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Translocation of single stranded DNA through the α -hemolysin protein nanopore in acidic solutions

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Abstract

The effect of acidic pH on the translocation of single-stranded DNA through the α -hemolysin pore is investigated. Two significantly different types of events, i.e., deep blockades and shallow blockades, are observed at low pH. The residence times of the shallow blockades are not significantly different from those of the DNA translocation events obtained at or near physiological pH, while the deep blockades have much larger residence times and blockade amplitudes. With a decrease in the pH of the electrolyte solution, the percentage of the deep blockades in the total events increases. Furthermore, the mean residence time of these long-lived events is dependent on the length of DNA, and also varies with the nucleotide base, suggesting that they are appropriate for use in DNA analysis. In addition to be used as an effective approach to affect DNA translocation in the nanopore, manipulation of the pH of the electrolyte solution provides a potential means to greatly enhance the sensitivity of nanopore stochastic sensing.

Keywords

acidic pH; α -hemolysin; nanopore; single-stranded DNA; translocation

Introduction

Nanopore stochastic sensing is currently an active research area, characterized by highly-sensitive, rapid, and multi-functional detection capabilities [1]. In nanopore sensing, the passage of analytes of interest through a nano-channel (or pore) at an fixed applied potential cause current modulations. The mean residence time and amplitude of the recorded events allows to determine the identity of the analyte, while the frequency of occurrence of the current modulations could be used to find its concentration [2]. In addition to the development of ultrasensitive sensors for a wide range of substances [3–17], these nanometer-sized channels offer exciting new possibilities for studying covalent and non-covalent bonding interactions [18–20], investigating biomolecular folding and unfolding [21, 22], probing enzyme kinetics [23], as well as analyzing and even sequencing DNA molecules [24–32]. The hypothesis for DNA sequencing in a nanopore is that when a single-stranded DNA (ssDNA) sample is electrophoretically driven through the pore, it is possible to read its base sequence if each nucleotide of the polymer produces a characteristic current modulation. Since kilobase length DNA can be read directly without amplification or use of

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Supporting Information Available. Additional graphs. This material is available free of charge online.

costly reagents such as enzymes and fluorescent tags, the nanopore approach can significantly reduce the sequencing cost, and has emerged as one of the most promising technologies to achieve the “\$1000 genome” goal set by the U.S. National Institutes of Health [33]. However, due to the rapid DNA translocation velocity through the nanopore, accurate detection of single nucleotide bases via the electrophoretically driven nanopore approach has not yet been achieved with the currently available single-channel recording technique [34].

To increase the nanopore resolution for nucleotide differentiation, three major approaches have been used in the past decade to slow DNA translocation. These include modification of the structures of both nanopores and DNA molecules [35–37], and manipulation of experimental physical conditions [38–42]. Note that the molecular transport and binding kinetics inside a channel are strongly dependent on the nature of the pore, the species passing through the channel, as well as the experimental conditions. It has been reported that enhanced translocation of ssDNA molecules through α -hemolysin protein channels could be achieved by manipulation of the internal charge within the pore [35]. By attaching chemical tags to DNA bases [36], or immobilization of DNA polynucleotides with streptavidin [37], prolonged DNA residence time in the pore could be obtained. Furthermore, experimental physical conditions such as temperature, voltage bias, viscosity, electrolyte, and application of an alternating electric field were also found useful to control the DNA translocation rate [38–42]. Other approaches include use of a host compound [43], and a polymerase [44].

It should be noted that nanopore experiments are usually carried out at or near physiological pH. The effect of pH on DNA transport through solid-state nanopores has been well studied. For example, Li and co-workers used high pH buffers to study transport of ssDNA through silicon nitride nanopores and found that ssDNA translocated through the pore more rapidly in a solution at a higher pH [45]. Timp and co-workers investigated the influence of acidic pH on transport of double-stranded DNA (dsDNA) and reported that acidic pH destabilized the double helix, facilitating DNA translocation [46]. Although the detection of single-stranded and double-stranded DNA in the α -hemolysin (α HL) pore at alkaline pH has been attempted [47], thus far, to the best of our knowledge, there have been no systematic studies of the effect of pH on DNA translocation through the biological protein nanopore, especially at acidic solutions. Here, we report that acidic electrolyte solutions could be used as an effective means to affect DNA translocation in the α HL protein pore.

2 Materials and Methods

Materials and Reagents

The wild type α HL protein was synthesized by coupled *in vitro* transcription and translation as described previously [48]. Except the 3-mer ssDNA (sequence: TTT, HPLC purification) which was obtained from Sigma Aldrich, all the other ssDNA samples (desalting) were purchased from Integrated DNA Technologies, Inc. (Coralville, IA). Lipid 1,2-diphytanoylphosphatidylcholine was obtained from Avanti Polar Lipids (Alabaster, AL). Teflon films were purchased from Goodfellow (Malvern, PA). All of the other reagents were purchased from Sigma Aldrich. All the ssDNA polymers were dissolved in HPLC-grade water (ChromAR, Mallinckrodt Baker). The concentrations of the stock solutions were 4 mM for each of the DNA samples. All the electrolyte solutions used in this work were prepared in HPLC-grade water, which contained 1 M NaCl and 10 mM NaH_2PO_4 , with the pH of the solutions adjusted to 3.0 – 7.5.

Planar Bilayer Experiments

The single-channel recording procedure has been described elsewhere [41]. Briefly, a planar bilayer chamber was divided into two compartments, *cis* and *trans*, by a Teflon septum. A

lipid bilayer of 1,2-diphytanoyl-sn-Glycero-3-phosphocholine was formed on a $\sim 120\ \mu\text{m}$ -diameter aperture in the teflon film by using the Montal-Mueller method [49]. The experiments were performed under a series of symmetrical conditions with a 2.0 mL solution comprising 1 M NaCl and 10 mM NaH_2PO_4 , with the pH of the solutions adjusted to 3.0 – 7.5 at $22 \pm 1\ ^\circ\text{C}$ unless otherwise stated. Both the αHL protein (with the final concentration of $0.2\text{--}2.0\ \text{ng}\cdot\text{mL}^{-1}$) and the ssDNA sample were added to the *cis* chamber compartment, which was connected to “ground”. The applied potential was +120 mV. Currents were recorded with a patch clamp amplifier (Axopatch 200B, Axon instruments, Foster city, CA, USA). They were low-pass filtered with an external four-pole Bessel filter at 30 kHz and sampled at 125 kHz by a computer equipped with a Digidata 1440 A/D converter (Molecular Devices). The final concentrations of ssDNA samples were $10\ \mu\text{M}$ each. At least three separate experiments were carried out for each DNA sample.

Data Analysis

Data were analyzed with pClamp 10.1 (Molecular devices), QuB (www.qub.buffalo.edu), and Origin 6.0 (Microcal, Northampton, MA) software. Current modulation events that caused at least 70% blockade of the open channel were taken into consideration to calculate the corresponding mean residence values and amplitude values. It has been well established that the events with less than 70% blockades are attributed to residence only in the channel vestibule or the collisions of the ssDNA molecules by the pore mouth [32]. Two significantly different types of events were observed for DNA's transit in the αHL pore at pH 5.0 or below: short-lived events with mean residence times of $\sim 80\ \mu\text{s}$; and long-lived events with mean residence times of $\sim 400\ \mu\text{s}$ or larger. Conductance values were obtained from the amplitude histograms after the peaks were fit to Gaussian functions. Mean residence time (τ_{off}) values of the short-lived events were obtained from the dwell time histograms by fitting the distributions to Gaussian functions, while those of the long-lived events were obtained by fitting the dwell time distributions to single exponential functions by the Levenberg-Marquardt procedure. Between 3,000 and 25,000 events were recorded in each of the single channel recording experiments. All the results were reported as mean values $\pm$ standard deviation.

3 Results and Discussion

It has been reported that stochastic gating of the αHL channel might occur more frequently at a lower pH [50]. To demonstrate the feasibility of investigating the pH effect on DNA's transport using the wild type αHL pore, a control experiment was first performed in a 1 M NaCl solution at pH 3.0. Our experimental result (Supporting Information, Fig. S1) showed that in the absence of DNA, the background current modulations of the αHL pore were rarely observed (~ 4 events/min), thus suggesting that channel gating would not interfere with the study of DNA transport under our experimental conditions. The experiments were then carried out at +120 mV with $(\text{dA})_{20}$ in 1 M NaCl solutions having various pH ranging from 3.0 to 7.5. As shown in Figs. 1 and 2, at pH 6.0 and pH 7.5, only one major type of short-lived translocation events was observed with mean residence times of 83 ± 11 , and $70 \pm 10\ \mu\text{s}$, respectively. In sharp contrast, in all of the other three examined acidic electrolyte solutions, two significantly different types of events with different blockage amplitudes (i.e., deep blockades and shallow blockades) were observed. The deep blockades show a narrow range of amplitudes (with a $\sim 98\%$ mean channel block) and a large mean duration value but with a broad distribution of residence times, while shallow blockades present a small mean residence time and a wide range of current blockage amplitudes (from $\sim 70\%$ to almost full channel block). Although the deep blockades and shallow blockades overlapped to some extent regarding the blockage amplitudes, these two types of events were well defined and could be readily separated from the event density plot (Supporting Information, Fig. S2). More than 95% of the deep blockades had at least 97% channel block and lasted larger than

0.5 ms, whereas more than 95% of the shallow blockades showed residence times of less than 0.5 ms and caused ~80% to ~90% blockage of the open channel (Supporting Information, Fig. S2). To minimize the potential interference from the shallow blockades, only the events with duration larger than 0.5 ms and having more than 97% channel block were included to analyze the mean residence times of the deep blockades. To calculate the percentage of the deep blockage events in the total events in such experiments, the frequency of the deep blockage events (f_{deep}) and the overall frequency of all the DNA events (f) are calculated. Then, the percentage was obtained by dividing f_{deep} by f . Table 1 illustrates the results of the statistical translocation properties of (dA)₂₀ in five different pH solutions, including the mean residence times and residual currents of the deep blockades and shallow blockades, as well as the percentage of deep blockades in the total events. It is apparent from this table that with a decrease in the pH value of the electrolyte solution, the percentage of deep blockades increases. At pH 3.0, 19.5 ± 1.0 % of the total events belonged to the deep blockades with a mean residence time of $1210 \pm 156 \mu\text{s}$. Furthermore, as pH decreases, the mean residence time of the shallow blockades is almost unchanged, while that of the deep blockades increases significantly until pH 4.0, after which the event mean duration does not vary much. As an important aside, we noticed that, when the pH of the solution decreased from 7.5 to 3.0, the overall frequency of (dA)₂₀ events first increased and then decreased.

As mentioned in the introduction, to utilize the nanopore technique for analyzing DNA, it is imperative to slow down DNA translocation since in this case, high measurement bandwidths are not necessary for the detection of current modulations induced by the DNA translocation through the nanopore. The major advantages of lower bandwidth measurements include smaller data storages and significantly reduced measurement noise, which allow more convenient data analysis and an enhanced resolution to the discrimination of various polynucleotides. The mean residence time of the deep blockage events of (dA)₂₀ at pH 3.0 or pH 4.0 was ~ 17 – 18 folds larger than that of (dA)₂₀ events at pH 7.5. Therefore, in an electrolyte solution having a lower pH, the nanopore resolution to DNA analysis should be improved if the mean residence time of these deep blockage events are dependent on the polymer length. Moreover, as the pH of the electrolyte solution decreased from 7.5 to 3.0, the open channel current increased by 14.7% (from 97.7 ± 1.4 pA to 112.1 ± 2.4 pA). The pH dependence of the open channel current has been observed by Kasianowicz and Bezrukov, which was proposed to be attributed to the reversible binding of protons to ionizable residues inside the channel [50]. The increase in the open channel current offers the potential to increase the S/N ratio, thus providing a further improvement in the capability of the nanopore to differentiate polynucleotides.

To investigate whether these deep blockades are caused by the translocation of (dA)₂₀ through the αHL pore or rather they are attributed to the sticking of these DNA polymers to the channel or binding of one or more bases of the polymer to the protein pore for long periods of time with intermittent short periods of rapid translocation, two additional polydeoxyadenine samples with different lengths were examined. As shown in Fig. 3, with an increase in the DNA length, the mean residence time of the deep blockades increased, thus clearly suggesting that these deep blockades were not due to the tangling of the (dA)₂₀ molecule to the channel, but rather caused by the slower threading of the DNA molecule through the pore. Note that it has been well established that the current blockage events having the residence time not dependent on the polymer length are due to the polynucleotides' collision with or binding to the pore, while those with the dwell time sensitive to the sample length depict actual DNA translocations [38, 39]. To further demonstrate that the deep blockades were attributed to translocation, voltage dependence study was performed. Our experimental results showed that the mean residence time of these

events decreased as the voltage increased (Fig. 4). Taken together, the combined results provided evidence that the deep blockades were indeed related to translocation.

To investigate whether an acidic electrolyte solution could be used as an effective approach to increase the nanopore resolution for DNA analysis instead of the commonly used buffer solution at/near physiological pH, three additional 20-mer ssDNA samples, including (dT)₂₀, (dCdT)₁₀, and (dAdG)₁₀, were examined with the wild-type α HL pore in 1 M NaCl at pH 3.0. As was found for (dA)₂₀, all of the three additional DNA molecules produced both deep blockades and shallow blockades. The mean residence times and amplitudes of the deep blockades and shallow blockades as well as the percentage of deep blockage events for these three 20-mer DNA samples are summarized in Table 2. From the table, we can see that, in terms of the deep blockades, the residence times of ~65 to 287 μ s/base for various polynucleotides were typically at least ~20 folds larger than the well-documented values of ~1 to 3 μ s/base with the translocation of 100-mer DNA polymers through the WT α HL channel in the 1 M KCl solution with/near physiological pH at room temperature [25]. Therefore, at a lower pH, various DNA polymers indeed translocate through the wild-type α HL pore at a slower velocity. The different event mean residence time and amplitudes as well as different percentages of deep blockades produced by different nucleotides (Tables 1 and 2) may permit the convenient discrimination among the four DNA molecules examined.

Due to the enhanced resolution, the feasibility of utilizing nanopores to analyze very short polynucleotides in acidic solutions was then investigated. A 3-mer ssDNA sample with a sequence of TTT was examined in the wild-type α HL pore in a 1 M NaCl solution with a pH of 3.0. The control experiment was carried out in 1 M NaCl at pH 7.5. Note that such a short DNA sample has not yet been able to be detected by using the electrophoretically-driven nanopore approach before. As shown in Fig. 5, the 3-mer DNA sample indeed produced current modulations at pH 3.0, while no DNA events could be identified at pH 7.5. The ability to detect short DNA molecules offers the potential to use nanopore technique as a rapid effective approach to detect and characterize DNA samples. The study of other short (e.g., 2-mer, 4-mer, etc.) DNA molecules in the α HL pore at pH 3.0 is currently in progress.

It is worthwhile to probe the underlying reasons why DNA samples produce two significantly different types of events (i.e., deep blockades and shallow blockades) in acidic solutions, and why DNA polymers translocate through the α HL pore at a slower velocity but with a larger frequency at acidic pH than at physiological pH. One possible cause is that the change in the pH of the solution affects the charge selectivity of the protein pore. Bayley and co-workers' research showed that when the pH of the electrolyte solution decreased from 11.0 to 7.5 and then to 5.0, the charge selectivity of the wild-type α HL pore changed from cation-selective to weakly anion selective, and then to anion selective [51]. Therefore, a decrease in the pH can enhance DNA translocation due to the enhanced electroosmotic flow, which was supported by our experimental result that a ~8 fold increase in the event frequency was observed when the pH of the solution decreased from 7.5 to 4.0. This is similar to the observation made for the translocation of a 92-nt single-stranded DNA through the (M113R)₇ pore vs. the wild-type pore, in which a ~10 fold increase in the event frequency was found [35]. Note that, in this case, the (M113R)₇ pore was anion selective, while the wild-type α HL pore was weakly anion selective. Recently, Wong and Muthukumar reported that the charge selectivity of the α HL pore could be changed by using a pH gradient across the protein channel. Further, they showed that the effect of electroosmotic flow on the event mean residence time and the event frequency was monotonic [52]. However, in our experiment, we found that with the decrease of the pH of the solution from 7.5 to 3.0, the frequency of (dA)₂₀ events increased until pH 4.0, and then decreased significantly (~5 fold reduction from pH 4.0 to pH 3.0) (Table 1). Therefore, the electroosmotic effect is not the only factor which would affect transport of DNA. And hence,

another likely interpretation is that the change in the pH of the electrolyte solution affects the net charge of nucleic acids. It is well-known that at physiological pH, DNA has a net negative charge since the phosphate components of the DNA backbone are deprotonated, whereas the purine or pyrimidine bases are in a neutral form. It has been estimated that the effective charge per nucleotide is substantially less than 1 electron charge in nanopore experiments [53, 54]. With a decrease in the pH of the solution, the DNA bases can accept the protons in the electrolyte, leading to a reduction in the effective negative charge of the DNA molecule. And thus a slower DNA migration in the nanopore could be expected at a fixed applied potential, e.g., +120 mV in this work. Take (dA)₂₀ for example, the pK_a value of atom N-1 of adenine is ~3.6 [55]. When the pH of the solution decreases to pH 4.0, ~21.5% of the adenine nucleobases of (dA)₂₀ will be protonated, while the protonated form of adenine nucleobases will be ~80% at pH 3.0. Furthermore, given that the nucleosides had different protonation states at these two pHs, a ~5 folds reduction in the frequency of (dA)₂₀ events was not unreasonable when the pH of the solution decreased from pH 4.0 to pH 3.0. To support the notion that the protonation of the nucleosides affects the event frequency, the interaction of (dT)₂₀ with the α HL pore was investigated at pH 4.0 and pH 3.0. Since the pK_a value of atom N-3 of thymine is ~9.8 [55], the pyrimidine bases of (dT)₂₀ at pH 3.0 and pH 4.0 had the same protonation states and both should be protonated. Therefore, unlike the observation made for (dA)₂₀, the frequency of (dT)₂₀ events should be monotonically increases with the electro-osmotic effect this time. As we expected, our experiments showed that the frequency of (dT)₂₀ events indeed increased by ~4 folds when the pH of the solution decreased from 4.0 to 3.0. The fact that pH affects the charge of nucleic acids was also noticed by Timp and co-workers [46]. In the study of the pH influence on dsDNA permeation through solid-state nanopores, they found that the protonation of the nucleosides destabilized the double helix, thus facilitating DNA translocation.

However, the constant impact of both the net charge of the DNA molecule and the charge selectivity of the protein pore on molecular transport (e.g., the event residence time) [20, 51] could not explain our observation that two quite different types of events were produced when DNA translocated through the α HL pore in acidic solutions. It has been well established that a single polymer molecule may pass through a nanopore in two different configurations (unfolded and folded) [21, 52, 56, 57]. Recently, Li and co-workers found that the folded and unfolded configurations of a 3kb ssDNA polymer produced events with quite different residence times when they translocated through a solid-state nanopore [45]. More recently and more comparable to the ssDNA samples used in our experiments, by arresting a 25-mer single-stranded poly(A) inside the α HL pore, Lin et al. were able to study the helix-coil transition kinetics of poly(A) since the polymer produced events with two distinct current blockage levels, which were proposed to be attributed to the coil and helix structures of poly(A) [58]. Although we can't rule out the possibility that the long-lived events might be attributed to the channel conformational change (e.g., channel collapse) induced by the DNA-protein pore interaction, we are leaning toward the interpretation that the occurrence of two types of events in acid solutions is due to the pH effect on DNA secondary structure. It has been well established that a solution with a lower pH can promote the formation of ssDNA secondary structure [59]. In fact, it is well known that a parallel duplex could be formed in poly(dA) below pH ~4 [60]. In order to translocate such a DNA molecule through the pore, the helical structure of the DNA must be disrupted [25, 61], and thus a longer residence time could be expected. In contrast, the shallow blockades may be attributed to the translocation of the unfolded (e.g., coiled) DNA molecules or the rapid entrance/exit of the helical DNA at the *cis* opening of the channel. In the latter case, after the helical DNA molecule enters the vestibule and moves toward the β -barrel, it fails to translocate through the limiting aperture but instead escapes immediately backwards and exits from the *cis* entrance of the pore. Further experiments and/or computational modeling are required to resolve the origin of these events.

4 Concluding remarks

The work presented here demonstrates that the translocation of single-stranded DNA through the α -hemolysin pore is strongly affected by the pH of the electrolyte solution. Besides the rapid translocation events (~ 4 to $10\mu\text{s}$ per base), another type of events with significantly prolonged residence times (~ 60 to $287\mu\text{s}$ per base) are observed at low pH. Furthermore, the open channel current increases with a decrease in the pH of the electrolyte solution, offering the potential to increase the S/N ratio and hence providing a further improvement in the capability of the nanopore to differentiate polynucleotides. As stated in the introduction, nanometer-sized channels have been used for the development of stochastic sensors for the detection of various substances presenting pharmaceutical, clinical, environmental, and biological interest due to their high sensitivity and resolution. These include organic molecules [3], anions [4], cations [9], terrorist agents [62], enantiomers [10,11], peptides [23], proteins [13–16], DNA, etc. Our experiments demonstrated that the sensitivity of a nanopore sensor could also be greatly improved by reducing the pH of the electrolyte solution (Supporting Information, Fig. S3). Therefore, in addition to be used as an effective approach to affect DNA translocation in the nanopore, manipulation of the pH of the electrolyte solution may find useful application in stochastic sensing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

αHL	alpha-hemolysin
dsDNA	double-stranded DNA

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Figure 1.

Translocation of (dA)₂₀ in the wild-type αHL pore in 1M NaCl solutions having various pH values: (A) pH 7.5; (B) pH 6.0; (C) pH 5.0; (D) pH 4.0; and (E) pH 3.0. (*Left*) Representative single channel current recording traces; (*Middle*) Typical shallow blockades shown at expanded time scales; and (*Right*) Typical deep blockades shown at expanded time scales. The experiments were performed at +120 mV. Dashed lines represent the levels of zero current.

Figure 2.

Blockage characteristics of (dA)₂₀ in the wild-type αHL pore at pH 3.0. (A) Scatter plot of event amplitude vs. residence time; (B) event amplitude histograms, showing two types of blockades (deep and shallow); and (C) and (D) event residence time histograms of the shallow blockades and deep blockades, respectively. I_r/I_o in Figs. 2A and 2B is normalized blockage residual current, which was obtained by dividing the average blockage residual current of an event by the average open channel current. The experiments were performed at +120 mV.

Figure 3. Effect of DNA length on the mean residence time of deep blockades, suggesting that these events are caused by DNA's threading through the α HL pore. Experiments were performed at +120 mV with the wild-type α HL pore in a 1 M NaCl solution at pH 3.0.

Figure 4. Effect of the applied voltage on the mean residence time of deep blockades. Experiments were performed with the wild-type α HL pore in a 1 M NaCl solution at pH 4.0.

Figure 5.

Single channel recordings of a 3-mer ssDNA at (A) pH 7.5; and (B) pH 3.0, showing the viability of utilizing acidic electrolyte solutions for the detection of short DNA samples. Experiments were performed at +120 mV with a DNA sample (sequence: TTT) in the wild-type α HL pore in 1 M NaCl.

Effect of pH on the translocation of (dA)₂₀ through the wild-type αHL pore. Each experimental value represents the mean of three replicate analyses ± one standard deviation. Experiments were performed at +120 mV in 1M NaCl solutions with various pH values.

Table 1

pH	Mean residence time (μs)		Mean current blockage (%)		Percentage of deep block	Event frequency (s ⁻¹)	Open channel current (pA)
	Shallow block	Deep block	Shallow block	Deep block			
7.5	70 ± 10	n.o. ^a	87.7 ± 1.5	n.o. ^a	n.o. ^a	17.0 ± 3.9	97.7 ± 1.4
6.0	83 ± 11	n.o. ^a	77.5 ± 1.9	n.o. ^a	n.o. ^a	27.9 ± 5.7	94.2 ± 2.4
5.0	86 ± 11	350 ± 52	84.3 ± 2.0	97.2 ± 0.5	1.2 ± 0.3	66.1 ± 10.1	92.1 ± 3.9
4.0	116 ± 28	1250 ± 111	85.6 ± 1.5	98.4 ± 0.3	18.5 ± 4.4	133.2 ± 35.2	108.3 ± 1.4
3.0	80 ± 10	1210 ± 156	83.0 ± 3.2	98.8 ± 0.2	19.5 ± 1.0	26.8 ± 9.3	112.1 ± 2.4

^a could not be obtained.

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Table 2

Summary of statistical translocation properties of three ssDNA samples in the wild-type αHL pore at pH 3.0. Each experimental value represents the mean of three replicate analyses ± one standard deviation. Experiments were performed at +120 mV.

ssDNA sample	Mean residence time (μs)		Mean current blockage (%)		Percentage of deep block
	Shallow block	Deep block	Shallow block	Deep block	
(dT) ₂₀	190 ± 23	1295 ± 215	92.1 ± 0.3	98.1 ± 0.5	41.9 ± 3.9
(dAdG) ₁₀	195 ± 20	5740 ± 254	80.7 ± 2.0	94.6 ± 0.1	49.8 ± 1.9
(dCdT) ₁₀	166 ± 15	2746 ± 266	87.8 ± 1.2	98.6 ± 0.4	48.2 ± 16.1