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Biopolymer Entering into and Escaping from a Nanometer Pore

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Abstract We theoretically investigated negative entropy S of biopolymer which passes through a nanometer pore(such as α-hemolysin), especially entering process and escaping process, on the basis of which we also studied biopolymer entering-pore time τ_{ent}, biopolymer entering mean velocity v_{ent}, biopolymer escaping-pore time τ_{esc}, and biopolymer escaping mean velocity v_{esc}, respectively. Our results illustrate that the entering and escaping processes of biopolymer depend on its negative entropy, and entering process is more difficult than escaping process for biopolymer translocation. This tremendous difference between the two processes will offer a useful engineering hint for single macromolecule identification.

Keywords Biopolymer; Nanometer pore; Negative entropy; Entering; Escaping

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1 Introduction

Sliding biopolymer entering into a nanometer biological pore is one of the fundamental and complex life processes including protein transport from the endoplasmatic reticulum to the Golgi apparatus, DNA and RNA translocation through nucleus pores, viral DNA injection into a host cell and drug delivery to cells[1—3]. Based on those processes, nanopore-related biotechnological applications, *i.e.*, fast nanopore DNA sequencing, nanopore sensor application and nanopore analytic detection, have also been paid extensive attention and excellent outcomes have been obtained[4—11], but the mechanism of biopolymer sliding process and the bottleneck of nanopore identification are still a challenging topic in polymer physics and nanotechnology.

In the past decades, researchers have built some theoretical models, which provide useful insights for understanding the basic feature of biopolymer threading a pore[12—15]. Muthukumar[16], Sung[17], Slonkina[18], and Ding *et al*.[19] utilized classical statistic theory and nucleation theory to study biopolymer translocation through a nanopore where the monomer friction across the pore is proportional to the length scaling of the biopolymer. For a long biopolymer, the whole translocation time τ-N^2 without external field, and τ-N in a strong external field[16]. Recently, Muthukumer *et al*.[20] also presented a modeling algorithm to the simulation of single biopolymer molecules sliding through protein channels on the basis of a combination of Langevin dynamics for coarse-grained models of biopolymers and the Poisson-Nernst-Planck formalism for ionic current. To characterize biopolymer translocation, the whole process can be divided into entering, passing, and escaping processes[18,21]. These results illustrate that the entropy barrier(EB) of biopolymer in whole translocation process can be overcome[16,17]. Negative entropy can be regarded as the conformational scaling of the biopolymer order and the fundamental component of the biopolymer system free energy F[22], threaded-biopolymer for entering pore process S_{ent} and escaping pore process S_{esc} were paid less attention. Along with the EB mechanism, we aim at the biopolymer terminational process, that is, the entering and escaping processes. To gain some insight into these processes with real experimental conditions, we selected α-hemolysin(α-HL) and considered the length scaling of biopolymer is longer than the length of α-HL in this article. The order of the selected threaded-biopolymer was evaluated by negative entropy, which was numerically calculated based on previous researches and

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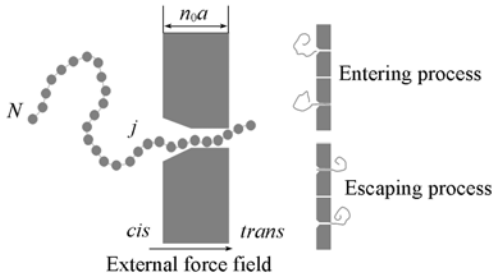
classical statistics[23].

For linear biopolymer in a good solvent, it can be represented as one chain containing N monomers. So its contour length $L=Na$, where a is the monomer-monomer equilibrium distance. As to the nanopore, it is regarded as an approximate cylindrical tube of length $l_0(=n_0a)$ in agreement with the dimensions of the narrowest part of the α-hemolysin membrane channel[4,24]. In a real experiment, a and n_0 are about 0.4 nm and 12, respectively. It is assumed that the interaction between the biopolymer and the pore is ignored and the chemical potential inside the pore is uniform in this paper. The process of biopolymer translocation, from C is side to *trans* side under external force field, is assumed to start as soon as the leading monomer enters the nanopore and to end when the end monomer leaves the nanopore, as shown in Scheme 1. Terminational process of biopolymer translocation includes entering process and escaping process.



Scheme 1 Schematic representation of an *N*-monomers biopolymer entering into a nanometer pore

j is the number of entering-pore monomers. The right insets represent the entering and escaping processes of biopolymer translocation.

2 Material and Methods

Polymer extended model[21] was adopted for the simulation of biopolymer translocation, that is, a biopolymer molecule with N monomers threading a pore of length $l_0(=n_0a)$ can be regarded as biopolymer with $N+n_0$ monomers translocating a 2-dimensional hole embedded in membrane. The nanopore length n_0a is shorter than the biopolymer length Na in consistence with Miller's experiment[4,5]. Since the energy gained by a single monomer is much larger than its thermal energy, the biopolymer cannot return when its first monomer enters the pore. In the thermal equilibrium state, the negative entropy of the biopolymer is obtained by using classic nucleation theory for biopolymer solution[16].

The negative entropy, $S=(F-U)/T$, is a fundamental component of the Helmholtz free energy(F) and a key quantity as it constitutes the correct criterion of order which is mandatory for determining the relative biopolymer structures[22]. T is the absolute temperature and U is the average free energy, which can be regarded as a constant for a given biopolymer. It is relatively convenient to calculate the energy, F_j, which is signed on system configuration j in terms of microscopic interaction for a given biopolymer. And negative entropy can be calculated based on Boltzman probability $P_B(j)$ that is equal to $\exp[-F_j/k_BT]/Z$, where k_B is Boltzman constant, T is the absolute temperature, and Z is the partition function. So the negative entropy S can be formally expressed as follows.

$$S=k_B\sum_j P_B(j)\ln[P_B(j)] \tag{1}$$

For simplicity, in this paper, the negative entropy is replaced with average negative entropy $\langle S\rangle$.

$$\langle S\rangle=\left\langle k_B\sum_j \frac{\exp\left(\frac{-F_j}{k_BT}\right)}{Z}\ln\left[\frac{\exp\left(\frac{-F_j}{k_BT}\right)}{Z}\right]\right\rangle \tag{2}$$

For threaded-biopolymer, we think that the negative entropy landscape is associated with the number of monomers and has independent contributions from different regions. Therefore, if the biopolymer inhabits the nanopore, the whole negative entropy is given by

$$\Im=\sum_{i=1,2,3}\langle S_i\rangle \tag{3}$$

where S_i is negative entropy of the biopolymer on *cis* side(i=1), pore(i=2) and *trans* side(i=3). Based on classical statistics, the partition equation, Z, for biopolymer of j monomers in different regions can be separately obtained by $Z\sim j^{\zeta-1}$. ζ is a parameter to describe the properties of threaded biopolymer chain, which is equal to 0.5, 0.69 and 1 for Gaussian, self-avoiding and rod-like chains, respectively[23]. So, the average negative entropy of biopolymer can be expressed by $-k_B\ln(j^{\zeta-1})$. Here, it should be point out that the chemical potential difference between neighbor monomers, $\Delta\varepsilon$, is also one of the key factors to determine the value of negative entropy, but it doesn't determine the order tendency of biopolymer in the whole translocation. Since the gyration radius of threaded-biopolymer is much larger than that of nanopore, the part of biopolymer in nanopore is usually regarded as rod like chain, that is, ζ=1 and then $S_{i=2}(j)$=0. Provided that the biopolymer on *cis* side and

trans side keeps the same biopolymer chain model, the average negative entropy of the biopolymer in entering process and escaping process is expressed as follows.

$$\Im = \begin{cases} (1-\xi)k_{\mathrm{B}}\ln(N-j) & j \leqslant n_0 \quad \text{Entering process} \\ (1-\xi)k_{\mathrm{B}}\ln(j-n_0) & N \leqslant j \leqslant N+n_0 \quad \text{Escaping process} \end{cases} \tag{4}$$

These equations elucidate that the order scale of slided-nanopore biopolymer is dependent on the whole monomers of the biopolymer N, nanopore n_0 and monomers in different regions j, and independent of chemical potential difference mismatched between sides of nanopore.

3 Results and Discussion

In order to explore the mechanism of biopolymer translocation through a selected-nanopore, we suppose the nanopore length n_0a=4.8 nm and $n_0 \leqslant N$, which is in agreement with the *Staphylococcus* α-hemolysin (α-HL) protein length[4,5]. Under this precondition, the result is shown in Fig.1. The negative entropy landscape for every case is symmetric in whole translocation process[Fig.1(B), Inset] except n_0=0. When the first monomer reaches *trans* side or the end monomer escapes *cis* side, the biopolymer has a conformation transition, which may be induced by environment factors and interaction between the biopolymer and the nanopore. Corresponding analysis allows us to sketch an inverted-bowl entropy landscape for the passing process. W is a measure of the length effect of channel to some extent, whereas the depth, H, is correlated with the entropy barrier that is approximate to $\ln(N-n_0)$. That is, the longer the biopolymer is, the higher the entropy barrier is. This brings forward challenge for us to find some approaches to overcome the entropy barrier that is theoretical obstacle for developing fast biopolymer identified-technique. Further analysis illustrates that the biopolymer has a highest order state when the mid monomer occupies middle position in the channel, but these stable states quickly break under the external force filed such as osmotic pressure, electrical field. Fig.1(A) shows biopolymer entering scenario. In entering process, the order tendency of biopolymer is dependent on its length and independent of the pore length. And minus slope of negative entropy reflects that the order of biopolymer decreases in entering process, the degree of order progression is going down with increasing the biopolymer length. That is to say, it is difficult for a long biopolymer to enter a nanopore. And the biopolymer has much smarter conformational variation in solution.

However, the situation completely changes when biopolymer escapes the nanopore. The order of biopolymer at escaping stage obeys the same rule whatever its length is, as shown in Fig.1(B). Its order routine can be regarded as inversion of the entering process for every escaping case. The biopolymer conformation in *cis* side can be regarded as the renascence of biopolymer in *trans* side after leaving nanopore for all scenarios. Due to the same reason, long biopolymer is easy to escape the nanopore. Further researches for the biopolymer ordering routines will become inconsistent if we change the nanopore length.

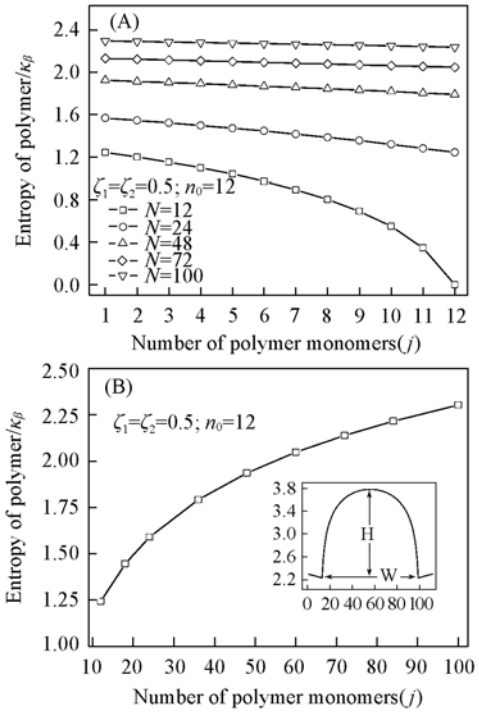


Fig.1 Plots of Negative entropy(in unit of k_{B}) for different biopolymers entering into fixed length nanopore against monomer number variation j

(A) Entering process; (B) escaping process. Inset, whole landscape of negative entropy for selected biopolymer entering into a selected nanopore. N=100, n_0=12.

To farther build the relation between negative entropy and biopolymer translocation, we have used entropy variable to reconsider translocation probability distribution function $\Psi(j,t)|_{i=1,2,3}$ in which the center of j monomer is found probability in the region i=1,2,3 at time t. The general master equation is expressed as[25]

$$\frac{\partial}{\partial t}\psi(j,t)=\sum_{j'}\left[\Lambda(j\mid j')\psi(j',t)-\Lambda(j'\mid j)\psi(j,t)\right] \quad (5)$$

where $\Lambda(j|j')$ is transition probability per unit time. If we consider only the adjacent monomer contributes these probabilities, that is, $\Lambda(j|j')\Psi(j'|t)=\lambda^{+}_{j-1}\Psi(j-1,t)+\lambda^{-}_{j+1}\Psi(j+1,t)$ and $\Lambda(j'|j)\Psi(j|t)=\lambda^{+}_{j}\Psi(j,t)+\lambda^{-}_{j}\Psi(j,t)$. In other words, λ^{+}_{j-1} is transition probability coefficient for adding one monomer to j monomers and λ^{-}_{j} is coefficient for removing one monomer from j monomers. And the relation between them is: $\lambda^{-}_{j}\sim\lambda^{+}_{j-1}\exp(S_j-S_{j-1})/k_B$[16,17]. Thus, the uniform expression of the evolution equation for probability $\Psi(j,t)$ in different regions can be written as[26,27],

$$\frac{\partial}{\partial t}\psi(j,t)=\lambda^{+}_{j-1}\frac{\partial}{\partial j}\left[\frac{1}{k_B}\frac{\partial S(j)}{\partial j}\psi(i,t)+\frac{\partial}{\partial j}\psi(j,t)\right] \quad (6)$$

where the first term is drift contribution that usually dominates the whole biopolymer translocation process. The transition probability $\lambda^{\pm}$ is usually independent of j and can be replaced by $1/\lambda_0$[28].

By analyzing the three processes, we can obtain entering time τ_{ent}, passing time τ_{pas} and escaping time τ_{esc}, respectively. And the whole translocation time τ is obtained, *i.e.*, $\sum\tau_i$, where i is ent, pas, esc, respectively. Using Fokker-Plank theory[29] and equation (6), with transition probabilities and ignoring some constants, the general time expression of three processes is written as follows.

$$\tau_i=\lambda_0\int_a^b dj\exp\left[k_B^{-1}S_i(j)\right]\int_0^j dn\exp\left[k_B^{-1}S_i(n)\right] \quad (7)$$

where parameters a and b represent boundary values which are a=0, b=n_0; a=n_0, b=N–1; and a=N, b=N+n_0 for entering process, passing process and escaping process, respectively. Therefore, the translocation time can be easily obtained. In fact, whatever the pore length is, the passing time, τ_{pas}, is approximately given by mean first passage time without considering the pore length scaling. Muthukumar has shown its results that $\tau_{pas}\sim N/\Delta\varepsilon$ for $N\Delta\varepsilon>1$(strong external field) and $\sim N^2$ for $N\Delta\varepsilon<1$(free external field)[18]. These results illustrate that the passing time of a long biopolymer($n_0/N\leqslant 0.1$) dominates the translocation time for short nanopore, which is in agreement with previous experimental data[4—6]. Although the chemical potential gradient $\Delta\varepsilon$ is one of the crucial factors for the whole process of biopolymer sliding throughs a nanopore, it can be regarded as constant coefficient contribution depending on real experimental condition. So, the biopolymer entering-pore time τ_{ent} and biopolymer escaping pore time τ_{esc} are all investigated without considering the chemical potential gradient $\Delta\varepsilon$ in this paper. Based on equations (4) and (7), the entering or escaping time is obtained from the following equation, respectively,

$$\begin{aligned}\tau_{ent}&=\lambda_0\int_0^{n_0}\left\{\exp\left[(1-\xi)\ln(N-j)\right]\int_0^j\exp\left[(1-\xi)\ln(N-n)\right]dn\right\}dj\\ \tau_{esc}&=\lambda_0\int_N^{N+n_0}\left\{\exp\left[(1-\xi)\ln(j-n_0)\right]\int_0^j\exp\left[(1-\xi)\ln(j-n_0)\right]dn\right\}dj\end{aligned} \quad (8)$$

So the biopolymer entering mean velocity $\langle v_{ent}\rangle$ and biopolymer escaping mean velocity $\langle v_{esc}\rangle$ are given by

$$\begin{aligned}\langle v_{ent}\rangle&=n_0\alpha/\tau_{ent}\\ \langle v_{esc}\rangle&=n_0\alpha/\tau_{esc}\end{aligned} \quad (8)$$

Fig.2 shows τ_{ent} and v_{ent} for biopolymer entering a selected-nanopore n_0=12. τ_{ent} is approximately linear increase with adding the biopolymer length. In other words, the entering process becomes more difficult for long biopolymer. v_{ent} curve also illustrates this result. The main reason for these landscapes can be elucidated by Fig.1(A).

Compared with those the entering scenario, τ_{esc} is much smaller than τ_{ent}, and v_{esc} is far larger than v_{ent}, as shown in Fig.3. τ_{esc} decreases and v_{esc} increases with the increase of the biopolymer length. The longer the biopolymer is, the shorter the τ_{esc} is. From their slopes, v_{esc} approaches a constant value for long biopolymer($n_0/N\leqslant 0.1$). For the whole translocation process, the entering process of biopolymer is more

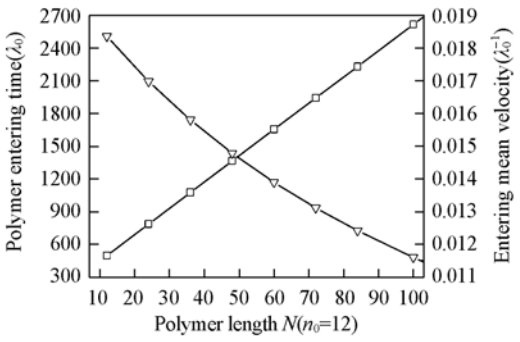


Fig.2 Plots of biopolymer entering pore yime(□)(in unit of λ_0) and nopolymer rntering pore mean velocity(▽)(in unit of λ_0^{-1}) versus biopolymer length N(n_0=12).

difficult than its escaping process which is like a thread passes through the eye of a needle. So, to overcome entropy barrier and to make biopolymer slide through nanopore easily and smoothly, the suitable external force field is usually applied. Currently, the main strategy of nanopore identification is to analysize the electrical characters of a single macromolecule passing through the nanopore. For DNA translocation through α-HL, at any given time, more than one base hold the pore, so electrical character is affected by all holded-bases. Thus, it is very difficult to obtain the best base resolution, especially for DNA termination. To improve this resolution, much work has been done. The exciting progress is artificial solid-state nanopore[30], which is so robust and short that only one base holds it.

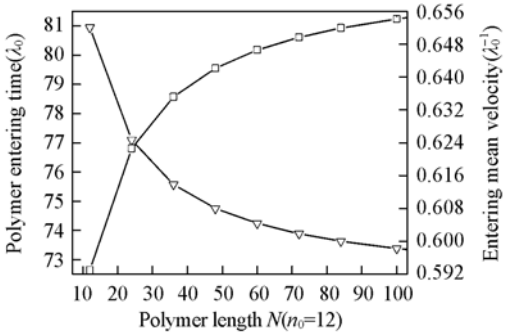


Fig.3 Plots of biopolymer escaping pore time(□)(in unit of λ_0) and biopolymer escaping pore mean velocity(▽)(in unit of λ_0^{-1}) *versus* biopolymer length N(n_0=12)

4 Conclusion

In summary, we have investigated the negative entropy landscape of biopolymer for sliding through a nanometer scale pore, especially for entering and escaping processes. Our research elucidates that negative entropy, as an effective means, can be applied to evaluating translocation process and ordering routine of biopolymer. For a selected nanopore, entering process of biopolymer is more difficult than escaping process of biopolymer. τ_{ent} and τ_{esc} deduced according to previous research also testify this result, which is in excellent agreement with previous experiments. This research puts forward some challenges for us to develop an optimal nanopore device for single molecule identification. we think that further and highlighted studies in this field is combining nanopore with other advanced technology by taking full advantages of the tremendous difference between τ_{ent} and τ_{esc}.

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