Single Polymer Molecules in a Protein Nanopore in the Limit of a Strong Polymer-Pore Attraction

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The capture and release of single poly(ethylene glycol) molecules by the alpha-Hemolysin pore are observed as time-resolved reversible steps in ion conductance. The capture on rate, inferred from the step frequency, decreases monotonically with polymer size. However, the polymer residence time shows a crossover behavior, first increasing and then decreasing with molecular weight. Our interpretation is that, in the case of polymers which are too large to be accommodated within the pore, the out-of-the-pore part of the molecule pulls on the trapped part, thus acting as an entropic spring.

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Progress in the physics of polymer partitioning into nanopores is important for many areas of science and technology. Recent experimental [1] and theoretical [2] work suggests that not only the quantitative but also the qualitative picture of polymer-nanopore interactions is still elusive. In what concerns ion conduction in nanostructures [3], among many questions of interest are the effects of water ordering by the surface, electrostatic and hydrodynamic interactions of ions with the channel walls and nonconducting inclusions, their hydration under the nanoscale confinement, etc.

Here we examine time-resolved blockages of ion current through the alpha-Hemolysin channel by single poly(ethylene glycol) (PEG) molecules exchanging between the channel pore and dilute bulk solution. To maximize polymer-pore attractions, promoted by salt [4], we use the extreme potassium chloride concentration, 4 M, which is close to the salt solubility limit in water at 23 °C [5]. This changes polymer partitioning *qualitatively*, strengthening it by orders of magnitude and slowing down exchange kinetics.

We find that the amplitude of the blockage depends on the polymer molecular weight. For smaller polymers, the dependence is consistent with a prediction based on the PEG effect on bulk solution conductivity [6]. For larger polymers, the effect on conductance deviates from such a prediction, suggesting that a fraction of the polymer molecule stays outside the channel. We also find that the residence time of a polymer molecule in the channel is a nonmonotonic function of polymer size. For molecular weights increasing from 600 to 3000, larger polymers stay in the channel longer. However, there is a crossover: The residence time reaches its maximum of ~100 ms for PEG3000 and starts to decrease with increasing molecular weight. As the polymer size reaches that of PEG8000, the residence time drops by about 2 orders of magnitude.

Example of traces of ion currents through single alpha-Hemolysin channels in the presence of differently sized PEGs in the membrane-bathing solution are shown in Fig. 1. The downward arrows indicate the instants of spontaneous channel insertion. Polymer concentrations and time scales are chosen to facilitate comparison of blockage events. Fast blockages by PEG600 [Fig. 1(a)] required a finer time scale and, therefore, a larger polymer concentration to show several representative events. Solution *p*H was chosen as 7.5 to stabilize the channel conformation and to minimize the noise of reversible protonation of the channel's residues [7]. It is seen that polymer molecules induce stepwise current fluctuations whose amplitude and duration depend on the polymer molecular weight.

The degree of blockage grows with the size of a polymer molecule, but the blockage is never complete. Figure 2 summarizes the results of statistical analysis of blockage amplitudes. PEG600 reduces current by about 50%, PEG4000 and larger polymers by 95%. The solid line compares the effect on channel conductance with the prediction for a PEG-induced decrease of bulk solution conductivity [6]:

$$\frac{\sigma}{\sigma_0} \cong (1-\phi)\exp\left(-2.7\frac{\phi}{1-\phi}\right), \qquad (1)$$

where σ/σ_0 is the ratio of bulk solution conductivity in the presence and absence of PEG and ϕ is the polymer volume fraction.

To perform this comparison, we assume that the channel pore captures the whole polymer molecule in a way that the polymer volume fraction in the pore can be calculated as $\phi = M_p(W)/\alpha M_w$. Here $M_p(W)$ is the mass of a single polymer molecule (in kilograms) with molecular weight W, α is the ratio of partial specific volumes of water and PEG $\alpha = \bar{v}_w/\bar{v}_p \cong 1.13$, and M_w, the total mass of water in the polymer-free pore, is the only adjustable parameter. The solid line in Fig. 2 is drawn according to Eq. (1) with $M_w = 6 \times 10^{-24}$ kg. This mass corresponds to 6 nm^3 of water, which is close to the volume of the stem part of the channel—the nearly cylindrical part which contributes

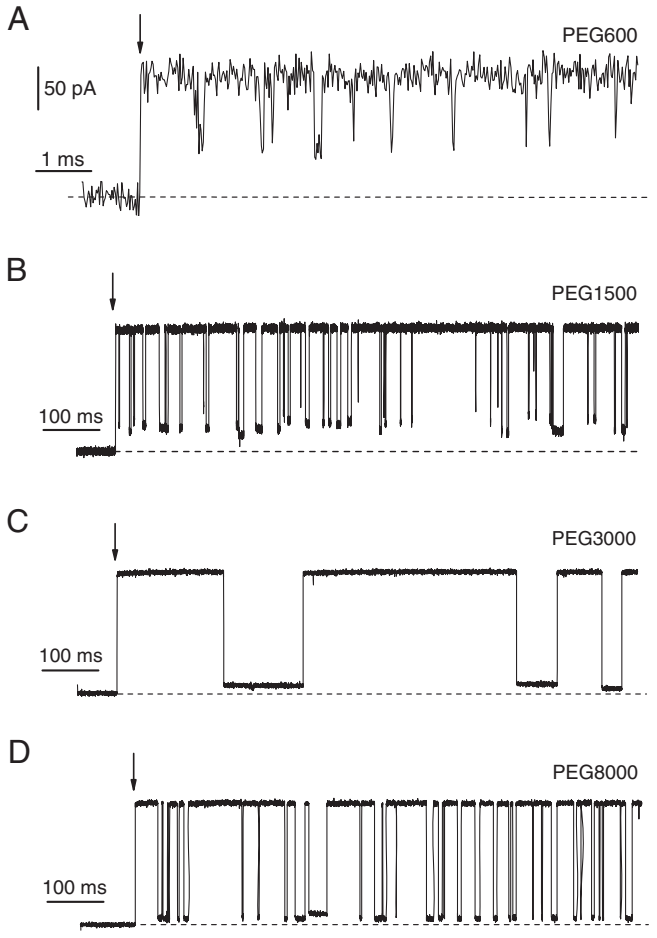

FIG. 1. In the presence of PEG, the ion current through the alpha-Hemolysin channel exhibits stepwise transitions between completely open and partially blocked states. The amplitude, frequency, and duration of these transient steps depend on the polymer molecular weight. (A) 400 μM PEG600, time averaging 5 μs; (B) 40 μM PEG1500, time averaging 30 μs; (C) 2 μM PEG3000, time averaging 100 μs; (D) 20 μM PEG8000, time averaging 100 μs. Note the change in the time scale from (A) to (B). Bilayer lipid membranes of 20 pF capacitance were formed from diphytanoyl phosphatidylcholine (Avanti Polar Lipids, Alabaster, AL, USA) at a room temperature of $23 \pm 2\,^{\circ}\mathrm{C}$ as previously described [4]; membrane-bathing aqueous solution contained 4 M KCl at *p*H 7.5 buffered by 5 mM Tris with PEG (polyethylene glycol standard, Fluka, GmbH, Germany) added from the "*trans*" side of the membrane; alpha-Hemolysin was added from the "*cis*" side of the membrane; applied voltage was 40 mV, negative from the side of protein addition.

most to the total channel resistance [4]. Equation (1) fits the three data points for the smaller polymers satisfactorily. For polymers larger than PEG1500, the experiment gives less conductance decrease than predicted. Our interpretation is that, starting from PEG2000, the polymers are too large to be completely accommodated in the cylindrical part of the channel. Consequently, their volume fraction in the pore is smaller than $M_p(W)/\alpha M_w$.

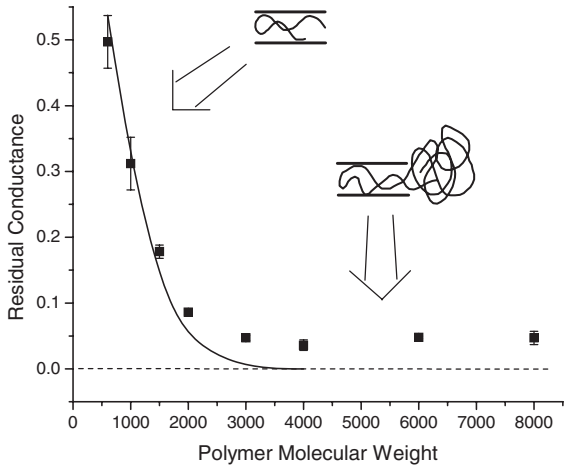

FIG. 2. The relative conductance of the channel in the polymer-blocked state shows that the blocking efficiency of PEG increases with its molecular weight saturating to about a 95% level for PEG4000 and larger. The solid line is a fit by Eq. (1) to the data for the smaller PEG600, PEG1000, and PEG1500. The volume fraction ϕ is set proportional to the polymer molecular weight with a common adjustable parameter described in the text.

The agreement between the blockage amplitudes for small polymers and their effect on bulk solution conductivity suggests that only one polymer molecule is involved in each blockage event. Additional support for a monomolecular reaction is provided by the analysis of on rates. We collect statistics of the time intervals between the end of a blockage event and the onset of the next one to find the average values. Figure 3(a) illustrates the procedure for PEG1500. The inset in Fig. 3(b) shows the average time interval τ_{on} as a function of polymer concentration. To minimize the natural spread in the properties of individual channel-forming molecules [4], the data in the inset were taken from an experiment with the same single channel, where polymer concentration was increased by consecutive additions of more concentrated PEG solutions. The inverse proportionality between τ_{on} and PEG concentration indicates that the blockage is a monomolecular reaction.

The on rate coefficient, defined as $k_{\mathrm{on}} = 1/(\tau_{\mathrm{on}}[c_p])$, where $[c_p]$ is the polymer concentration in the membrane-bathing solution, is given in Fig. 3(b) as a function of polymer size. Though the data (collected from different channels) show significant spread, it is clear that the decrease in the on rate is moderate. It is compatible with the decrease in the polymer diffusion coefficient in the bulk $D(W) \sim W^{-3/5}$ (the solid line through the data). For 1 μM polymer concentrations, the average frequency of events drops from about 2 per second for PEG600 to about 0.5 per second for PEG8000. Thus, the entropic part of the interaction, which is the leading term at small salt concentrations [4,8,9] and which allows pore sizing by polymer partitioning [10], is not seen here.

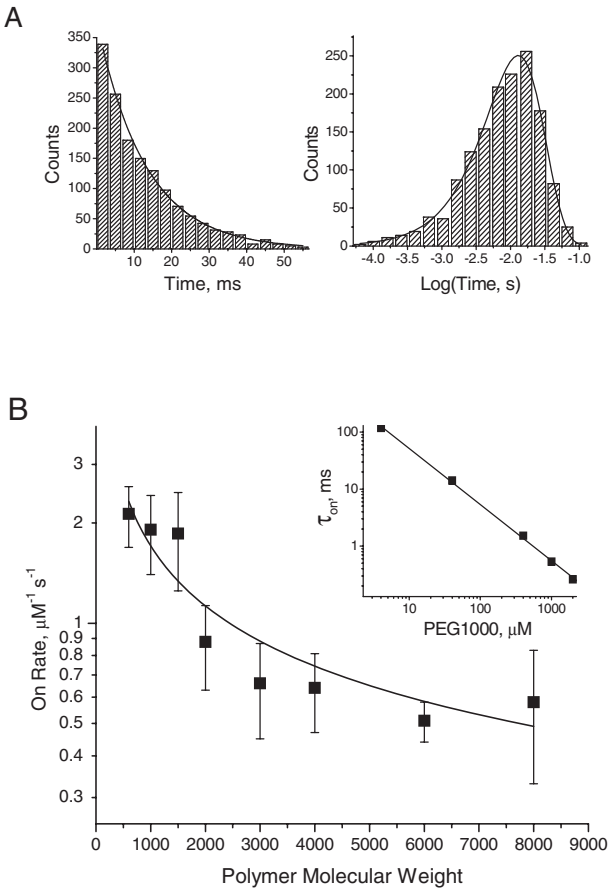

FIG. 3. The on rate coefficient of polymer capture by the channel is a surprisingly weak function of polymer molecular weight. (A) The time between successive blockages is distributed exponentially as shown for 40 μM PEG1500. Both linear (left) and logarithmic [15] (right) plots show excellent fits to a single exponential (solid lines) with characteristic time $\tau_{on} = 13$ ms. (The total number of events was 1470.) In the case of a logarithmic time axis, the histogram is logarithmically binned. Therefore, the single exponential fitting curve is $\propto t \exp(-t/\tau_{on})$; it exhibits a maximum at $t = \tau_{on}$. (B) Results of the on rate analysis for all polymers. Data for at least 3 single channels reconstituted in separate experiments were used for the plot. Inset: The average time between successive blockages is inversely proportional to the PEG1000 concentration.

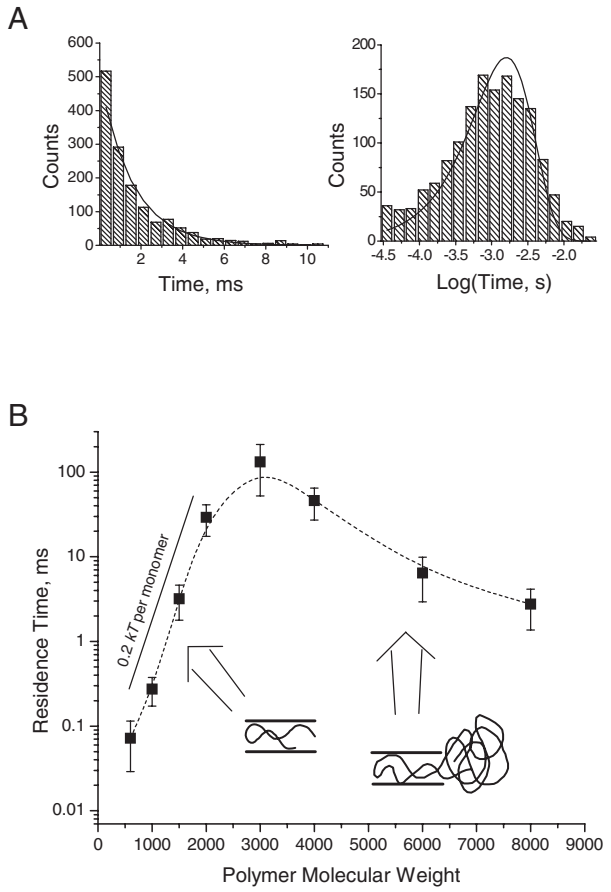

FIG. 4. The residence time of a captured polymer vs polymer molecular weight shows a crossover. (A) The residence time distributions demonstrate statistically significant deviations from a single exponential (solid lines) with characteristic time 1.6 ms in both linear (left) and logarithmic (right) plots. Both longer and shorter time tails are present. Statistics were collected from the same track of 1470 events as in Fig. 3(a). (B) The data for all polymers obtained through the single exponential fitting illustrated in (A). The decreasing portion of the curve corresponds to polymers that are too large to be accommodated by the channel. We hypothesize that the free part of the polymer molecule acts as an entropic spring.

In a related study—molecular dynamics simulations of the flow of macromolecules into a slit of nanometer dimension with strongly attracting walls [11]—a similar weak dependence of the flow parameters on polymer molecular weight was found. However, it is difficult to make a direct comparison with our results, because the simulation study was performed under strongly nonequilibrium conditions. Also importantly, in experiments reported here, we used dilute polymer solutions where nonideality effects [12] are virtually nonexistent. The largest concentration used was 0.5%, which is well below the c^* concentration for these PEGs.

The entropic interaction kicks in, rather surprisingly, in the off rate of polymer capture. The average time of polymer residence in the channel as a function of polymer molecular weight (Fig. 4) shows a crossover behavior. For PEG600 to PEG3000, the residence time increases with molecular weight. The initial increase in τ_{off} is close to exponential. Using simple reasoning that involves escape from a deep potential well whose depth $\Delta F(N)$ is proportional to the number of monomers N, we can write

$$\tau_{off} \sim \exp\left(\frac{\Delta F(N)}{kT}\right), \qquad \Delta F(N) = \chi N. \tag{2}$$

Defining the number of monomers within a PEG molecule

as $N = W/44$, from the initial slope of the curve (solid line), we infer $\chi \approx 0.2$ kT.

The residence time reaches a maximum at PEG3000 and then starts to decrease with polymer size. This maximum roughly coincides with the onset of the deviation of the residual conductance seen in Fig. 2. We believe that both observations are consequences of the finite length of the channel which is not long enough to accommodate all monomers of the larger PEGs. The part of the polymer molecule that protrudes from the channel (see the cartoon in the right lower corner in Fig. 4) acts as an entropic spring that pulls on the trapped part of the molecule. Indeed, any deformation of a polymer molecule, which reduces the number of its free-space conformations and thus decreases its entropy, is counteracted by an "entropic force." For PEG8000, the free energy of spring extension can be roughly estimated from the reduction of the residence time as $F_{\text{entropic}} \approx kT \ln(\tau_{\text{off PEG3000}}/\tau_{\text{off PEG8000}})$ and is close to 4 kT. For a hypothetical extension $R = 5$ nm, this yields a force of ~6 pN. This result is in reasonably good agreement with the pulling-out force estimated according to the bead-spring model (e.g., Ref. [13])

$$f \approx \frac{3kT}{Na^2} R, \tag{3}$$

with $a \simeq 0.35$ nm [14] and $N \simeq 180$ (for PEG8000), which gives ~3 pN.

Before summarizing our main findings, we note that partitioning of water-soluble polymers into ion channel pores has long been used for their sizing [10]. In the present study, we went to the extreme 4 M KCl. This changed polymer partitioning qualitatively and allowed us to observe time-resolved PEG-induced blockages to identify the following: (i) Amplitudes of the blockages (Fig. 2) suggest that for small polymers the conductance of the PEG-containing channel scales similarly with the conductivity of bulk PEG-containing solutions. Based on this, we conclude that the nanoscopic volume of water in the alpha-Hemolysin pore behaves quite macroscopically with respect to conduction of ions. (ii) The anomalously weak dependence of the capture on rate on polymer molecular weight (Fig. 3) eludes even qualitative explanation. One possibility could be that the entropic repulsion associated with polymer capture is almost completely suppressed by the strong polymer-pore attraction. The low absolute value of the on rate might also imply that some kind of critical fluctuation is necessary for polymer entry. (iii) The sharp increase of the residence time in the pore with PEG molecular weight for small polymers (Fig. 4) allows us to quantify the salt-induced attraction. The estimate is 0.2 kT per monomer. The crossover at higher polymer sizes, together with the leveling off of the blockage amplitude (Fig. 2), suggest that the channel is not spacious enough to accommodate larger polymers and that the out-of-the pore part of the polymer molecule acts as an entropic spring.

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