TRANSVERSE CONDUCTANCE MEASUREMENTS OF SINGLE DNA MOLECULES

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ABSTRACT

We describe recent studies monitoring the transport of single DNA molecules through nanochannels longer than the DNA's contour length. We integrated electrical probes to monitor the ionic conductance transverse to the long axis of extended DNA molecules. In this configuration, the transport-driving and measurement electric fields were decoupled. Large electric fields can be applied to obtain greater signals without driving DNA transport at velocities resulting in short residence times in the electrically sensitive measurement area. In order to verify that the transient conductance responses were due to DNA translocations, we imaged fluorescently-stained DNA while simultaneously measuring the transverse conductance.

KEYWORDS: Nanofluidics, Nanopore, DNA, Single-Molecule

INTRODUCTION

Beginning with the translocation of DNA passing through α-hemolysin pores [1], the use of nanometer-scale apertures to detect single macromolecules has been an active area of research. Solid-state pores (fabricated by focused electron or ion beam milling through membranes of materials such as silicon nitride, silicon oxide, and graphene) have been explored as a more robust alternative to protein pores in lipid bilayer membranes. In either case, entry of an analyte molecule into the nanopore causes ionic current perturbations that provide identifying information vis-à-vis event amplitude and duration. Techniques based upon this response have been proposed for use in the detection and sizing of polynucleotides, peptides, and synthetic polymers, or even in sequencing DNA [1-3]. However, current methods are too noisy and lack the resolution necessary to fully realize these applications, and significant refinements of the process will be needed [2]. We present here one strategy for advancing measurements based on this general phenomenon.

THEORY

The application of a voltage across a nanopore or nanochannel in electrolyte solutions results in a baseline ionic conductance. When a macromolecule of sufficient size enters one of these nanoconduits by diffusion, migration, or pressure-driven flow, the ionic conductance is perturbed, either increased or decreased depending on the ionic strength of the solution, surface charge, and other factors. The degree to which conductance is perturbed and the duration of the perturbation event can indicate the cross-section and length of the macromolecule, respectively. Other factors such as polymer chemistry can also influence the amplitude and duration. In particular, threading a large biopolymer into a nanoconduit requires a change from a randomly oriented globular conformation to an extended one. This process introduces a significant variability to the event duration and limits the precision of polymer sizing. To eliminate this source of error we have decoupled the threading of biopolymers and the ionic conductance perturbation measurement into separate processes. Polymeric analytes are threaded into a nanochannel that is sufficiently long to fully contain the extended length of the molecule. Once in the extended conformation, electrical measurements are made transverse to the long axis as the molecule migrates past an orthogonal pair of electrical probes [4].

EXPERIMENTAL

Nanochannels were fabricated by focused ion beam (FIB) milling trenches in planar quartz substrates with widths and depths as small as 10 nm [5]. To form fluidic nanochannels, the trenches were sealed by fusion bonding of a coverslip. We investigated devices having probes located at different locations along the transport nanochannel. In one device, the distance between the transport nanochannel entrance and the probes (9.5 μm) was less than the extension length of the λ-phage DNA (17 μm) such that the molecule would still be in the process of threading when electrical measurements were made. In a second device, this distance was increased to 26.6 μm, greater than the DNA's contour length, and the molecule would be fully threaded prior to and during electrical measurement. In both devices the distance from the probes to the channel exit was greater than the contour length. We performed a series of experiments in which λ-DNA was prepared in a 1 M KCl solution at concentrations of 0.25-1 μg/mL, and electrokinetically driven through the nanochannel. Conductance between the transverse probes was measured with an Axopatch 200B patch clamp amplifier. In a series of similar experiments, λ-DNA was fluorescently stained with YOYO-1 intercalating dye to allow simultaneous optical and electrical monitoring. For the latter experiments, solutions were prepared in 125 mM KCl, a lower electrolyte concentration necessary to maintain intercalation of the dye. Images of stained DNA passing by the transverse probes were recorded at 100X magnification on an inverted microscope using a high gain CCD camera (Cascade II, Photometrics). The times at which electrical events occurred were compared to the times of corresponding optical events. The integrated intensity of fluorescence from each

molecule of DNA was determined from the captured images and used as a measure of DNA size. A statistical analysis comparing the coincidence of optical and electrical measurements was performed.

RESULTS

Figure 1 shows the event amplitudes and durations observed for measurements made in 1 M KCl solutions, using the two different devices described above. Measurements made during threading (Fig. 1a) have a longer average duration and a wider, skewed distribution than those made on fully threaded DNA (Fig. 1c). The device in which measurements were made during threading had significantly lower resistance transverse probes, and showed proportionally higher-amplitude events (Fig. 1b). An average event duration of 13 ms, estimated extension length of 13.8 μm, and electric field of 820 V cm^{-1} indicate a mobility of 1.3×10^{-4} cm^2 V^{-1} s^{-1}, which is consistent with literature values [6-7]. Electrical events in 1 M KCl were recorded as an enhancement of the baseline current, whereas axial measurements at this ionic strength have shown a current blockade. In contrast, events in 125 mM KCl gave a current blockade. Simultaneous transverse and fluorescence measurements in 125 mM KCl gave optical and electrical events that could be matched with small temporal error, confirming that the electrical events are due to DNA translocations (Fig. 2).

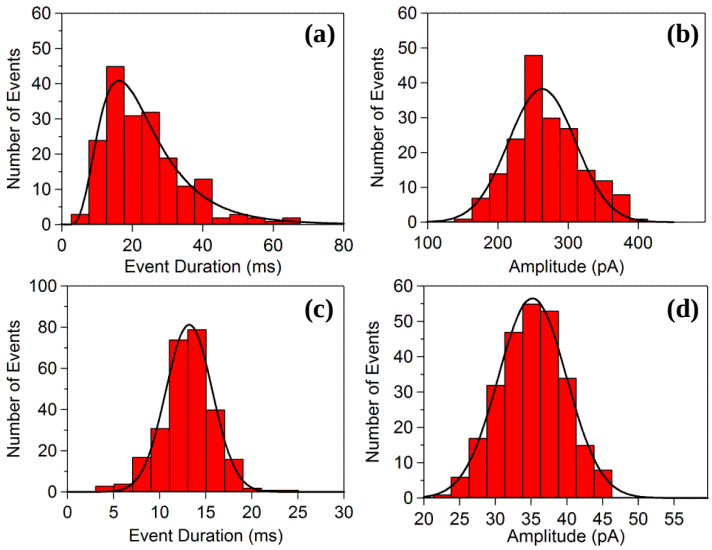

Figure 1: Event duration and current amplitude histograms for (a,b) threading DNA and (c,d) fully confined DNA. Each of the histograms was fit with a normal distribution, with the exception of that for event times in (a), which was fit to a lognormal distribution.

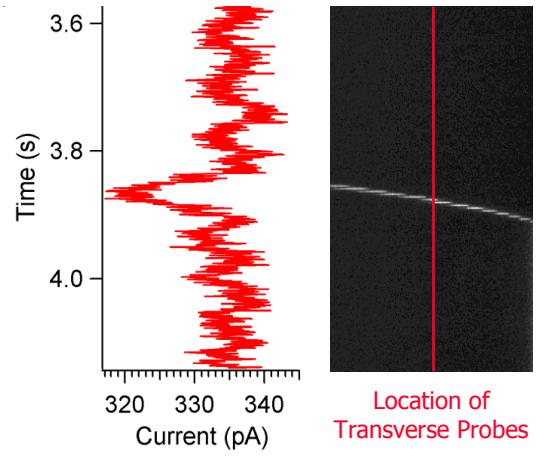

Figure 2: Simultaneous electrical and optical detection of fluorescently stained DNA. Current across the probes vs. time is shown on the left and a series of fluorescence microscopy images of the transport nanochannel at the corresponding times are shown on the right.

DISCUSSION

The distribution of durations of electrical perturbation by DNA translocation is wider and more skewed when DNA is measured while threading into the nanoconduit. This is similar to results of nanopore experiments in which molecular threading is a significant contribution to the translocation dynamics [8]. This effect is believed to be due to the unraveling of randomly oriented globular DNA, and its separation from the measurement process improves the ability of event duration to predict analyte size. Smaller DNA fragments usually did not induce any electrical signal at all in the lower ionic strength solution. The polarity of electrical perturbation switches from enhancement to blockade when KCl concentration is changed from 1 M to 0.125 M, implying a zero-perturbation crossover point somewhere between the two concentrations. Further improvements to signal interpretability may be obtained by optimizing the ionic strength of the carrier solution.

CONCLUSIONS

Transverse electrical measurement of confined single DNA molecules was demonstrated. Making measurements after the molecule is fully threaded into the nanoconduit allows a decrease of variability in the measurement event duration, improving the ability to determine the length of measured DNA molecules. The suitability of these planar devices to simultaneous optical and electrical measurements has allowed us to unambiguously determine that the observed conductance perturbations result from DNA transport past the orthogonal probes.

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