Driven DNA Transport into an Asymmetric Nanometer-Scale Pore

Sarah E. Henrickson,[1] Martin Misakian,[2] Baldwin Robertson,[1] and John J. Kasianowicz[1,*]

[1]*Biotechnology Division, National Institute of Standards and Technology, Gaithersburg, Maryland 20899-8313*

[2]*Electricity Division, National Institute of Standards and Technology, Gaithersburg, Maryland 20899-8113*

(Received 14 January 2000)

To understand the mechanism by which individual DNA molecules enter nanometer-scale pores, we studied the concentration and voltage dependence of polynucleotide-induced ionic-current blockades of a single α-hemolysin ion channel. We find that the blockade frequency is proportional to the polymer concentration, that it increases exponentially with the applied potential, and that DNA enters the pore more readily through the entrance that has the larger vestibule. We also measure the minimum value of the electrical potential that confines a modified polymer inside the pore against random diffusion and repulsive forces.

PACS numbers: 87.15.Kg, 87.14.Gg

Polymer transport through nanometer-scale pores occurs in many biological processes including protein translocation [1], DNA transfer from virus to host cells [2,3], and gene transduction between bacteria [4]. Theoretical analyses of polymer partitioning into simple model geometries provide valuable insight into the physics of DNA confinement in structures with biologically relevant length scales [5,6]. However, experimental methods have only recently permitted the detection of either several polymers [7–11] or single polymers [12–14] in narrow proteinaceous pores [15].

Individual DNA molecules driven electrophoretically through a solitary channel formed by the *Staphylococcus aureus* α-hemolysin protein (αHL) [16–20] can be detected using single channel recording techniques [12]. Polynucleotides thread through the αHL channel as extended chains; their time of flight through the pore is proportional to the polymer's contour length; and the polymerase chain reaction (PCR) method confirms that single-stranded, but not blunt-ended double-stranded, DNA traverses the pore [12]. Subsequently, other groups have used PCR to show that double-stranded DNA can pass through an ion channel that has a larger aperture [21]. These experimental findings are in part the basis for several theoretical analyses of driven [22–24] and passive [24,25] polymer transport through a narrow pore.

To understand the mechanism by which individual DNA molecules enter and thread through a nanometer-scale pore, we need to determine the interaction between DNA and the pore. This can be done by varying all the physical variables that have an effect on the transport of the DNA through the pore and measuring the rate of transport as a function of the variables. The interaction between K^+ and Cl^- ions and a single α-hemolysin ion channel is partially characterized by measurements of the total ionic current as a function of the applied potential; the current is nonlinear in the applied voltage and is partially rectified [26]. The same kind of result must be true for any ion, in particular for a polynucleotide. Thus, to gain some understanding of the threading process and the interaction between a polynucleotide and the channel, we measure the rate of the polynucleotide entering the pore as a function of the magnitude and direction of the applied potential and as a function of the concentration of the polynucleotide.

Details of the experimental methods for reconstituting αHL into planar lipid bilayer membranes and for detecting polynucleotides threading through it are described elsewhere [12,18]. Briefly, a solvent-free diphytanoyl phosphatidylcholine lipid membrane is formed on a 60 μm diameter hole in a 17 μm thick Teflon partition that separates two halves of a Teflon chamber. The chambers contain identical aqueous solutions (1 M KCl, 10 mM HEPES, pH 7.5). The αHL is added to one side (cis) and, after a single channel forms in the membrane, excess protein is removed. A transmembrane electric field is applied via two Ag-AgCl electrodes, and the current is converted to voltage using a patch clamp amplifier (Axopatch 200B, Axon Instruments). The signal is electronically filtered (f_c = 20 kHz) and digitized at 50 kHz using an analog-to-digital converter (Digidata 1200 or 1321, Axon Instruments). A negative potential drives anions from the cis to the trans side. Single-stranded homopolymeric DNA (Midland Certified Reagants, Midland, TX) was initially suspended in 10 mM Tris-HCl, 1 mM EDTA, pH 7.5, and added in small aliquots ($<$1% of each chamber volume) to either the cis or trans chambers. For shorter polynucleotides that block the ionic current for a relatively brief time, an event is defined as a blockade if it reduces the magnitude of the current through a fully open single channel by more than 4 standard deviations of the open-channel current noise. For longer homopolymers [e.g., 100 nucleotide long poly(dT)] that block the current for a relatively long time, we used an additional criterion to define an event. Specifically, events that persisted less than 40 μs were not counted.

In the absence of polynucleotides, the current through the single channel is stable (Fig. 1, far left). Adding single-stranded DNA (ssDNA) to either the cis or trans side causes transient current blockades, provided the applied

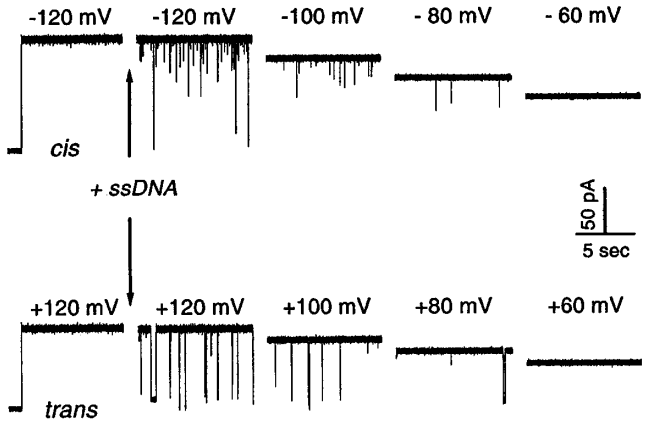

FIG. 1. Interaction of polynucleotide with a single αHL channel. Far left: In the absence of polymer, the (K^+ and Cl^-) ionic current through the channel is quiescent. Top: When the applied potential has the polarity to drive negative ions from the cis side into the pore, adding 400 nM single-stranded DNA (ssDNA), specifically 30-nucleotide long 5′-biotinylated poly(dC), to the cis side causes transient blockades of the ionic current. The number of polymer-induced blockades per minute decreases as the magnitude of the potential is decreased, and it vanishes when the polarity is changed (not shown). Bottom: Current when 800 nM ssDNA is added to the trans side and the applied potential has the polarity to drive negative ions from the trans side into the pore.

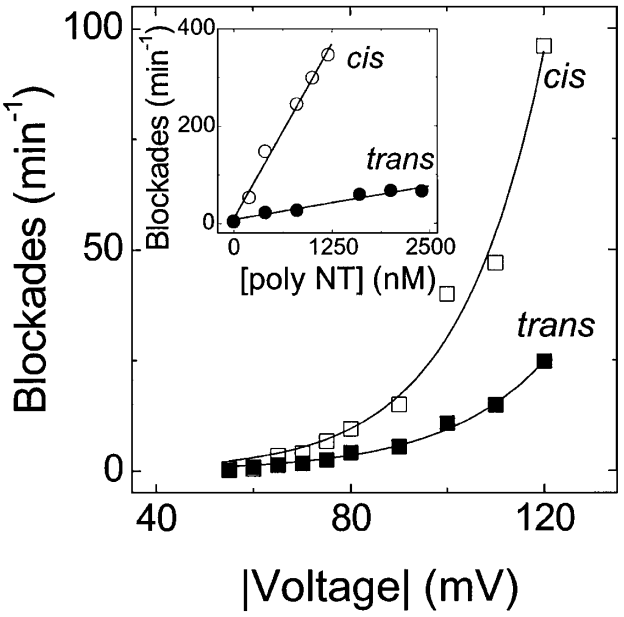

FIG. 2. Voltage and concentration dependence of the rate of blocking the current by a polynucleotide. The number of blockades per minute depends markedly on the magnitude and direction of the applied potential V and on which side polynucleotide is added. Main graph: The rate versus the magnitude $|V|$ when 400 nM bT-poly$(dC)_{30}$ is added to the cis side (open squares), and when 800 nM is added to the trans side (solid squares). The polarity of V in each case is such that negative ions are driven from that side into the pore. The solid lines are least-squares fits of a Van't Hoff-Arrhenius or transition-state relation (see text) to the data. Inset: The blockade rate is proportional to the concentration of polymer added to the cis side when $V = -120$ mV or to that added to the trans side when $V = +120$ mV. At a given concentration, polymer added to the cis side is about six times more likely to transiently block the pore than when it is added to the trans side.

potential drives anions from that side into the pore. Decreasing the magnitude of the applied potential decreases the frequency of polymer blockades (Figs. 1 and 2). The ssDNA we used is bT-poly$(dC)_{30}$, a 30-nucleotide long homopolymer of deoxycytidylic acid biotinylated at the 5′ end. Qualitatively similar results are obtained using a variety of nonbiotinylated homopolymers of poly(dC), poly(dA), and poly(dT) (not shown). At fixed DNA concentration and for $|V| = 120$ mV, the number of blockades is greater for cis- than for trans-side polymer addition. The difference is actually more pronounced than shown in Fig. 1 because there the polymer concentration in the trans side is twice that in the cis side.

The blockade rate R is described by a Van't Hoff-Arrhenius or transition-state relation $R = \kappa\nu \times \exp[-(U^\ddagger - \Delta U)/kT]$, where κ is a probability factor, ν is the frequency factor, $U^\ddagger$ is the activation energy or barrier height [27], ΔU is the reduction in the energy barrier due to the applied potential, and kT is as usual. The energy difference due to the transmembrane potential V that drives polynucleotides through the channel is $\Delta U = ze|V|$, where z is the magnitude of the effective total number of elementary charges on the polymer and e is the magnitude of the elementary charge [15,28]. The rate in a vanishing applied potential is $R_o = \kappa\nu \times \exp(-U^\ddagger/kT)$. It follows that $R = R_o \exp(ze|V|/kT)$, where R_o is independent of V. Fitting the last equation to the data in Fig. 2 with $kT/e = 25.7$ mV yields $z \approx 1.9$ and $R_o \approx 1.5 \times 10^{-2}$ min^{-1} for polymer addition on the cis side, and $z \approx 1.4$ and $R_o \approx 4 \times 10^{-2}$ min^{-1} for addition on the trans side. Because each nucleotide has one phosphate group that in the polymer has one negative charge, the total number of negative elementary charges on poly$(dC)_{30}$ is 30. This is much larger than the effective number z. Most of the charges on the polymer are either effectively screened by counterions or are not in a region of the channel where the electric field is relatively large. On average, only about 1.5 or 2 of the nucleotides interact strongly with the channel at one time.

To obtain a value for the activation energy, we assume $\kappa = 1$ and estimate the frequency factor ν by a simple barrier penetration calculation [29], which gives $\nu \approx CDA/\ell$, where C is the bulk concentration of the polymer, D is its diffusion coefficient, A is the cross sectional area of the channel, and ℓ is the width of the barrier. If $C = 400$ nM (Fig. 2, cis), $D = 10^{-7}$ cm^2 s^{-1}, $A \approx 3 \times 10^{-14}$ cm^2 [19], and $\ell \approx 10^{-6}$ cm (the length of the channel [19]), then $\nu \approx 40$ min^{-1}, and $U^\ddagger \approx 8kT$. These estimates are uncertain because κ, ℓ, and D in the pore are uncertain [10].

Polynucleotide entry into the αHL channel is a rare event. However, this is in part a consequence of the polymer's relatively low concentration (<2500 nM), not because of a small cross section for interaction between the polymer and the pore. An upper bound for the polynucleotide flux to the pore can be estimated by assuming the polymers are point particles diffusing to a perfectly adsorbing disk of diameter d and by using $J = 4DdC$ [30]. Using the previous values of D and C with $d \approx 2$ nm [10,19] gives $J = 3000\ \text{min}^{-1}$. The results in Fig. 2 (inset) show that for $V = -120$ mV and [ssDNA] = 1000 nM, about 300 blockades min^{-1} occur. Evidently, under these experimental conditions, about 1/10 of the cis-side ssDNA-pore collisions result in current blockades and by inference, polymer translocation [12].

There are several candidate mechanisms that might account for the asymmetry in the observed blockade rate. (i) The interactions shown schematically in Fig. 3 might qualitatively account for asymmetric polynucleotide partitioning into the pore. Electrostatic repulsion by negatively charged side chains on the trans side (e.g., aspartic acids at positions 127 and 128 [19,20]) reduces the concentration of DNA there. That decreases the DNA flux in the pore when the applied voltage is positive. (ii) Electrostatic attraction by positively charged amino acid side chains on the cis side (e.g., the lysine at position 8 [19]) might increase the concentration of DNA there. That would increase the DNA flux through the pore when the applied voltage is negative. (iii) The asymmetry might occur because the larger vestibule on the cis side presents a smaller entropic barrier for polymer entry compared to that on the trans side [5]. All of these involve asymmetry in the rate of polynucleotide entering the pore. (iv) A different candidate for the observed asymmetry might be that the polymer is driven through the pore so much more rapidly when it enters from one side than from the other that it does not get counted. The present experiments do not distinguish between these candidates.

A threshold value of applied potential may be needed to drive a polynucleotide into either channel mouth (Fig. 2). Similarly, when a polynucleotide can enter but not traverse the pore, there is a minimum transmembrane potential that will keep the polymer in the pore against thermal agitation and repulsive forces (Fig. 4). Avidin (neutravidin, Pierce Biochemicals), which binds strongly to biotin, binds also to the biotinylated poly(dC)$_{30}$ and is too large to enter the pore. A short time after a transmembrane potential is applied, Avidin:bT-poly(dC)$_{30}$ complex added to the cis side partially blocks the channel and does so virtually indefinitely. Once the capped polynucleotide occludes the pore, the potential is slowly changed to determine the minimum voltage that confines the polymer in the pore. The escape of the Avidin:bT-poly(dC)$_{30}$ causes the abrupt current

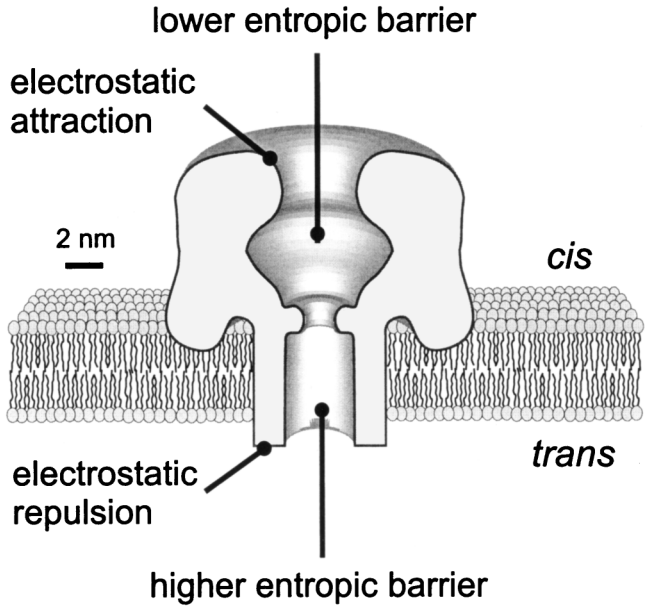

FIG. 3. Candidate locations for polynucleotide-pore interactions. Polynucleotide entry from the cis side into the pore may be favored over that from the trans side because of the relatively large vestibule (the entropic barrier is lower for larger confinement volumes) and/or electrostatic attraction (or repulsion) caused by charged amino acid side chains in key regions. A major barrier for polynucleotide entry may also be the physical constriction that is shown schematically in this representation of the channel's crystal structure [19].

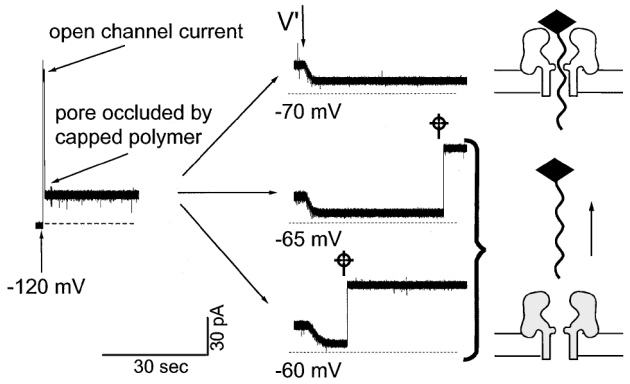

FIG. 4. Minimum transmembrane potential required to balance the thermal agitation and repulsive forces on a capped polynucleotide, i.e. an Avidin:bT-poly(dC)$_{30}$ complex, occluding the channel. When $V = -120$ mV, it drives the complex into the channel from the cis side, thereby reducing the single-channel current. When $V > -70$ mV, the complex backs out of the pore. The downward arrow at V' indicates the time at which the potential was reduced by hand from -120 mV to a lesser magnitude. The cartoons on the right-hand side indicate imagined relative positions of the capped polynucleotide in the αHL channel for different applied potentials, although poly(dC)$_{30}$ may not completely span the pore. The Celtic crosses indicate DNA escaping from the channel following the reduction of the potential. Experiments with different length bT-poly(dC) in the presence of Avidin demonstrate that polymers that contain less than about 20 nucleotides do not cause long-lived blockades (at -120 mV), which correspond to capped polymers occluding the pore (not shown).

increase at −65 to −70 mV indicated by Celtic crosses in Fig. 4. For trans-side additions, the escape occurs at +55 to +50 mV (not shown). The interactions between the channel and either the polynucleotide or Avidin are not separately determined. Changing the physical properties of the cap may change the potential required to confine the capped polymer in the pore. If the cap is flexible enough to partition into the cis antechamber but too large to traverse the transport barrier (Fig. 3), a lower potential may be sufficient to keep the polymer confined. When the polymer structure inside the pore is known, the capped polymer may be useful as a molecular ruler for probing the structure of the pore.

In summary, we have shown that the ability of single-stranded DNA to enter the nanometer-scale pore depends not only on the DNA concentration and the applied voltage but also on the structure of the pore, which is different on the cis and trans sides. The asymmetry in the polymer ion flux through the pore is consistent with the asymmetry in the monovalent ion (e.g., K^+ and Cl^-) flux. However, unlike the monovalent ion flux, the polymer ion flux and thus the blockade rate increase exponentially with the magnitude of the applied voltage (Fig. 2). The transition-state expression for the rate fits the data well. The best-fit value for the effective number of negative charges on poly(dC) entering the pore from the cis side is 1.9, and that entering from the trans side is 1.4. The values are much smaller than the total number of negative charges on the polymer. Evidently, only a small part of the polymer interacts with the pore at a time. This suggests that the polymer threads through the pore rather than going through as a random coil.

We thank Mr. S. Lee for technical assistance and Dr. H. Bayley for the kind gift of αHL. This work was supported in part by the NIST Advanced Technology Program and NAS/NRC (J. J. K.).

*Corresponding author.
Email address: john.kasianowicz@nist.gov

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