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Observation of DNA transport through a single carbon nanotube channel using fluorescence microscopy†

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DNA transport through a single multiwall carbon nanotube (MWNT) channel was directly observed *via* fluorescence microscopy.

Here we report fluorescence microscopy observations of DNA transport through a membrane containing a single MWNT. Our results suggest that when the radius of gyration (R_g) of a DNA molecule is larger than the channel radius, the molecule will be trapped at the channel entrance but ultimately transported through the channel upon application of a sufficiently high membrane potential. In contrast, when R_g is smaller than the channel radius such behavior is not observed.

The transport properties of DNA through nanoporous media are of special interest because of their connection to DNA separation by gel electrophoresis[1–3] and entropic traps.[4,5] For example, it is known that DNA transport in gel matrices is controlled by both the pore radius of the gel (P_E) and R_g of the DNA. Whereas DNA in its compact form can freely pass through the gel in the case of $P_E > R_g$, DNA must reconfigure to a more linear form when $P_E < R_g$.[1–3] This structural change is entropically unfavorable, and it is the basis for entropic traps in which DNA is unable to escape through openings smaller than R_g if little or no external driving force is applied. When a large driving force is present, entropically trapped DNA must stretch or unwind itself before passing through the restricted opening. This process has been observed by fluorescence microscopy when DNA passes through gels[6] or rectangular entropic traps.[4,5]

DNA transport through channels having cylindrical symmetry has previously been studied using single nanopores formed within Si_3N_4 membranes,[7] the pore-forming membrane protein α-hemolysin,[8,9] and a 200 nm-diameter pore formed in poly(dimethylsiloxane) (PDMS).[10] In all of these studies DNA transport was observed using the Coulter counting technique, in which presence of DNA within a nanopore is signaled by a decrease in pore conductance.[8] Significant results concerning pore structural modification, DNA sequence recognition, and hairpin detection have been reported. Theoretical modeling of the Coulter counting results has also recently appeared.[11] Here, we explore an alternative means for monitoring DNA transport through nanopores: time-resolved fluorescence microscopy.

The use of fluorescence for monitoring the motion of molecules in nanoporous media is a well-established methodology. For example, it has been used to study the random diffusion of DNA through a two-dimensional array of spherical cavities interconnected by circular holes. The results of this study suggested that DNA transport in a nearly free diffusional field is limited largely by the entropic barrier imposed by the short, circular holes connecting neighboring cavities.[12] We chose to base our fluorescence study on 30–100 nm radius nanopores defined by MWNTs because the uniform inner wall and relatively long tubular shape of MWNT channels are highly desirable characteristics of a good structural model for transport studies.[13,14] Additionally, the surface charge of the MWNT channel is effectively zero,[13] suggesting that this system is appropriate for studying electrophoretic transport in the absence of electroosmosis.

Membranes containing a single MWNT channel (radius: 77 nm) were immobilized on a Si/Si_3N_4 support as shown in Fig. 1a.[13] Cyclic voltammagrams (CVs) of the channels were measured to determine their length.[13] In addition, CVs also showed that the MWNT channels were stable after applying a membrane potential (E_M) of ±5 V. This is an important result, because under certain conditions it is possible to anodically dissolve MWNTs.[15] An illustration of the experimental apparatus used for fluorescence microscopy studies is shown in Fig. 1b.‡ The nanotube channel was oriented vertically, and a shallow groove in the cover glass prevented the surface of the section from directly contacting the glass. A DNA solution was added to the inner chamber, and a buffer solution was added to the outer chamber. Next, a membrane potential was applied across the MWNT channel, and then the channel was observed using an inverted microscope capable of capturing both optical and fluorescence images. Typically, 40 successive fluorescence images were recorded at regular intervals of ~1 s (exposure time: 300 ms). The electrochemical and microscopy data described in this communication were obtained from a single MWNT channel, but similar results were obtained using two other membranes prepared according to the same procedure.

Fig. 2a is an optical image of a typical membrane section containing a single nanotube. The black dot indicated by the arrow is the MWNT, and the circular feature is the aperture of the Si_3N_4 support membrane. Fig. 2b is a series of fluorescence images capturing the transport of phiX174 RF I DNA (5386 bp, circular) through the nanotube over a period of 14 s. The R_g of this molecule (87 nm), calculated according to the appropriate equations[16] and assuming a 0.34 nm length-per-base-pair and a 50 nm persistence length,[17] is a little larger than the channel radius (77 nm). In the membrane potential range of +0.6 to +1.0 V, two transport modes were observed for the 87 nm-DNA: sometimes DNA molecules first paused in or near the MWNT channel before passing through, and sometimes transport proceeded without this initial pause. Fig. 2b shows an example

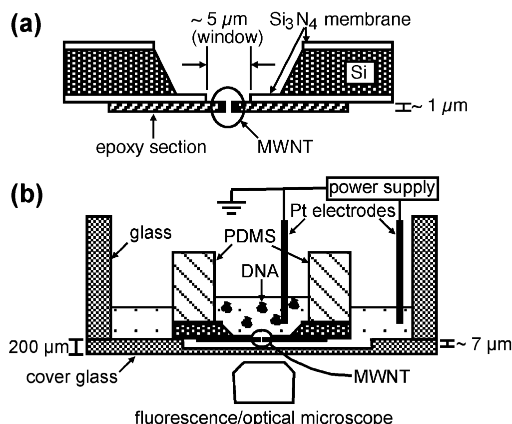

Fig. 1 Schematic illustrations of the experimental apparatus.

† Electronic supplementary information (ESI) available: movie clip showing DNA transport through a single carbon nanotube channel. See http://www.rsc.org/suppdata/cc/b3/b302511j/

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of the former transport mode. In this experiment, the potential was scanned linearly from 0 to + 1.0 V at 0.1 V s^{-1}, and then maintained at +1.0 V thereafter. Immediately after the final potential of +1 V is attained (0 V) no fluorescence is observed, but gradually it increases in intensity around the MWNT channel (1–4 s), reaches a maximum intensity (4 s), and then the fluorescence gradually disappears (10–14 s). The increase in the fluorescence intensity (1–4 s) suggests that DNA molecule(s) are entering the channel and/or accumulating at the entrance of the channel, and the decrease in intensity (10–14 s) indicates passage of DNA through the MWNT and into the outer chamber of the cell. Because DNA transport is not observed when the potential is reversed, we infer that the driving force is electrophoretic. Electroosmotic transport is unlikely, because the interior wall of the MWNT is uncharged. This situation is in contrast to other materials used for nanochannel fabrication such as glass.[4] The pause time of a single transport event ranged from 1 to 14 s for nine observations, and the maximum fluorescence intensity varies from one event to another, suggesting that aggregates of the DNA may also be involved in some transport events.

Similar transport behavior was observed for Lambda Phage DNA (48,502 bp, linear), which has a significantly larger R_g (370 nm) than the MWNT channel, when the constant potential applied after the initial voltage scan was higher than +1.0 V. No transport of the 370 nm-DNA was observed at positive potentials less than +1.0 V during a total observation time of 5 min. This suggests that, compared to the smaller 87 nm-DNA, the 370 nm-DNA requires a higher electric field to achieve the necessary structural deformation required to overcome entropic trapping.[1,2,4]

The transport behavior of LITMUS 28 DNA (2823 bp, circular), which has an R_g (63 nm) that is smaller than the channel radius, is different than the two larger DNA molecules. Specifically, there was no pause observed for the 63 nm DNA, and DNA rapidly passed through the channel upon application of a membrane potential in the range of +0.8 to +1.0 V. This result indicates that when DNA is smaller than the channel diameter, it spends less time in the entropically trapped state.

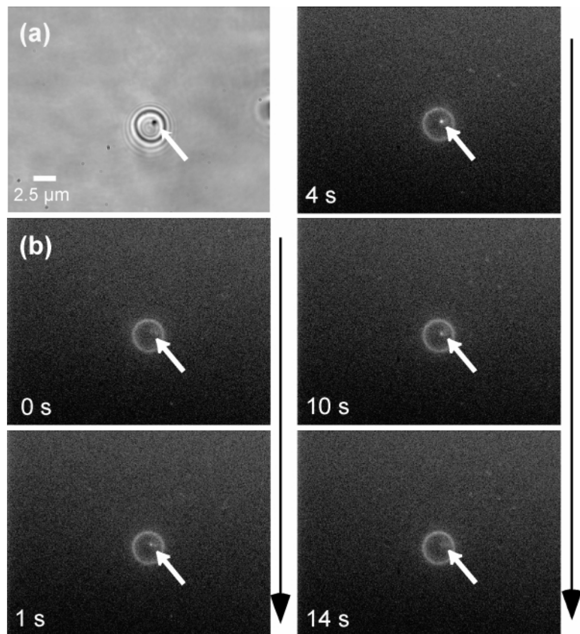

Fig. 2 (a) An optical image of the MWNT channel. (b) A series of fluorescence images showing transport of phiX174 RF I DNA through a MWNT channel. The inner radius and length of the channel were determined by TEM and CV, respectively, and found to be 77 nm and 0.84 μm. Note that the circumference of the aperture of the Si_3N_4 support membrane has trapped some fluorescent dye. The fluorescence images were obtained at E_M = +1.0 V, and the five images shown were extracted from a series of 25 obtained over a period of 25 s.

At present, we are not able to capture clear images of DNA molecules after they exit the channel, and quantitative flux measurements are therefore difficult to estimate. Sometimes, it was possible to observe a blurred fluorescent dot rapidly moving away from the channel exit, but acquiring more quantitative data obviously demands imaging detectors with better time-resolution and sensitivity.

To summarize, we have described DNA transport through a single carbon nanotube channel by fluorescence microscopy. The MWNT channel is a good model system for studying mass transport behavior of DNA through a well-difined cylindrical nanopore. We attribute differences in the transport behavior of DNA having different sizes to entropic trapping.

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Notes and references

‡ The nanotube-containing section (Fig. 1a) was set on a cover glass (no. 2, 25 × 25 mm; Corning, Acton, MA) containing a shallow groove (~7 μm deep, ~3 mm wide, and 1 cm long). The two half cells consisted of two coaxial chambers: the inner chamber was fabricated from a reservoir of PDMS (Sylgard 184, Dow Corning, Midland, MI) attached to the back surface of the support, and the outer chamber was prepared from a glass tube affixed to the cover glass using PDMS glue. The DNA solutions of LITMUS 28, phiX174 RF I DNA (New England Biolabs, Beverly, MA), or Lambda Phage DNA (Sigma, St. Louis, MO) contained 0.1 μg mL^{-1} DNA, 1.5×10^{-8} M YOYO-1 (dye : base pair ratio = 1 : 10), 0.01 M Tris-HCl (pH 8), and 1 mM EDTA. The membrane potential across the MWNT channel was controlled using a DC power supply (model E3620A, Hewlett-Packard) connected to a Pt electrode in each of the two chambers. All potentials are referenced to the electrode in the inner chamber. The inverted microscope (TE 300, Nikon, Tokyo, Japan) was equipped with a two-dimensional CCD array detector (SenSys 1401E, Photometrics, Tucson, AZ) and a dichroic filter block (Model XF115–2, Omega Optical, Brattleboro, VT).

1 G. W. Slater, J. Rousseau, J. Noolandi, C. Turmel and M. Lalande, *Biopolymers*, 1988, **27**, 509.
2 S. Magnúsdóttir, B. Åkerman and M. Jonsson, *J. Phys. Chem.*, 1994, **98**, 2624.
3 C. Heller, *Electrophoresis*, 2001, **22**, 629.
4 J. Han and H. G. Craighead, *Science*, 2000, **288**, 1026.
5 J. Han and H. G. Craighead, *Anal. Chem.*, 2002, **74**, 394.
6 K. Minagawa, Y. Matsuzawa, K. Yoshikawa, Y. Masubuchi, M. Matsumoto, M. Doi, C. Nishimura and M. Maeda, *Nucleic Acid Res.*, 1993, **21**, 37.
7 J. Li, D. Stein, C. McMullan, D. Branton, M. J. Aziz and J. A. Golovchenko, *Nature*, 2001, **412**, 166.
8 H. Bayley and C. R. Martin, *Chem. Rev.*, 2000, **100**, 2575.
9 D. W. Deamer and D. Branton, *Acc. Chem. Res.*, 2002, **35**, 817.
10 O. A. Saleh and L. L. Sohn, *Nano Lett.*, 2003, **3**, 37.
11 C. Y. Kong and M. Muthukumar, *Electrophoresis*, 2002, **23**, 2697.
12 D. Nykypanchuk, H. H. Strey and D. A. Hoagland, *Science*, 2002, **297**, 987.
13 L. Sun and R. M. Crooks, *J. Am. Chem. Soc.*, 2000, **122**, 12340.
14 T. Ito, L. Sun and R. M. Crooks, *Anal. Chem.*, 2003, **75**, 2399.
15 T. Ito, L. Sun and R. M. Crooks, *Electrochem. Solid-State Lett.*, 2003, **6**, C4.
16 V. A. Bloomfield, D. M. Crothers and I. J. Tinoco, *Physical Chemistry of Nucleic Acids*, Harper & Row, New York, 1974.
17 P. J. Hagerman, *Ann. Rev. Biophys. Biophys. Chem.*, 1988, **17**, 265.