Dynamics of Polyelectrolyte Transport through a Protein Channel as a Function of Applied Voltage

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We study the transport of dextran sulfate through a protein channel as a function of applied voltage. Below 60 mV, the chain’s entrance to the pore is hindered by an entropic barrier; above 60 mV, the strong local electric field forces the chain entrance. The effective charge of the polyelectrolyte inside the pore is reduced. We observe two types of blockades which have durations that decrease when the applied voltage increases. The shortest is a straddling time between the polyelectrolyte and the pore; the longest is the translocation time. The translocation time obeys an exponential dependence upon applied voltage.

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The first observation of the passage of one single strand DNA through a protein channel, α-hemolysin, in a planar lipid bilayer was observed in 1996 [1]. This pore is asymmetric [2] and stable [3–5]. The sensitivity of techniques for the electrical detection of the molecule translocation through single protein channels has been used in many applications [1,6–9]. These include fundamental studies of confined neutral polymer [10] or charged polymer chains [11,12]. Recent developments include the transformation of nanopores into a manipulation tool and force apparatus by techniques of active control [13,14] and the study of the translocation coupled to the protein unfolding [8].

There is not yet a complete description of the process of polyelectrolyte translocation through a narrow pore but several theories describe the possible phenomena. The blockade rate \( f \) is in general described by a Van’t Hoff-Arrhenius law: \( f = f_0 \exp(|V|/V_0) \) where \( f_0 \propto \nu \exp(-U^*/kT) \) is the zero voltage event frequency governed by an activation barrier \( U^* \) (\( \nu \) is a frequency factor), and \( |V|/V_0 = ze|V|/kT \) is a barrier reduction factor due to the applied voltage \( V \), acting on \( ze \), the effective charge of the polyelectrolyte [15]. The barrier \( U^* \) is often of entropic origin as is the case for neutral polymer chains, but can be of electrostatic origin because of surface charges, or dielectric effects, or possibly both if some kinds of confinements of the polyelectrolyte counterions are involved. Two alternate theories of charged confined chains predict that the chain’s dynamics are dominated by either its mobility or the free-energy barrier. In the first case the translocation time \( \tau \) is inversely proportional to the applied force, i.e., to the transmembrane voltage [16–19], \( \tau \propto 1/V \). In the second case, one expects an exponential dependence on the applied voltage [15], \( \tau \propto t_0 \exp(V/V_z) \). Several parameters have been studied, in particular, the influence of the degree of polymerization \( N \) and applied voltage on the translocation time and the event frequency. The predictions of the theoretical models [18,20] concerning the influence of the degree of polymerization are confirmed by the experiments [1,12,21,22] and the simulations [23,24]. The behavior depends on the ratio \( r/L \) of the polyelectrolyte size over the nanopore length. Experiments show the exponential dependence of the frequency of pore blockades upon applied voltage [8,25,26]. The same behavior is observed at high voltage with a smaller slope [26]. Presently, only a few experiments of the translocation of polyelectrolyte through protein [1,12] or artificial [22,27–29] nanopore have been performed. The translocation time has been found to be inversely proportional to the applied voltage [1,12,27]. A quadratic voltage dependence of the translocation velocity has also been observed [21] in other experiments. Recently, the dynamics of translocation of a polyelectrolyte through a nanopore has been investigated by molecular dynamics using a coarse grained model [24]. The simulations show two different regimes for the probability of translocation as a function of the applied voltage. Both regimes are approximated by an exponential fit. An exponential dependence of the translocation time upon voltage is found below a crossover for which the energy barrier disappears completely in agreement with the experimental data [26]. All-atom molecular dynamics simulation of DNA translocation through synthetic nanopores have also revealed a nonlinear dependence of the DNA translocation velocity on transmembrane bias [30]. Similar results were obtained for RNA translocation through carbon nanotubes [31].

In our experiments, the polyelectrolyte is the dextran sulfate sodium (\( M_w = 8 \) kDa or \( M_w = 500 \) kDa) of 31 monomers, each bearing two negative charges. It is added to the cis compartment in 1M KCl or 0.1M KCl, 5mM HEPES pH 7.4 buffer for a final concentration of 0.5 mg/mL. Experiments on the dynamics of the polyelectrolyte transport were performed by varying the transmembrane voltage \( V \). Lipid bilayers were prepared using a previously described method [18]. The channels were inserted by adding 0.30 nmol of monomeric α-hemolysin in the cis compartment. The ionic current through one...
channel was detected with an Axopatch 200B amplifier. Data are filtered at 10 kHz and acquired at 200 kHz with the DigiData 1322A digitizer coupled with Clampex software (Axon Instruments). The measurements of the transients are based on the statistical analysis of the current traces using IGOR PRO software (WaveMetrics Inc.). Single-channel current traces are obtained between 40 and 155 mV (Fig. 1). A decrease in the applied voltage results in the open pore current decreasing from 165 ± 12 pA down to 41 ± 2.5 pA and we observe a decrease in the event frequency [Figs. 1(a)–1(c)]. Below 40 mV, no events are observed. The mean open pore current is plotted as a function of applied voltage, which indicates a linear behavior and a measured pore conductance, $G = 1069 \pm 15$ pS [Fig. 1(d)], in good agreement with previous experiments [32]. To separate the current blockades caused by dextran sulfate molecules swelling in the pore from the noisy pore current, a statistical analysis of each current transient is performed [32]. To separate the current blockades caused by dextran sulfate [34] at low ionic strength from 40 mV to 155 mV (Fig. 1). A decrease in the applied voltage results in the open pore current decreasing from 165 ± 12 pA down to 41 ± 2.5 pA and we observe a decrease in the event frequency [Figs. 1(a)–1(c)]. Below 40 mV, no events are observed. The mean open pore current is plotted as a function of the applied voltage [Figs. 1(a)–1(c)]. Below 40 mV, no events are observed. The mean open pore current is plotted as a function of the applied voltage [Figs. 1(a)–1(c)]. Below 40 mV, no events are observed. The mean open pore current is plotted as a function of the applied voltage [Figs. 1(a)–1(c)]. Below 40 mV, no events are observed.
the long time is clearly more convenient (Fig. 3). We have also performed experiments with a high molecular weight dextran sulfate 500 kDa at 0.5 mg/ml (upper panel). Distribution of blockade time $T_t$ for different dextran sulfate molecular weight (lower panel). The dextran sulfate is added to the cis compartment in 0.1M KCl, pH = 7.4 buffer, at a final concentration of 0.5 mg/ml.

The limiting stage of the translocation process could thus be the search of suitable conformations for entering the pore but not the transport of the molecules through the channel [28].

We have estimated the effective charge of the polyelectrolyte inside the pore from the voltage dependence of the translocation time (Fig. 3), $V_c = 58 \pm 3.6$ and $z_{pore} = 0.44 \pm 0.03$. The effective charge is much lower than the one deduced from the charge density of the dextran sulfate chains using Manning theory. If the dielectric constant of pore environment is that of water, the global condensation of negative charges of dextran sulfate inside the channel is expected to be around 85%, yielding $z_{bulk} = 7.5$. With the experimental value $z = 0.44 \pm 0.03$, the global condensation is found to be much larger: around 99%. The low value of the effective charge observed experimentally could be associated with an increased condensation of the counterions due to the confinement of the charges in the medium of low dielectric constant [37]. In the theory of Zhang and Shklovskii [37], the effective charge of the chains in neutral pore is in practice related to the normalized ionic current $\langle I_B \rangle / \langle I_0 \rangle$, where $\langle I_0 \rangle$ is the ionic current in the empty pore and $\langle I_B \rangle$ the mean blockade current. This ratio contains all the free-energy factors associated with the electrostatic interactions, screening effects, and confinement of the electric field in the pore. It represents the effective section of the pore available for ionic motion. In the range of applied voltage and salt concentration explored, the normalized ionic current is constant (Fig. 5).

We find, respectively, $\langle I_B \rangle / \langle I_0 \rangle = 0.23 \pm 0.04$ and...
due to the reduction of the cross section available for ion
process of ions inside the pore in the presence of a confined
field or ionic strength do not influence the conduction
with DNA, the mean blockade current depends on ionic strength and voltage [39], because the diameter of single strand chain (~1.4 nm) is higher than that of dextran sulfate (~0.4 nm). With DNA, the mean blockade current depends on ionic strength and voltage [39], and is probably due to the reduction of the cross section available for ion motion inside the pore.

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33. See EPAPS Document No. E-PRLTAO-100-015816 for statistical analysis of a current trace. For more information on EPAPS, see http://www.aip.org/pubservs/epaps.html.


