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Slowing DNA Translocation in a Solid-State Nanopore

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ABSTRACT

Reducing a DNA molecule's translocation speed in a solid-state nanopore is a key step toward rapid single molecule identification. Here we demonstrate that DNA translocation speeds can be reduced by an order of magnitude over previous results. By controlling the electrolyte temperature, salt concentration, viscosity, and the electrical bias voltage across the nanopore, we obtain a 3 base/μs translocation speed for 3 kbp double-stranded DNA in a 4–8 nm diameter silicon nitride pore. Our results also indicate that the ionic conductivity inside such a nanopore is smaller than it is in bulk.

A nanopore-based sensor can detect single DNA molecules, and nanopore sensing represents a potential future technology for rapid DNA sequencing. Since Kasianowicz et al.[1] demonstrated that individual DNA molecules could be electrophoretically driven through a single ~2 nm diameter α-hemolysin protein nanopore, several studies have clarified and extended the utility of this nanopore.[2−6] Recently, solid-state[7−14] nanopores have also been used to detect DNA molecules. The DNA nanopore translocation process has also been investigated theoretically.[15−19] Several serious technical problems remain to be solved if the goal of rapid molecule characterization and sequencing is to be achieved in solid-state nanopores. One is that the measured DNA translocation speed of ~30 bases/μs requires an electronic sensing system at extremely high bandwidth, and the concomitant electronic noise poses serious limitations in electrically discriminating between bases. Below we demonstrate how the bandwidth requirements can be reduced by an order of magnitude by slowing down the molecule translocation speed.

The detection of a DNA molecule is performed by placing a nanopore chip between two separated chambers, electrically connected only by an ionic solution inside the nanopore. When a voltage is applied, a negatively charged DNA molecule in the vicinity of the nanopore will be captured by the electric field, and forced to pass through the nanopore from the negative (cis) side to the positive (trans) side. A molecule inside the nanopore causes a detectable ionic current blockade. Both the translocation time (dwell time, t_d) and the amplitude of the blockade (current drop, ΔI_b) are dependent on the solution conditions (ionic concentration, viscosity, and temperature), properties of the nanopore, bias voltage, and the passing molecule. DNA translocation is a very complex process, but it can be envisioned as resulting from a balance between the electric driving force and viscous drag. The nanopore electrical behavior in ionic solution seems to be an ohmic one, with the electrical current blockages proportional to the applied voltage[14]

$$\Delta I_b = \sigma V A_{DNA}/H \quad (1)$$

where σ is the solution conductivity, V the applied voltage across the nanopore, H the effective thickness of the nanopore, and A_{DNA} the hydrodynamic cross section of the translocating molecule. Using a simple equation of force balance between the electric force in the nanopore and the viscous drag over the whole molecule, one finds for the translocation time[7]

$$t_d = K\frac{\eta L_{DNA}}{\lambda V} \quad (2)$$

where η is the viscosity of the solution, λ and L_{DNA} are the linear charge density and length of the DNA molecule, respectively, and K is a constant of proportionality accounting for complex issues beyond the capabilities of the simple model. Equations 1 and 2 are coupled by the fact that σ will depend on η ($\sigma \sim 1/\eta$).[20] In addition, σ, η, and λ will depend on the temperature and the concentration of ions in the nanopore.

In this work we explore the various accessible experimental factors in eqs 1 and 2 for slowing DNA molecule

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transport through nanopores, namely, viscosity, bias voltage, salt concentration, and temperature. Our nanopores are fabricated in a free-standing 280 nm thick silicon nitride membrane supported by a 380 μm thick silicon substrate using a combination of focus ion beam milling and feedback-controlled ion beam sculpting.[8,21] Due to the fabrication process, individual nanopores may have different thickness, diameter, shape, or even surface charge in solution, all of which may result in various translocation characteristics. To avoid these problems, a single nanopore with a diameter of 4−8 nm was used for each experiment described below. All measurements are performed in a typical TE (10 mM Tris, 1 mM EDTA) buffer (pH = 7.5), with different concentrations of KCl (0.5−3 M) and/or different concentrations of glycerol (0−50%). A linear 3 kbp plasmid (pSP65) was added to our cis chamber in a 10 nM final concentration. Ionic current signal through solid-state nanopores was measured and recorded using an integrated Axopatch 200B patch-clamp amplifier system (Axon Instrument) in resistive feedback mode. The 10 kHz low-pass Bessel filter in the Axopatch 200B was selected for all measurements in this work. At this setting, the whole measuring system was tested and calibrated with artificial current blockages, ideal square pulses, generated from a function generator (Agilent 33250A). The pulse widths generated were 20, 40, 60, 80, 100, 140, and 160 μs, and the pulse height was about 100 pA. The recorded data were analyzed with the same MatLab routines for real DNA translocation. When the pulse width is less than 100 μs, the pulse height will be attenuated, but the time durations (the width of half-height) remain correct. When the time duration measured in this work was less than 100 μs, the current blockage amplitude was corrected with this calibration. The current blockages, ΔI_b, and the translocation times, t_d, are extracted from the recorded data using custom Matlab routines. Unless otherwise mentioned, the bias voltage is set to 120 mV. For every DNA data set the number of recorded events is between 3000 and 12 000, and the ratios between the standard deviation and the mean are at most 15%, except for the low temperature−low voltage experiment where the error is about 25%.

Viscosity Study. We studied DNA translocation in different bulk solutions with viscosities between 1 and 5.3 cP. The viscosity of KCl-TE solution was changed by adding glycerol (0−50%), while keeping the KCl concentration constant at 1.5 M, and was measured using a model GV-2100 (Gilmont Instruments) drop ball viscometer. Plotting the peak positions of current drops and translocation times as a function of viscosity, our results in Figure 1a show the following: (1) the current blockades (ΔI_b, filled squares) decrease inversely proportional to the viscosity, which can be explained with eq 1 by the fact that conductivity, σ, is proportional to $1/\eta$; (2) the translocation times increase linearly with viscosity, as predicted by eq 2. The ratio of open pore current to current drop for all measured viscosities is about 45 ± 5 indicating the open pore current and current drop are actually modified in the same manner when the viscosity is changed (data not shown). The most important result from our viscosity study is that the translocation time

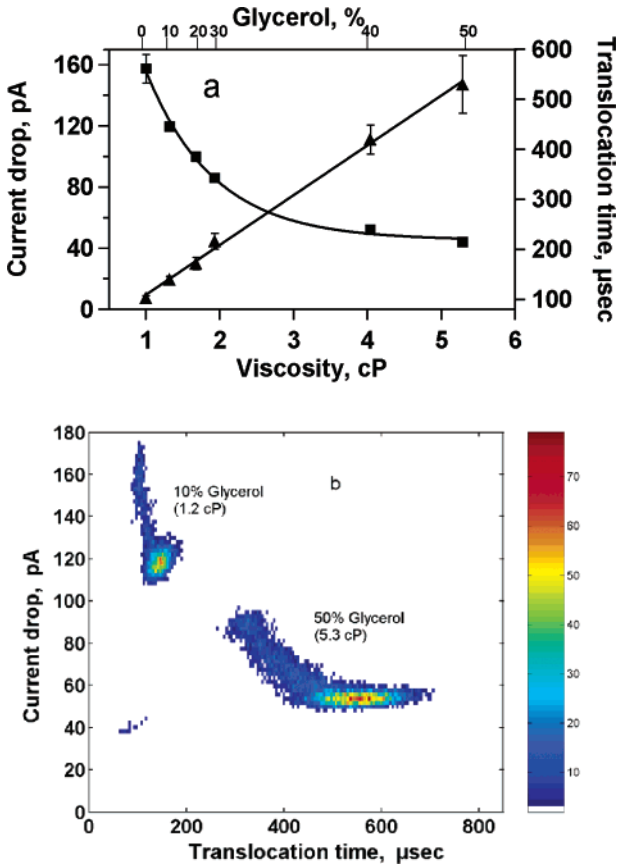

Figure 1. (a) Current blockage (■) and translocation time (▲) versus viscosity for 3 kbp DNA in 1.5 M KCl-TE solution at 120 mV bias voltage. The solid curves are fits for $\Delta I_b \sim 1/\eta$ and $t_d \sim \eta$. (b) The scatter plots for the addition of 10% and 50% glycerol to the ionic solution. The color scale represents the events density.

can be increased by increasing the solution viscosity. Increasing the viscosity about 5 times (adding 50% glycerol) increases the translocation time, and thus the temporal resolution, by about 5 times. Furthermore, the current blockage is still large enough for signal analysis.

The current blockages versus the translocation times for DNA translocation (the scatter plot) for 10% (1.3 cP) and 50% (5.3 cP) glycerol are shown in Figure 1b. These scatter plots show the same pattern as previously reported[7,14] for KCl solution with 0% glycerol: the DNA appears to exist in two folding states. The first one, characterized by peak positions for current drops of about 50 pA and translocation times of about 550 μs for 50% glycerol, and 120 pA and 140 μs for 10% glycerol, belongs to unfolded DNA molecule passing the nanopore. The second more distributed one (the tails of the density plot, Figure 1b) shows an increased current drop and a reduced translocation time, corresponding to folded DNA molecules.

Bias Voltage Study. Figure 2 shows the voltage dependency of DNA translocation through a ~6 nm silicon nitride pore. The measured current blockages are linear with applied voltage in the 20−100 mV range, and the translocation times are inversely proportional to the voltage. Although at the lowest voltage used (20 mV) the current drop is very small (about 22 pA), however, the DNA translocation signal can still be easily measured and analyzed.

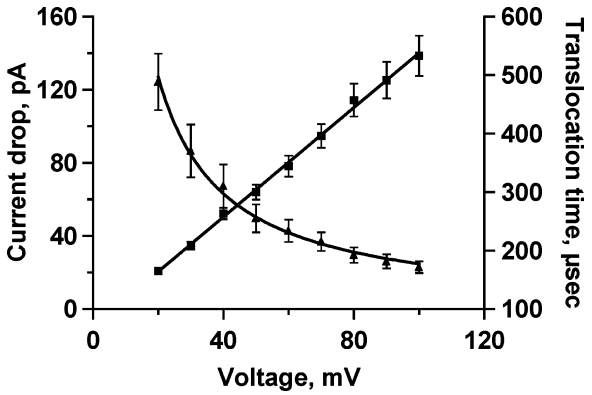

Figure 2. Current blockage (■) and translocation time (▲) versus applied voltage measured in 1.6 M KCl-TE buffer containing 20% glycerol. The solid curves represents fits where $\Delta I_b \sim V$ and $t_d \sim 1/V$.

These results are consistent with previously reported values for solid-state nanopores[7,14] and for the α-hemolysin[1,2,22,23] protein pores showing an inverse relationship between translocation time and applied voltage; however, they are in contradiction with results obtained for smaller diameter pores.[11] For an α-hemolysin nanopore, it has been suggested that there exists an energetic barrier that DNA needs to overcome before translocation can occur.[2,22,23] The height of this barrier is determined by both electrostatic interactions and geometrical restrictions in a confined volume.[23,24] DNA passes the protein pore only for applied voltages higher than 40−60 mV.[22,23] In our case, as shown in Figure 2, the DNA molecules pass through our silicon nitride pore with applied voltage as low as 20 mV. This suggests the energy barrier for our silicon nitride pores is lower than that for the protein pore, probably due to the increased diameter of the nanopore and reduced electrostatic interactions.

Salt Concentration Study. To study the salt concentration dependency of DNA translocation, the KCl concentration of TE buffer was changed from 0.5 to 3 M. In this range, the macroscopic conductivity (measured with a VWR Traceable expanded-range conductivity meter) is not linear with respect to KCl concentration, as saturation is observed in the solution's conductivity as salt concentration becomes high (Figure 3a). When the KCl concentration is increased from 1 to 3 M, the bulk conductivity is increased from 95 to 145 mS/cm. However, both the open pore current (Figure 3a, squares) and current blockage (Figure 3b, squares) change linearly with KCl concentration.

The saturation of the bulk conductivity above 1 M KCl can be explained by increased interionic effects at high KCl concentration, which increases the resistivity of the ionic solution. The linear relation between the ionic current and KCl concentration in a silicon nitride nanopore is consistent with recent work done by Stein et al.,[25] in which the authors found that the conductance in nanochannels is approximately linear to KCl concentration between 0.1 and 1 M. In this salt concentration range, the ionic current, I, is directly proportional to ion concentration n, as a limiting case for the Levine relationship,[26] $I \sim n(1 + C)$, where C is the correction to the bulk conductivity. The fact that both the

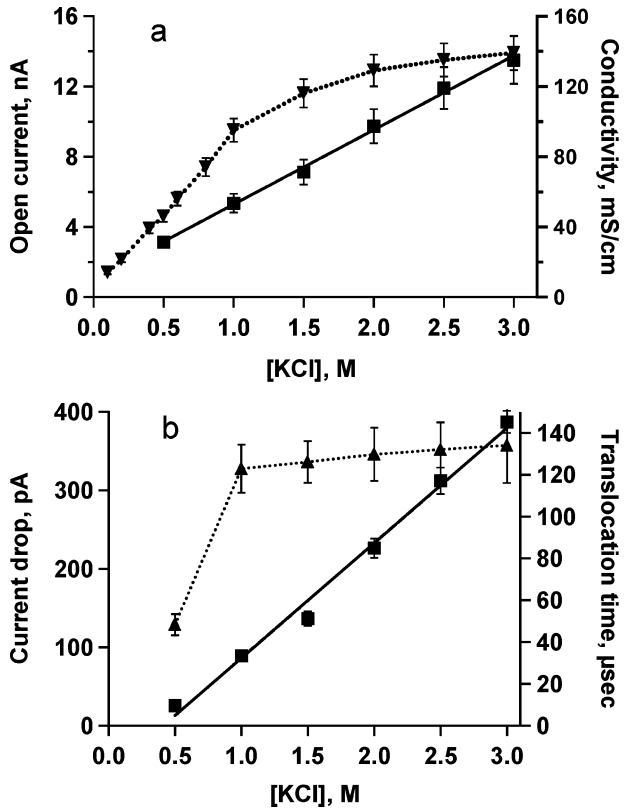

Figure 3. (a) Open current (■) (without DNA added) and bulk conductivity (▲) change as a function of salt concentration at 120 mV bias voltage. (b) Current drop (■) and translocation time (▲) change as a function of KCl concentration. The solid curves are linear fits of ΔI_b and t_d to KCl concentration.

open pore current and current drop are linear versus KCl concentration suggests the KCl concentration in a 4−8 nm silicon nitride nanopore is lower than the bulk, and the mechanism of ion transport through a nanopore is the same with or without DNA inside the nanopore. The difference between the conductivity in a nanopore and in bulk is still under investigation.

For the KCl concentration range studied, the translocation time is essentially constant except 0.5 M (Figure 3b). DNA molecules seem to be saturated with counterions so a higher concentration only slightly increases the translocation time by decreasing the DNA charge (the well-known screening effect of positive ions).

Temperature. We studied the temperature behavior of DNA translocation in TE buffer, containing 1.6 M KCl and 20% glycerol at two different temperatures, 22 and 4 °C. The temperature was kept constant using a HCC-100A (BioScience Tools) temperature controller. In contrast with the results reported from biological nanopore experiments,[2,27] which showed a strong dependency of translocation time related on temperature,[2,27] at low temperature the DNA translocation pattern through a silicon nitride nanopore is not strongly modified. When the temperature was decreased from 22 to 4 °C, the current blockage decreased from 210 to 140 pA and the translocation time increased from 165 to 280 μs (Figure 4), less than a factor of 2. This behavior supports the supposition of a stronger dependency of protein

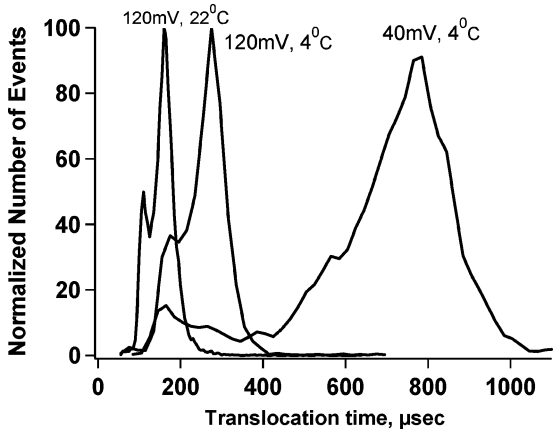

Figure 4. Translocation time is increased when the temperature is decreased. At low voltage (40 mV) and low temperature (4 °C) in 1.6 M KCl-TE containing 20% glycerol, the translocation time is about 800 μs.

pore properties on temperature, not major changes in DNA or bulk transport properties. Temperature can affect conductivities, mobility, viscosity, or the pore channel itself. Again, we measured the same ratio of open pore current to current drop for different temperatures which suggests the open pore current and current drop are modified in the same manner by temperature changes. Also, for our silicon nitride nanopores the transport properties seem to remain relatively constant, whereas the properties of the bulk such as viscosity increase with decreasing temperature are the main contributors to the temperature dependence on translocation time and current drop.

To demonstrate the goal of increasing the translocation time, a DNA translocation experiment was carried out in a 1.6 M KCl-TE solution, containing 20% Gly, at 40 mV and 4 °C. Although the recorded current drop was small, about 40 pA, the signal was easily discernible from noise. The translocation time was about 800 μs (Figure 4), almost 1 order of magnitude higher than the typical translocation time recorded for a same DNA molecule passed through a same nanopore at room temperature, bias voltage 120 mV and 1 M KCl buffered bulk.[7] The first small peak for every histogram (Figure 4) corresponds to folded DNA molecules translocating through the nanopore and is characterized by a shorter translocation time.

The DNA translocation speed through 4−8 nm silicon nitride nanopores is about 30 base/μs without glycerol, the same as measured under similar conditions.[7,14] By adding 50% glycerol, the translocation speed can be slowed by a factor of 5.5. Decreasing the bias voltage to ∼20 mV slows the DNA translocation speed by a factor of 3, and decreasing the temperature of our measuring system to 4 °C, slows it by a factor of ∼2. Combining all of these factors while maintaining a good signal-to-noise ratio, the DNA translocation speed can be slowed by a factor of 10, or an order of magnitude. The strategies used in this work slow DNA molecules; however, it also slows conducting ions which decreases the current blockage signal. Our future strategy will be slowing down only the DNA molecules not the ions.

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