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Electrostatic Control of Polymer Translocation Speed through α -Hemolysin Protein Pore

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Abstract

The electrostatic origins behind the speed of translocation of a uniformly charged flexible macromolecule through α -hemolysin (α HL) protein pores under a voltage are investigated using variations in pH and electrolyte concentration. We have measured durations of successful threading of poly(styrenesulfonate) through α HL at two different pH conditions, pH 4.5 and pH 7.5, under various salt concentration conditions. Salt concentrations in the donor (*cis*) and the recipient (*trans*) compartments influence the polymer translocation dynamics differently, depending on pH. At both pH 4.5 and pH 7.5, decreasing the *cis* salt concentration, $c_{s,cis}$, results in faster polymer translocations. On the other hand, a decrease in *trans* salt concentration, $c_{s,trans}$, retards the polymer transport process at pH 4.5, while at pH 7.5 the translocation time is observed to be independent of $c_{s,trans}$. We present a theoretical model to calculate the translocation times from the free energy of the polymer along the translocation process to describe our experimental results. We show that the charge density of the polymer inside the nanopore is significantly affected by $c_{s,cis}$, explaining the *cis* salt effect on the speed of polymer translocation. The *trans* salt effects are attributed to the electrostatic interaction between the polymer and the exit portion of the α HL pore, which is determined by the pH of the *trans* compartment. At low pH where the net charge of the end of the α HL is positive, the attractive electrostatic interaction in *trans* becomes stronger, as $c_{s,trans}$ decreases, resulting in delays in translocation process.

Graphical abstract

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.macromol.6b01663. Additional data for effect of polymer-pore interaction on the mean polymer translocation time and for changes in q upon choosing different k_0 values (PDF)

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Notes

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INTRODUCTION

Electrophoretic transport of charged macromolecules through nanometer-scale biological pores or solid-state nanopores, typically called “polymer translocation”, has been an active field of research in view of characterizing chemical or physical identities of synthetic and biological polymers as well as studying fundamental properties of single macromolecules under confined states.^{1–25} The experimental arrangement is the following. Transport of single macromolecules driven by an externally applied electric field from one side of a thin membrane to the other side through a nanopore causes temporal blockades of the electrolyte current flowing through the pore. Durations and depths of the ionic current blockades are collected from the recorded current traces and interpreted to obtain information on the analytes.

Despite considerable efforts in the past two decades, details of physical mechanisms of polymer translocation processes are not yet fully understood. The whole process, including polymer–pore encounters and polymer threading through a nanopore, involves not only the drift motion of the polymer chain but also complex convective flows through the pore, chain entropy contributions, and local pore–polymer interactions. These contributing factors are in turn governed by the circumstances of the system such as salt concentration, temperature, pH, and the applied voltage bias as well as the specificity of the nanopore. Studying effects of these contributing factors on polymer translocation process through systematically designed experiments will facilitate further understanding of the physical mechanisms of polymer translocation.

In an effort to understand the principles of polymer translocation process, we have recently studied the effects of salt concentrations in donor (*cis*) and recipient (*trans*) compartments on the frequency of electrophoretic polymer captures, R_c , using α -hemolysin protein pores.²⁶ Unlike the results on silicon nitride (SiN) nanopores reported earlier,²⁷ upon changes in the salt concentrations, effects of electrostatic interactions between the polymer molecules and the entrance of the protein pore are dominant in polymer capture process under a low salt concentration condition over the additional electrophoretic forces induced by the salt asymmetry between *cis* and *trans*, resulting in nonmonotonic dependence of R_c as a function of *cis* salt concentration.

In the present study, in order to fully understand the salt concentration effects on the polymer translocation process, we extend our work to investigating salt concentration effects on polymer translocation dynamics by measuring durations of successful polymer translocations through α HL protein pores. The pore has about 1.4 nm of the inner diameter

at the narrowest point,²⁸ allowing only single-file translocations for single-stranded polynucleotides and flexible synthetic polyelectrolytes.

EXPERIMENTAL SECTION

The lipid molecules 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine and α -toxin from staphylococcusaureus (α HL) for forming lipid bilayers and protein pores are purchased from Avanti Polar Lipid Inc. (Alabaster, AL) and Sigma-Aldrich (St. Louis, MO), respectively. The sodium salts of poly(styrenesulfonate) (NaPSS) standards with narrow polydispersities (PDI < 1.2) are purchased from Scientific Polymer Product, Inc. (Ontario, NY).

Free-standing lipid bilayer membranes are prepared using the method reported by Montal and Mueller²⁹ on an aperture in 12.5 μ m thick Teflon membrane (Hansatech Instruments Ltd. UK) that separates two Teflon chambers, each about 0.5 mL. Both chambers are filled with potassium chloride (KCl) solutions with 10 mM 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) in deionized water (18 M Ω cm) with pH adjusted by adding potassium hydroxide (KOH) or hydrochloric acid (HCl). After a lipid bilayer prepared, $\sim$ 10 ng of α HL is added into one side of the Teflon membrane, set as a donor compartment (*cis*), to form single protein pores. Formation of a single pore can be identified by observation of a rapid increase in ionic current as much as $\sim$ 100 pA in room temperature while 100 mV (positive in *trans*) is applied across the membrane. Once a single pore is prepared, *cis* or *trans* chamber is flushed with excess amount of HEPES buffer with a desired KCl concentration. The temperature of the system is controlled using a water circulating brass block surrounding the Teflon chambers.

0.1–1 nmol of NaPSS polymers is introduced into the *cis* chamber, depending on the applied voltage, pH, and salt concentration of the system. The electric potential difference between the two chambers is controlled using lab-made silver/silver chloride (Ag/AgCl) electrodes connected to Axopatch 200B integrated patch clamp amplifier (Axon Instruments, CA). A Digidata 1550 data acquisition system and Axoscope software (Axon Instruments) are employed to record the ionic current through the α HL pore at 250 kHz of data acquisition rate, and the recorded ionic current traces are analyzed using MATLAB (The MathWorks, Inc., Natick, MA) to capture the current blockade events.

As well-known from the earlier studies, and also as seen in Figure 1a, two distinct levels of partially blocked ionic currents are observed for translocations of single stranded-DNA or NaPSS molecules through a α HL pore due to the existence of a spacious vestibule and a narrower β -barrel regions inside the pore.^{2,3,19,30} Shallower current blocking levels correspond to the occupation of the vestibule by a polymer chain and the deeper current levels correspond to the polymer threading through the β -barrel. Durations of the deeper current blocking states are collected as successful translocation times, termed τ_2 . Appropriate threshold for measuring τ_2 varies for different experimental conditions, such as temperature, salt concentrations, pH, and the applied voltage. We use 30–35% and 5–10% of the open pore currents as the current thresholds for pH 7.5 and pH 4.5, respectively, at 30 °C.

Polymer translocation process is intrinsically stochastic, resulting in typical distributions in τ_2 as seen in Figure 1b. In the present study, we use a log-normal distribution function to fit the histograms of τ_2 collected from the translocation experiments.

$$f(\tau_2; \tau_{2,c}, w) = \frac{1}{w\tau_2\sqrt{2\pi}} \exp\left[-\frac{(\ln\tau_2 - \ln\tau_{2,c})^2}{2w^2}\right] \quad (1)$$

with two fitting parameters $\tau_{2,c}$, the mean τ_2 , and w , the standard deviation. The most probable translocation times, $\tau_{2,\text{peak}}$, are obtained as $\tau_{2,\text{peak}} = \tau_{2,c} \exp(-w^2)$ and used as representative successful translocation times to interpret the experimental results.

RESULTS AND DISCUSSION

Cis Salt Concentration Effect on Polymer Translocation

Measuring durations of successful translocations, τ_2 , of NaPSS through an α HL pore show that changes in *cis* salt concentration, $c_{s,cis}$, significantly influence the dynamics of polymer translocation process. The histograms of τ_2 under two different pH, pH 7.5 and pH 4.5, are shown in Figure 2a for different $c_{s,cis}$ from 0.5 to 2 M, while the salt concentration in *trans* is maintained to be 1 M, for 35 kDa NaPSS ($M_w = 35$ kg/mol) at 140 mV and 16 kDa NaPSS ($M_w = 16$ kg/mol) at 100 mV, respectively. For both pH conditions, faster polymer translocations are observed at lower $c_{s,cis}$. In Figures 2b and 2c, $\tau_{2,\text{peak}}$ is plotted as a function of the applied voltage for different $c_{s,cis}$, showing the decrease in $\tau_{2,\text{peak}}$ with a decrease in $c_{s,cis}$ under the entire voltage range used in this study.

There can be many factors affecting the dynamics of polymer translocation upon change in $c_{s,cis}$. The candidates include the pore–polymer electrostatic interaction, electroosmotic flow induced by charges on the pore wall, polymer chain flexibility, and osmotic flow induced by the salt asymmetry between *cis* and *trans* compartments. The same trend in τ_2 upon changes in $c_{s,cis}$ at different pH conditions (Figure 2a) supports the idea that the electroosmotic flow is not significantly affecting the polymer threading through an α HL pore. Also, if the osmotic flow of water molecules through the pore induced by salt asymmetry influences the translocation dynamics, τ_2 should be dependent on the ratio of $c_{s,cis}/c_{s,trans}$, not only on $c_{s,cis}$ at pH 7.5 as seen in the *trans* effect section (see below). On the basis of the fact that decrease in $c_{s,cis}$ accelerates the polymer threading both at pH 7.5 and pH 4.5, we attribute the dependence of τ_2 on $c_{s,cis}$ to the change in the charge density of the polymer chain inside the α HL pore upon change in $c_{s,cis}$. It is known that the effective charge of polyelectrolytes is different from its nominal charge due to adsorptions of counterions on the polymer.³¹ Furthermore, it is reported that the degree of counterion adsorption depends on the chain length, ionic strength, and extent of polymer confinement.³² We expect that the effective charge density of a polymer chain during the translocation process, under highly confined state and strong electric field inside the nanopore, is largely influenced by the bulk salt concentration, resulting in changes in polymer translocation speed. For example, when *cis* salt concentration is lowered, the degree of counterion adsorption is decreased, causing an

enhanced electric force driving the polymer translocation, which is consistent with the experimental observations given in Figure 2.

To interpret our experimental results, we employ the theoretical model of Di Marzio and Mandell³³ and Muthukumar^{34,35} describing single-file polymer translocation through a narrow cylindrical channel, as illustrated in Figure 3. Using this model, a free energy change of the polymer chain along the translocation process can be formulated by considering an interaction energy between monomers and the channel wall, the polymer chain conformational entropy, and the electric potential contribution, and taking the number of monomers already entered into the channel, m , as the primary variable. Following Muthukumar's work,³⁵ the free energy of the polymer chain under electrophoretic translocation, for different values of m , is given by

$$\frac{F(m)}{k_B T} = -\frac{\epsilon m}{k_B T} - \frac{|q|V}{2M} \frac{1}{k_B T}, \quad 0 \leq m \leq M \quad (2)$$

$$\frac{F(m)}{k_B T} = M \left(-\epsilon - \frac{|q|V}{2} \right) \frac{1}{k_B T} - \frac{|q|V}{k_B T} (m - M), \quad M \leq m \leq N \quad (3)$$

$$\frac{F(m)}{k_B T} = -\epsilon (N + M - m) \frac{1}{k_B T} - \frac{|q|V}{2M k_B T} [M^2 - (m - N)^2] - \frac{|q|V}{k_B T} (m - M), \quad N \leq m \leq N + M$$

(4)

where N is the degree of polymerization, M is the length of the channel in the unit of the monomer length, ϵ is an interaction energy between a monomer and the channel wall, q is charge per monomer, and V is the applied voltage. We note here that the chain conformational entropy term is ignored due to its negligible contribution in comparison with the other two terms, viz., pore-polymer interaction and electric potential contributions.

Using the Fokker-Planck formalism, the average translocation time τ can be calculated from the free energy profile given by eqs 2–4³⁵

$$\tau = \frac{1}{k_0} \int_0^{N+M} dm \int_0^m dm' \exp \left[\frac{F(m)}{k_B T} - \frac{F(m')}{k_B T} \right] \quad (5)$$

where k_0 is a rate constant, which is a phenomenological parameter reflecting the monomer friction against the pore. Here we make comparisons between the mean τ obtained from eq 5

with appropriate parameters and $\tau_{2,\text{peak}}$ values measured from the experiments. Equation 5 allows us to confirm that the slopes in the plots of mean τ vs the polymer length N , for a given M are determined by the electric force factor, $qV/k_B T$, independent of the pore–polymer interaction, ϵ (see the Supporting Information). Since the pore–polymer interaction is not homogeneous along the pore length, we fit the experimentally obtained $d\tau_{2,\text{peak}}/dN$ with the theory so that the result is not dependent on the value ϵ chosen. To do this, we measure τ_2 for different molecular weights of NaPSS at pH 7.5 under different applied voltages, from 140 to 180 mV, and the *cis* concentrations, 0.75, 1, and 1.5 M.

In Figure 4, the $\tau_{2,\text{peak}}$ values measured in the experiments are plotted as a function of N , for different values of voltage difference. Having two unknown parameters, k_0 and q , in the calculation of the mean τ , we obtain the relative changes in q for a certain value of k_0 from the fitting of the experimental results with theory, for different applied voltages and *cis* salt concentrations. The dashed curves in Figure 4 are fits to the model for $M = 15$, acknowledging the length of the β -barrel of α HL pore as 5 nm and the average size of a NaPSS monomer as 0.33 nm, and $\epsilon = 0$, when $q = 0.01$ is assumed for $V = 140$ mV and $c_{s,\text{cis}} = 1$ M which gives $1/k_0 = 5.41 \times 10^{-4}$ ms, for example. Here, k_0 is assumed to be the same for different V and $c_{s,\text{cis}}$. The effective polymer charge densities inside the nanopore during the translocation from the fittings are plotted in Figure 5 for different voltages and $c_{s,\text{cis}}$ conditions when $1/k_0 = 5.41 \times 10^{-4}$ ms is used. It is clear that the effective charge parameter q increases with the applied voltage, V . This is consistent with the simulation results reported in ref 33 as mentioned above. Closer inspection of the ionic current data for different applied voltages also supports this conclusion as seen in the inset showing the distributions of the averaged blocked ionic currents for the translocation events, $\langle I \rangle$, with respect to the open pore current, I_0 . Increased $\langle I \rangle/I_0$ for larger V means that there are more free, unbound ions flowing through the pore upon the presence of a polymer chain inside the nanopore, corresponding to the larger value of q . In addition we find that the effective charge density of the polymer chain is larger at lower *cis* salt concentrations. The larger q causes an enhanced electrophoretic force exerted on the polymer chain inside the pore. As a result, the polymer transports through the pore faster in lower *cis* salt concentrations, explaining the $c_{s,\text{cis}}$ effect on the translocation dynamics as seen in Figures 2 and 4. We note that the changes in q with $c_{s,\text{cis}}$ and V are qualitatively same regardless of the selection of k_0 (see the Supporting Information).

Trans Salt Concentration Effect on Polymer Trans-location

To study *trans* salt concentration ($c_{s,\text{trans}}$) effects on the polymer translocation process, we measure the successful translocation times, τ_2 , for different $c_{s,\text{trans}}$ while $c_{s,\text{cis}}$ is fixed. The τ_2 histograms for different $c_{s,\text{trans}}$ at pH 7.5 and pH 4.5 for 35 kDa NaPSS under 160 mV and for 16 kDa NaPSS under 120 mV, respectively, are given in Figure 6a. Here the interesting observation is that the changes in τ_2 upon varying $c_{s,\text{trans}}$ are different at different pH conditions; the translocation time is observed to be independent of $c_{s,\text{trans}}$ at pH 7.5 (left), while at pH 4.5 the NaPSS translocation process is observed to be slower with decrease in $c_{s,\text{trans}}$, which is shown as a shift in the τ_2 distribution to larger values (right). The increase in τ_2 as $c_{s,\text{trans}}$ is decreased for fixed $c_{s,\text{cis}}$ is the opposite of the effect seen in τ_2 as $c_{s,\text{cis}}$ is decreased for fixed $c_{s,\text{trans}}$. In Figures 6b and 6c, we show that these $c_{s,\text{trans}}$

effects on τ_2 are observed within the entire applied voltage range used in this study, from 130 mV to 180 mV at pH 7.5 and from 120 mV to 160 mV at pH 4.5.

We attribute the *trans* salt concentration effects to the electrostatic interactions between the polymer chain and the α HL pore in the *trans* compartment. For α HL pore, it is known that seven aspartic acid residues and seven lysine residues are located at the exit location of the β -barrel, having their side groups inward.²⁸ We have estimated, taking the pK_a values of the side groups of aspartic acid and lysine as 3.9 and 10.5, the net charge of the ring at the end of the β -barrel as a function of pH using the equation³⁶

$$Z = \sum_i N_i \frac{10^{\frac{pK_{a_i}}{10}}}{10^{\frac{pH}{10}} + 10^{\frac{pK_{a_i}}{10}}} - \sum_j N_j \frac{10^{\frac{pH}{10}}}{10^{\frac{pH}{10}} + 10^{\frac{pK_{a_j}}{10}}} \quad (6)$$

where i and j correspond to the positively chargeable and the negatively chargeable amino acid residues, respectively, and N is the number of the amino acid residues. The net charge of the ring at the end of the α HL pore is plotted as a function of pH in Figure 7a. On the basis of this, we can expect that during the polymer translocation process the monomers inside the β -barrel and also the monomers in the *trans* side are attracted by the net positive charge of the α HL pore at low pH (<6.0), as illustrated in Figure 7b. This explains the $c_{s,trans}$ effect on τ_2 at pH 4.5. When the *trans* salt concentration is lowered, the pore-polymer electrostatic attraction becomes stronger, retarding the motion of the polymer chain leaving the pore. This idea is also consistent with our observations that changes in $c_{s,trans}$ do not affect $\tau_{2,peak}$ at pH 7.5 (Figure 6) because, based on Figure 7b, the pore-polymer electrostatic interaction at the exit of the β -barrel is negligible at pH 7.5.

CONCLUSIONS

We have investigated the process of the electric-field-driven single-file translocations of the flexible polyelectrolytes NaPSS through α HL pores, especially to understand the effects of salt concentrations in donor (*cis*) and recipient (*trans*) compartments on the successful translocation time, τ_2 . The translocation time is observed to be decreasing as the salt concentration in *cis*, $c_{s,cis}$, is lowered at both pH 7.5 and pH 4.5. Using a theoretical model, we demonstrate that the charge density of the polymer inside the α HL pore is substantially increased with decreasing $c_{s,cis}$, leading to stronger electric force which drives the polymer threading motion. On the other hand, the effects of *trans* salt concentration, $c_{s,trans}$, on polymer translocations depend on the electrostatic characteristics of the α HL pore. At pH 4.5, where the net charge of the end of the β -barrel is estimated to be positive, τ_2 is found to significantly increase as $c_{s,trans}$ is decreased. In contrast, τ_2 is independent of $c_{s,trans}$ at pH 7.5. We argue that the delayed polymer threading as $c_{s,trans}$ is lowered in low pH is mainly due to the augmented attractive electrostatic forces between the negatively charged NaPSS monomers in the *trans* side and the positive charges at the pore exit.

We note that the results from this study are qualitatively different from the earlier work done by Wanunu and collaborators with double-stranded DNA (dsDNA) translocation through

silicon nitride (SiN) nanopores.²⁷ The speed of electrophoretic threading of dsDNA through a solid state SiN nanopore is dependent on the ratio between the salt concentrations in *cis* and *trans* sides. They reported that the translocation time of dsDNA becomes longer as $c_{s,cis}/c_{s,trans}$ decreases. This is qualitatively opposite to our results in terms of $c_{s,cis}$ effects on τ_2 . The discrepancy can be explained by the identity of the nanopore used in these studies. The relatively homogeneous distribution of negative charges on the wall of the SiN nanopore and on the rigid dsDNA backbone may induce substantial electroosmotic flow (EOF) in the opposite direction of the polymer threading whereas for the α HL pore, which has heterogeneous charge distributions, the effect of EOF on the polymer translocation is known to be minor. For the solid state nanopores, the asymmetric salt concentrations ($c_{s,cis}/c_{s,trans} < 1$) strengthen the EOF, and as a result, the polymer translocation can become slower upon decrease in $c_{s,cis}/c_{s,trans}$ for SiN nanopores.

The theoretical model used here is able to capture the essential features of electrostatics on the characteristics of translocation and provides insight into slowing down charged polymers through protein pores. Also, the much simpler experimental manipulations using variations in pH and electrolyte concentration demonstrated here might be an alternate methodology to synthesis of charge-modified protein pores using site-directed mutagenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(a) A typical ionic current trace recorded in the experiments of NaPSS translocation through an α HL nanopore (57.5 kDa NaPSS in 1 M KCl (pH 7.5), under 150 mV at 30 °C). The threshold current for measuring durations of the successful translocations is set to be 30% of the open pore current. (b) The most probable translocation times, $\tau_{2,\text{peak}}$, are obtained by fitting a histogram of the successful translocation times, τ_2 , using a log-normal distribution function.

Figure 2.

(a) τ_2 histograms for 35 kDa NaPSS ($M_w = 35$ kg/mol) under 140 mV/pH 7.5 (left) and 16 kDa NaPSS ($M_w = 16$ kg/mol) under 100 mV/pH 4.5 (right) for varying the $c_{s,cis}$ from 0.5 to 2 M KCl, while $c_{s,trans}$ is fixed at 1 M. The most probable translocation times, $\tau_{2,peak}$, of (b) 35 kDa NaPSS at pH 7.5 and (c) 16 kDa NaPSS at pH 4.5 are plotted as a function of the applied voltage for different $c_{s,cis}$ concentrations, showing decreases in $\tau_{2,peak}$ as $c_{s,cis}$ lowered for both pH conditions.

Figure 3. Illustration of a model for the electric-field-driven single-file polymer translocation through a narrow cylindrical channel.

Figure 4.

$T_{2,\text{peak}}$ from the experiments (points) and the average translocation times from the calculations (dashed lines) for *cis* salt concentrations of (a) 0.75, (b) 1, and (c) 1.5 M ($c_{s,\text{trans}} = 1$ M/pH 7.5/30 °C). The $1/k_0$ is chosen as 5.41×10^{-4} ms for the τ_2 calculations.

Figure 5.

Polymer charge density q used in the translocation time calculations of the plots in Figure 4 is given as a function of the applied voltage for different *cis* salt concentrations. Here, $q = 0.01$ at 140 mV for $c_{s,cis} = 1$ M is arbitrarily chosen, giving $k_0 = 5.41 \times 10^{-4}$ ms.

Figure 6.

(a) τ_2 histograms for 35 kDa NaPSS under 140 mV/pH 7.5 (left) and 16 kDa NaPSS under 100 mV/pH 4.5 (right) for varying the $c_{s,trans}$ from 0.25 to 1.5 M KCl, while $c_{s,cis}$ is fixed at 1 M. The most probable translocation times $\tau_{2,peak}$ are plotted as a function of the applied voltage for different *trans* salt concentrations for (b) 35 kDa NaPSS at pH 7.5 and for (c) 16 kDa NaPSS at pH 4.5.

Figure 7.

(a) Net charge of the ring at the end of α HL pore is plotted as a function of pH. The ring is composed of seven aspartic acids ($pK_a = 3.9$) and seven lysines ($pK_a = 10.5$). (b) At low pH, the positive charges at the end of the β -barrel attract the negatively charged polymer segments inside the β -barrel and also those in trans side. The extent of the electrostatic interaction is expected to be changing upon change in $c_{s,trans}$.