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Electrophoresis of poly(dT)$_{20}$ through α-hemolysin nanopore in high concentration potassium chloride solution

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Abstract: Experiments of poly(dT)$_{20}$ electrophoresis through α-hemolysin nanopores were performed to unveil the electrophoretic transport mechanism of DNA through nanopores in high concentration potassium chloride solution. It is found that there are two obvious current blockades induced by poly(dT)$_{20}$ translocation and collision events. Both blockade currents increase linearly with the applied bias voltage. However, the normalized blockade currents are almost kept the same although variable bias voltages are applied. The collision time of poly(dT)$_{20}$ in the luminal site of the pore remains constant for different voltages. The translocation speed of poly(dT)$_{20}$ through the nanopore decreases with the increase of bias voltage. It is because as the potential increases, the drag force on the homopolymer helps it to crumple into a cluster much easier due to the poor stacking of thymine residues compared with homopolymers consisting of other nucleotides. Molecular dynamics simulations further confirm the experimental results. Increasing the applied bias voltage can slow down the translocation velocity of the flexible poly(dT)$_{20}$, which favors increasing the precision of single molecule detection by using nanopores.

Key words: nanopore; transport speed; ionic current; electrophoresis

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Nanopore sequencing technique is considered to be the third-generation DNA sequencing technology which is characterized by high-throughput and low-cost sequencing capabilities[1]. In the nanopore approach, the electrophoretic translocation of DNA through the nanopore modulates the ionic current through the pore[2]. It is possible for the discrimination of nucleotides if adenine (A), cytosine (C), thymine (T), and guanine (G) produce unique residual current levels as they pass through the nanopore[3], which could offer the prospect of sequencing a human genome at the expense of $1 000 within 24 h. Solid-state nanopores have unique properties including well-defined geometries, dimensions and mechanical robustness. However, synthetic nanopores fabricated by the same procedure are often of low similarity, which is a critical obstacle for DNA molecule detection. While the geometry of α-hemolysin (α-HL) nanopores can be precisely kept the same, they have been the mostly used biological nanopores for advanced research applications[4]. Akeson et al.[5] demonstrated that α-HL nanopore could rapidly discriminate between adenine and cytosine segments. Meller et al.[6] demonstrated that the α-HL nanopore could distinguish between polynucleotides of similar length and composition. Khulbe et al.[7] showed that the translocation of a ssDNA molecule consisting of 50 adenine bases followed by 100 cytosine bases through an α-HL nanopore induced two characteristic currents. For precisely characterizing the sequences of the ssDNA, it is crucial to unveil the transport dynamics of ssDNA through the nanopore. Henrickson et al.[8] studied the voltage dependence of polynucleotide-induced ionic-current blockades of a single α-HL ion channel. They found that the blockade frequency increased exponentially with the applied potential. Normally, poly(dT)$_n$ (n indicates the number of deoxynucleotide) resembles most closely a freely jointed chain due to the poor stacking of thymine residues[9]. Studies of poly(dT)$_n$ transport dynamics through nanopores have not been finalized until now. Besides, most research in polymer detection experiments was performed in 1 mol/L potassium chloride (KCl) solution and seldom reported in high concentration KCl solution. Therefore, in this paper, our main concern is the study of the electrophoretic transport mechanism of poly(dT)$_{20}$ through α-HL nanopore in 2 mol/L KCl solution.

1 Experimental Method

The experimental setup is illustrated in Fig. 1. The α-HL nanochannel inserts in the phosphate lipid bilayer; the green and grey spheres represent the potassium and chloride ions. The planar bilayer chamber was divided into two compartments (*cis* and *trans*) by a Teflon septum. To form a bilayer, the 150 μm Teflon aperture was coa-

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ted by applying 5 μL of a 200 μg/mL solution of 1, 2-diphytanoyl-sn-glycero-3-phosphacholine (Avanti Polar Lipids, Alabaster, AL, USA) in spectroscopy grade decane, which was then evaporated using a light stream of nitrogen. The chambers on both sides of the aperture were filled with the ionic solution consisting of 2 mol/L KCl, 0.01 mol/L Tris-HCl and 0.001 mol/L EDTA, which was degassed, filtered, and adjusted to pH 8.0 at room temperature. A brush was used to dip into a 200 μg/mL solution of 1, 2-diphytanoyl-sn-glycerol-3-phosphacholine in spectroscopy grade decane and then brushed across the aperture till a stable bilayer was formed. Afterwards, 2.5 μL of 0.04 g/mL α-HL (Sigma-Aldrich) was added to the *cis* side of the chamber. A single α-HL channel was inserted into the lipid bilayer over approximately 3 to 240 min. Insertion was indicated by an abrupt current increase to 260 pA when a bias voltage of 120 mV was applied. Then, the excess α-HL was removed by perfusion with a fresh buffer to prevent further channel incorporation. The poly(dT)$_{20}$ with HPLC purification, purchased from Takara BIO Inc., was dispersed into the *cis* chamber. Under the action of the external electric field, the DNA homopolymers will be electrophoretically driven through the nanopore towards the *trans* side. The current responses of the nanopores, including open pore current I_0 and the blockade current ΔI_B induced by DNA inside the nanopore, were acquired using a resistive feedback amplifier (HEKA EPC10, HEKA Elektronik) at 200 kHz with low-pass filtering at 10 kHz through Ag/AgCl electrodes. All measurements were conducted inside a dark Faraday cage.

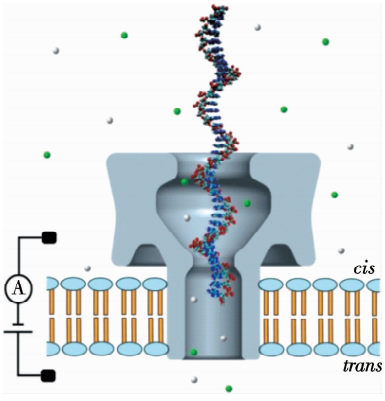

Fig. 1 Schematic of the experimental system setup

2 Results and Discussion

By adding 2.3×10^{-7} mol/L poly(dT)$_{20}$ into the *cis* chamber, numerous current blockades occurred due to the translocation of poly(dT)$_{20}$ through the α-HL channel. Fig. 2(a) shows the examples of the transient blockades as poly(dT)$_{20}$ is driven through the α-HL nanopore with different bias voltages applied. It is clear that there are more captured events as the bias voltage increases from 80 to 240 mV. In Fig. 2(b), the blockade rate R versus the applied bias voltage is plotted. It is found that the frequency of polymer blockades increases with the potential. Besides, the blockade rate increases exponentially with the applied potential which is consistent with the results obtained by Henrickson et al[8]. To further explain this phenomenon, molecular dynamics (MD) simulations were performed to calculate the potential energy as a double stranded DNA was translocating through a nanopore. A 10-layer graphene nanopore with a diameter of 2.5 nm was used to mimic the beta barrel of the α-HL. The system setup is similar as our previous work[10-11]. It is found that there is an energy barrel for DNA getting inside the pore (see Fig. 2(c), DNA resides inside the nanopore from 60 to 100 ps). Increasing the bias will help the DNA to break through the energy barrel and facilitate the capture of DNA by the nanopore.

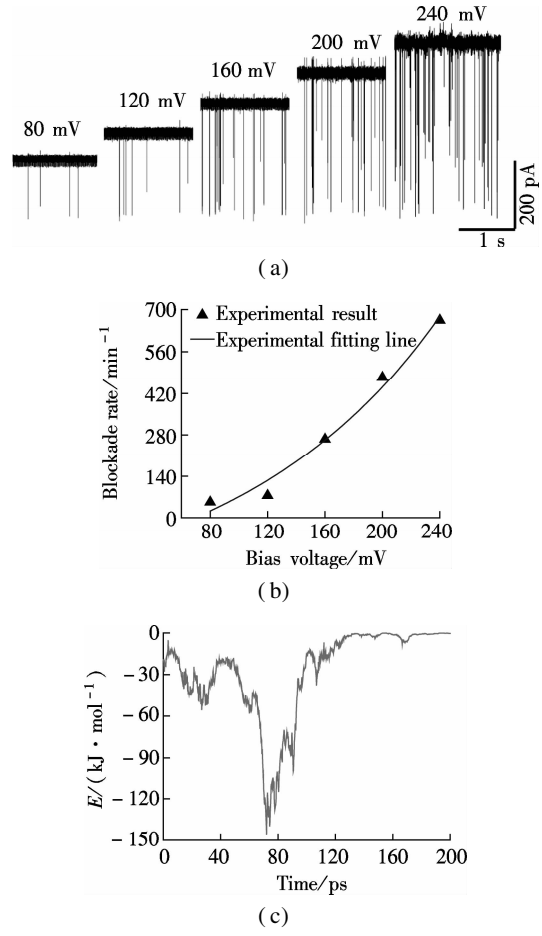

Fig. 2 DNA capture by α-HL nanopore. (a) Ionic current traces with different applied bias voltages; (b) The relationship between the frequency of polymer blockades and the applied bias voltage; (c) The potential energy of DNA during its transport from simulations

Fig. 3 shows the scatter plots of current blockage ΔI_B versus the dwell time when poly(dT)$_{20}$ passes through the α-HL nanopore. The main clusters of events are narrowly distributed in two blockade currents. The results are analyzed by plotting the histograms of current blockage distri-

butions in Figs. 4 (a) to (e) and dwell time distributions in Figs. 5(a) to (e). The events with a large current blockage amplitude, which is larger than 75% of the open pore current, are deemed to be the translocations[12]. The remaining low blockade events are regarded as collisions, which are mainly induced by a knock or an interaction with the luminal sites of the pore[13]. As demonstrated in Fig. 4, there are two conspicuous peaks for all histograms of current blockage once those histograms are fitted with the multi-peak Gaussian distribution function. The blockade amplitude of poly(dT)$_{20}$ exhibits two main blockade current states, which is very similar to the characteristic blockade signals monitored in previous experiments[9]. The first and second peak blockade current values are defined as ΔI_{p1} and ΔI_{p2}, which are mainly induced by the collisions and translocations of poly(dT)$_{20}$, respectively. Fig. 4 (f) plots the open pore current, the first peak blockade current and the second peak blockade current versus bias voltage. I_0, ΔI_{p1} and ΔI_{p2} all increase linearly with the applied potential.

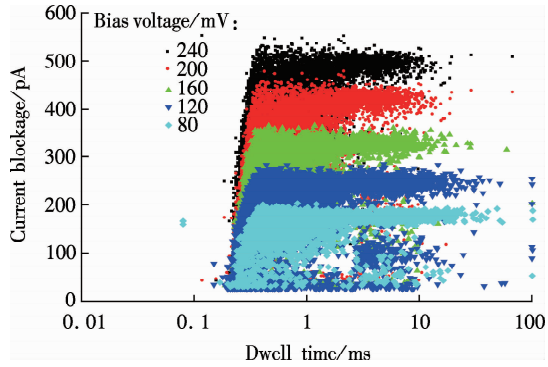

Fig. 3 Scatter plots of current blockage vs. dwell time for poly(dT)$_{20}$ transport events

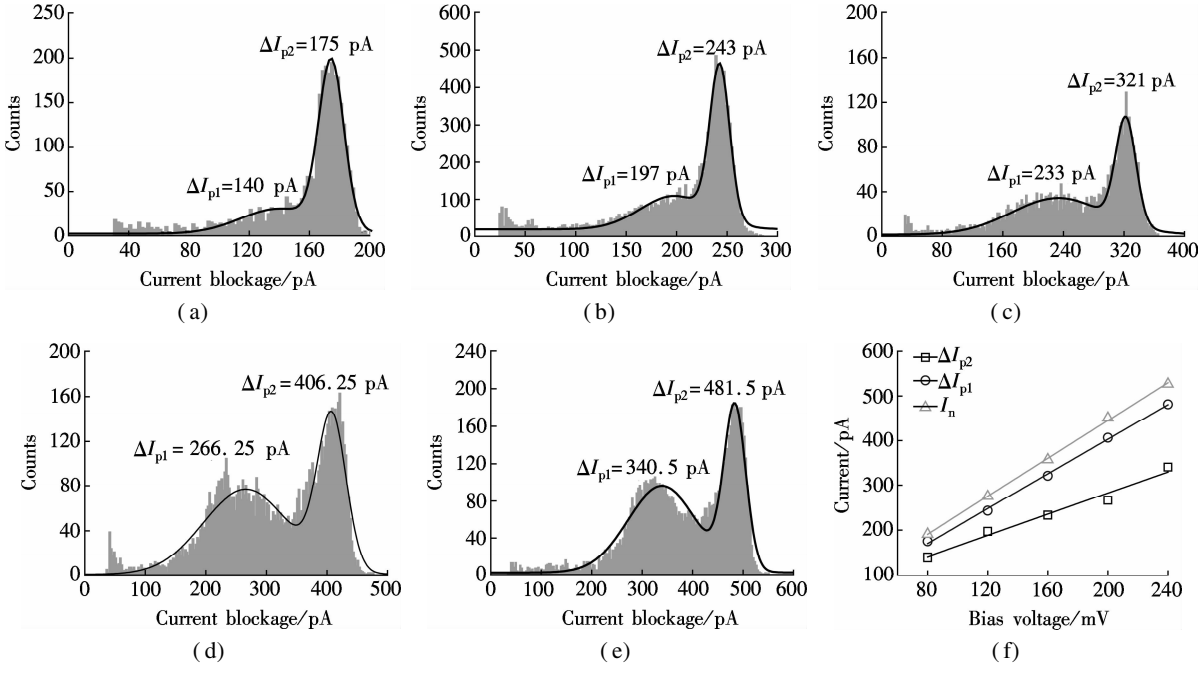

Fig. 4 Current blockage histograms for poly(dT)$_{20}$ transport through α-HL nanopores under different bias voltages. (a) 80 mV; (b) 120 mV; (c) 160 mV; (d) 200 mV; (e) 240 mV; (f) Open pore current, first and second peak blockade current vs. bias voltage

We also compute the normalized current blockage ($\Delta I/I_0$), which is shown in Fig. 6. It is found that the first peak and the second peak normalized blockade currents are not related to the bias voltage. $\Delta I_{p2}/I_0$ is around 90% and remains constant in all the studied potential. $\Delta I_{p1}/I_0$ is about 65% and varies slightly more than $\Delta I_{p2}/I_0$. This is reasonable and understandable since the first peak current blockage is mainly induced by the collisions of poly(dT)$_{20}$ with the vestibule of α-HL nanopore.

In Figs. 5 (a) to (e), by selecting events with ΔI_B larger than 75% of I_0 for data analysis, the histograms of translocation time for poly(dT)$_{20}$ transport through α-HL nanopore with different applied bias voltages are plotted (The histograms of collision time are not shown). Unlike the histograms of current blockage which can be fitted by the Muti peaks Gaussian distribution function, the histograms of translocation time cannot be well fitted by Gaussian curves but exponential curves with time constant t_d. The time constant values are 591, 681, 960, 1 080 and 1 138 μs for 80, 120, 160, 200 and 240 mV, respectively. Clearly, the dwell time for poly(dT)$_{20}$ translocating through the nanopore increases with the bias voltage as shown in Fig. 5(f), which is on the contrary to the results of Meller et al[14].

As reported in Meller's study, homopolymers consisting of denine acid were used, which were usually assumed to have a rigid secondary structure and the homopolymers were merely in contrast to poly(dT)$_{20}$ used in our study. As the bias voltage is small, poly(dT)$_{20}$ remains linear conformation like other rigid homopolymers.

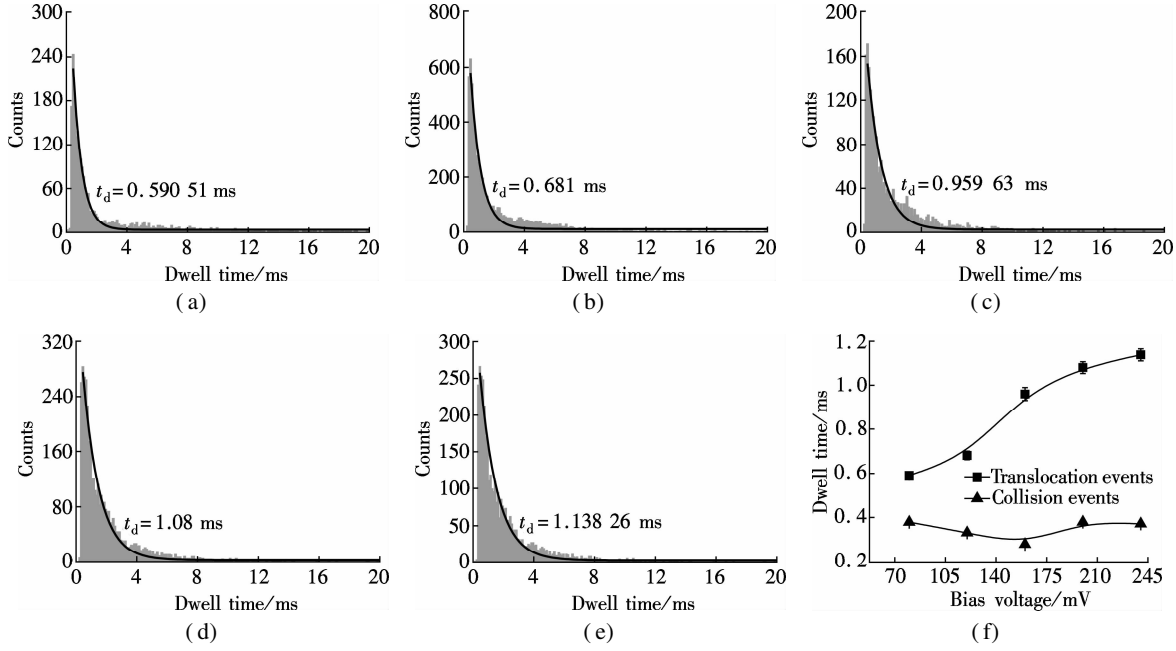

Fig. 5 Dwell time histograms for poly(dT)$_{20}$ transport through α-HL nanopore under different bias voltages. (a) 80 mV; (b) 120 mV; (c) 160 mV; (d) 200 mV; (e) 240 mV; (f) Poly(dT)$_{20}$ translocation and collision time vs. bias voltage

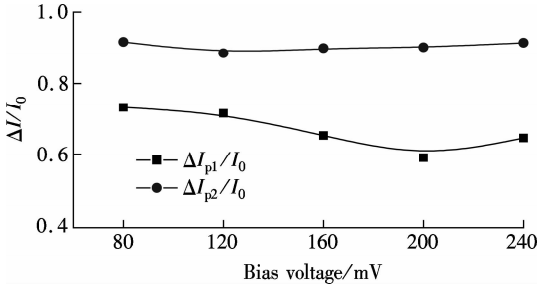

Fig. 6 Normalized blockade current ($\Delta I/I_0$) vs. bias voltage

However, as the potential increases, the drag force on the polymer is more likely to disrupt the conformation of poly(dT)$_{20}$ due to the poor stacking of thymine residues compared to homopolymers consisting of other nucleotides. Then, poly(dT)$_{20}$ may spend more time translocating through the nanopore. That is why the transport speed of poly(dT)$_{20}$ decreases with the increase of the bias voltage. In Fig. 5(f), the relationship between the collision time and bias voltage is also plotted. The collision time seems to be not related to the applied bias voltage. The constant t_d remains 350 μs which is much shorter than the translocation time. This further confirms that the events with ΔI_B smaller than 75% of I_0 are collisions but not translocations though these results have been reported earlier[12-13].

To further confirm our experimental results, molecular dynamics (MD) studies are performed. Classical molecular dynamics package GROMACS[15-16] was used to simulate the dynamics of the designed system as shown in Fig. 7(a). AMBER94[17] force field was used for the B-helix form poly(dT)$_{20}$ and the TIP4P model for water molecules. The salt KCl solution was kept at 2 mol/L concentration (matched the ionic concentration used in our experiment) in the whole simulation with the application of the periodical boundary conditions along the three axis directions. All simulations were performed at a NVT ensemble under constant temperature (300 K) and constant volume (20 nm (x) × 10 nm (y) × 10 nm (z)). V-rescale was used for temperature coupling and the particle-mesh-Ewald (PME)[18] algorithm was adopted to evaluate the electrostatic interaction. Other simulation details were explained in our previous studies[10-11]. The system was firstly simulated for 100 ps for equilibration. Then, another 500 ps was used to simulate the 20-nucleotide long homopolymer consisting of deoxythymidine acids translocation in the potassium chloride solution under the action of the 0.1 and 1.0 V/nm electric field. As shown in Fig. 7(b), poly(dT)$_{20}$ keeps the original structure as the electric field is 0.1 V/nm through the entire simulation. However, it sticks together as a cluster with 1.0 V/nm applied after the 500 ps simulation. As the electric field is larger, poly(dT)$_{20}$ will not be kept single-stranded molecule but become clustered gradually. This means that it is very difficult for poly(dT)$_{20}$ to translocate through the nanopore as the external bias is larger. Therefore, poly(dT)$_{20}$ may spend more time translocating through the nanopore based on the MD results above, which is consistent with the experimental results obtained in this work. Another possible reason is that as DNA is negatively charged, positive ions will bind to the surface of the DNA and form an electric double layer, which will significantly change the ionic distribution inside the pore as DNA is resident in the pore. Increasing the potential across the protein membrane, there will be more ionic

flux through the pore and the fast moving ions in the confined nanopore will also deform the DNA configuration, which can induce the DNA structure from the linear strand to an expanded state. The deformation of DNA also decreases the DNA transport speed when the voltage is increased.

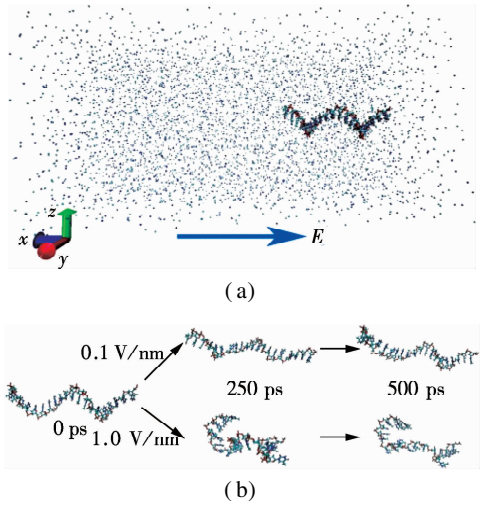

Fig. 7 Molecular dynamics study of poly(dT)$_{20}$ translocation in the 2 mol/L potassium chloride solution. (a) The system setup for the molecular dynamics study; (b) Configurations of poly(dT)$_{20}$ in the 2 mol/L KCl solution with 0.1 and 1.0 V/nm

3 Conclusion

The study presented here demonstrates that the translocation process of poly(dT)$_{20}$ through α-HL nanopore is strongly affected by the applied bias voltage in a high concentration potassium chloride solution. There are two obvious current blockades which are induced by the translocations and collisions of poly(dT)$_{20}$. The two kinds of current blockades both increase linearly with the applied bias voltage while the normalized blockade currents are not related to the bias voltage. It is also found that the transport time of poly(dT)$_{20}$ through α-HL nanopore increases with bias voltage. We have validated that it is reasonable and understandable although the result deviates from the result of Meller et al. [14] who used a homopolymer consisting of deoxyadenine and deoxycytosine acids in their studies. It is because as the potential increases, the drag force on the polymer can more easily disrupt the conformation of poly(dT)$_{20}$ due to the poor stacking of thymine residues compared to homopolymers consisting of other nucleotides. MD simulation results also verify the experimental results. As the electric field is larger, poly(dT)$_{20}$ will not be kept as a single stranded molecule but become clustered gradually. Besides, the collision time appears to be not related to the bias voltage applied. Our research makes an essential improvement which compensates for the deficiency of the mechanism of ssDNA transport through nanopores.

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高浓度氯化钾盐溶液中 poly(dT)$_{20}$ 在 α 溶血素纳米孔内的电泳动力学

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摘要: 为了揭示高浓度盐溶液中 DNA 穿过纳米孔时的动力学行为,实验研究了 poly(dT)$_{20}$ 电泳穿过 α 溶血素纳米孔的输运机理. 实验结果表明,阻塞电流主要由 DNA 的过孔事件和碰撞事件引起. 2 种事件引发的阻塞电流都随着外加电势的增加而线性增加. 尽管外加电势存在差异,归一化之后的阻塞电流幅值却都保持不变. 此外,poly(dT)$_{20}$ 的碰撞事件持续时间并不受电压影响,而过孔时间却随着外加电势的增加而相应延长. 这是由于胸腺嘧啶的堆叠效应较差,相比较其他碱基而言,在强电场力作用下,poly(dT)$_{20}$ 更容易发生蜷曲,导致过孔阻力增加. 分子动力学模拟也进一步对实验结果进行了验证. 研究结果表明,增加跨膜电势差有助于降低柔性 poly(dT)$_{20}$ 的过孔速度,从而提高单分子的检测精度.

关键词: 纳米孔;过孔速度;离子电流;电泳

中图分类号: TH789; Q786