quartz coverslip. We have recently demonstrated the use of FIB milling to fabricate

nanofluidic channels with dimensions as small as 3 nm [10]. Figure 1 shows an array with nanochannels having critical dimensions (width and depth) of ~100 nm. Experiments were performed in standard Tris-borate-EDTA (TBE) electrophoresis buffers with DNA molecules stained with the intercalating dye YOYO-1. Double-stranded phage DNA of known length (λ phage, 48.5 kbp; T4 phage, 165.6 kbp) were electrokinetically driven through the nanochannel arrays. Experiments were conducted on an inverted fluorescence microscope using 60X or 100X oil immersion objectives. Fluorescence was excited at 488 nm using an argon ion laser and emission was detected through a 520-nm emission filter with imaging using an electron multiplying CCD camera (Photometrics Cascade II). A number of events were recorded for both phage DNAs at different field strengths. Individual frames were analyzed using an automated analysis program written in Matlab.

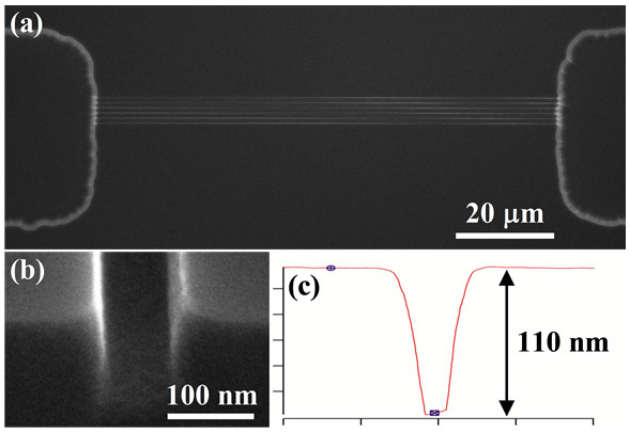

Figure 1: Metrology of nanochannels using scanning electron microscopy and atomic force microscopy (AFM). (a) Top-view of an array of 90-µm long nanochannels having critical dimensions of ~100 nm. (b) Tilted cross-section of a single nanochannel. (c) Line profile across a nanochannel measured using AFM.

RESULTS AND DISCUSSION

Figure 2 shows a typical series of frames captured during a single DNA translocation event; in this case, λ-DNA was driven through a 100-nm channel using a field strength of 190 V cm^{-1}. An accurate, automated determination of DNA position and extension at each time point was possible, given the high signal resulting from laser illumination and high gain detection. These values are used to determine the mobility of DNA [$2.46(\pm0.07)\times10^{-4}$ cm^2 V^{-1} s^{-1}, this value does not account for electro-osmotic flow counter to the direction of migration] as well as the intramolecular dynamics. A close inspection of Figure 2 reveals a decrease in the molecule's extension length during the course of transport. For λ-DNA driven using a field strength of 190 V cm^{-1}, the molecular relaxation can be adequately fit with a single exponential having a time constant of 27±10 ms (N=14). At higher electric field strengths, we have observed time constants as fast as 5 ms. These relaxation dynamics are two orders of magnitude faster than observed in previous related studies that measured near-equilibrium relaxations [11-13].

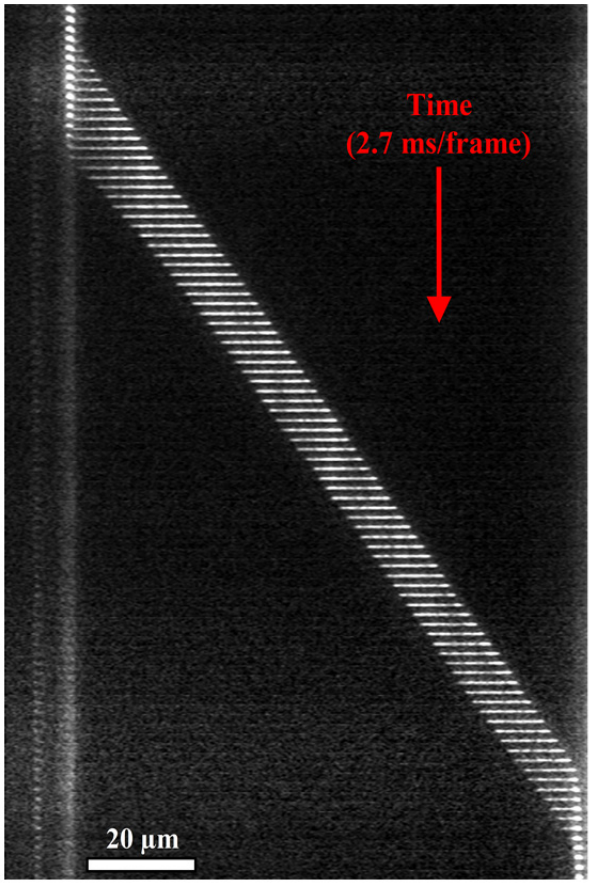

Figure 2: Series of frames showing the translocation of λ-phage DNA through a 100-nm channel. The electric field strength was 190 V cm^{-1}.

We hypothesized that these fast relaxations were the result of the large force applied to the DNA during threading, followed by the near instantaneous disappearance of any force gradient along the molecule once it was fully inserted into the nanochannel. The relaxation time constant of a polymer in solution, τ, is determined by its effective spring constant, k, and friction coefficient, ζ: $\tau=\zeta/k$. In the strong force regime of the DNA force-extension relationship, the spring constant increases approximately linearly with applied force. Assuming that the friction coefficient does not dramatically change as a function of initial extension then one expects that the time constant should be inversely proportional to the applied field strength. Figure 3 shows average relaxation curves measured for λ-DNA at a series of voltages, plotted using reduced variables. As expected, the λ-DNA curves collapse onto a single curve when plotted as a function of time×voltage×1/length (where polymer length is constant for the set of λ-DNA experiments). Also shown in Figure 3 is the collapse of the relaxation curves for T4-DNA. The effective spring constant of the DNA is not dependent on its length (when acted upon by a Lorentz force), whereas the friction coefficient is proportional to length. As a result, for DNA molecules of different lengths driven at the same voltage, the relaxation curves should collapse to a uniform curve when plotted as a function of time×1/length, as observed in Figure 3. We note that the conditions described above do not necessarily hold for relaxations

from lower tension initial conformations, for example, near equilibrium conditions.

CONCLUSION

The transport dynamics of single molecules of DNA translocating through nanofluidic channels were studied using fluorescence microscopy. Fast relaxation dynamics were observed and the results of a systematic study of the dynamics were consistent with a model in which DNA molecules are highly strained by the threading process. These fast dynamics are of fundamental interest and are relevant to "on-the-fly" sizing, mapping, or sequencing applications.

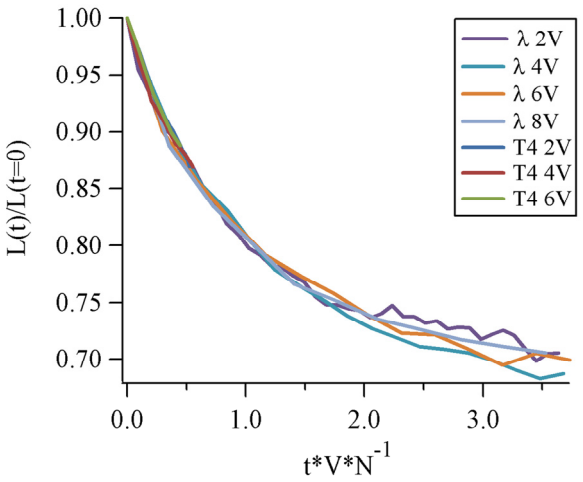

Figure 3: Plot of DNA relaxation using reduced variables. L(t) is the extension length at time t. L(t=0) is the extension length in the first frame in which DNA is fully threaded into the nanochannel. V is the applied voltage (in volts). N is the number of base pairs in the molecule (in kbp). Time is given in ms.

REFERENCES

[1] Reccius, C.H., Stavis, S.M., Mannion, J.T., Walker, L.P. and Craighead, H.G. Conformation, length, and speed measurements of electrodynamically stretched DNA in nanochannels. *Biophys. J.* 95, 273-286 (2008).

[2] Riehn, R., et al. Restriction mapping in nanofluidic devices. *Proc. Natl. Acad. Sci. USA* 102, 10012-10016 (2005).

[3] Branton, D., et al. The potential and challenges of nanopore sequencing. *Nat. Biotechnol.* 26, 1146-1153 (2008).

[4] Tsutsui, M.; Taniguchi, M.; Yokota, K. and Kawai, T. Identifying single nucleotides by tunnelling current. *Nat. Nanotechnol.* 5, 286-290 (2010).

[5] Foquet, M.; Korlach, J.; Zipfel, W.R.; Webb, W.W. and Craighead, H.G. Focal volume confinement by submicrometer-sized fluidic channels. *Anal. Chem.* 76, 1618-1626 (2004).

[6] Daoud, M. and de Gennes, P. G. Statistics of macromolecular solutions trapped in small pores. *J. Phys. (Paris)* 38, 85-93 (1977).

[7] Odijk, T. On the statistics and dynamics of confined or entangled stiff polymers. *Macromolecules* 16, 1340-1344 (1983).

[8] Marko, J.F. and Siggia, E.D. Stretching DNA. *Macromolecules* 28, 8759-8770 (1995).

[9] Smith, S.B.; Cui, Y. and Bustamante, C. Overstretching B-DNA: the elastic response of individual double-stranded and single-stranded DNA molecules. *Science* 271, 795-799 (1996).

[10] Menard, L.D. and Ramsey, J.M. The fabrication of sub-5-nm nanochannels in insulating substrates using focused ion beam milling. *Nano Lett.* 11, 512-517 (2011).

[11] Mannion, J.T.; Reccius, C.H.; Cross, J.D. and Craighead, H.G. Conformational analysis of single DNA molecules undergoing entropically induced motion in nanochannels. *Biophys. J.* 90, 4538-4545 (2006).

[12] Balducci, A.; Hsieh, C.-C. and Doyle, P.S. Relaxation of stretched DNA in slitlike confinement. *Phys. Rev. Lett.* 99, 238102 (2007).

[13] Reisner, W.; Morton, K.J.; Riehn, R.; Wang, Y.M.; Yu, Z.; Rosen, M.; Sturm, J.C.; Chou, S.Y.; Frey, E.; and Austin, R.H. Statics and dynamics of single DNA molecules confined in nanochannels. *Phys. Rev. Lett.* 94, 196101 (2005).

CONTACT

*J.M. Ramsey, tel: +1-919-9627492; jmramsey@unc.edu