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A chemical-induced pH-mediated molecular switch

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Abstract

The transmembrane protein α -hemolysin pore has been used to develop ultrasensitive biosensors, study biomolecular folding and unfolding, investigate covalent and non-covalent bonding interactions, and probe enzyme kinetics. Here, we report that by addition of ionic liquid tetrakis(hydroxymethyl)phosphonium chloride solution to the α -hemolysin pore, the α -hemolysin channel can be controlled open or closed by adjusting the pH of the solution. This approach can be employed to develop a novel molecular switch to regulate molecular transport, and should find potential applications as a 'smart' drug delivery method.

INTRODUCTION

Transmembrane protein ion channels (or pores) play an important role in selectively transporting molecules and ions essential for various signaling, physiological, and metabolic activities. In such a process, the protein pore acts as a signal transducer that senses chemical and physical stimuli, leading to the opening and closing of an ion channel¹ (also known as gating). Gating may be reversible or irreversible depending upon the intra/extracellular environment.^{2,3} Numerous pH gated,² voltage-gated,^{4,5} mechanical gated,^{6,7} and ligand gated^{1,8–10} ion channels have been found in nature. Gated channels offer potential as a useful tool for drug discovery and controlled drug delivery.⁸

Nanopore-based sensing technique provides a unique platform for studying and probing channel gating. Such studies may have potential applications in medicine, fuel cell development, and analytical chemistry.¹¹ Both biological ion channels embedded in planar lipid bilayers and artificial nanopores fabricated in solid state membranes have been used as sensor elements to detect analytes via successive single molecule events. When individual molecules pass through a nanometer-sized pore at a fixed applied potential bias in a high salt buffer solution (typically 1 M NaCl or 1 M KCl at or near physiological pH), the modulations of the ionic current flowing through the pore can be detected as random events.^{12–14} The compiled information from these events can be used to reveal both the identity and the concentration of an analyte: the former by its characteristic current signature, typically the residence time (τ_{off}) of the analyte coupled with the extent of the channel blockage (amplitude), and the latter from the frequency of occurrence ($1/\tau_{\text{on}}$) of the current modulations. The most widely used sensor element in nanopore sensing is a single transmembrane protein α -hemolysin (α HL) channel, which has a ~3 nm diameter *cis* entrance and ~2 nm diameter *trans* opening.¹⁵ The constriction within the α HL channel has a diameter of ~1.4 nm. α HL channels have been used as nanopore sensing elements for the

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detection of a wide variety of substances such as organic molecules,¹⁶ anions,¹⁷ cations,¹⁸ explosives,^{19,20} enantiomers,²¹ reactive molecules,²² proteins,^{23,24} DNA,^{25–28} peptides²⁹ and their cleavage products.³⁰

In our previous study, we introduced solutions of ionic liquids as advantageous supporting electrolytes for this nanopore technology.¹⁹ Specifically, the use of butylmethylimidazolium chloride (BMIM-Cl) solution instead of the commonly used NaCl/KCl solution as a supporting electrolyte enhanced the nanopore resolution, and also permitted the analysis of ions and molecules (e.g., liquid explosives and mono-valent cations²¹) that are difficult or even impossible to achieve using solutions of inorganic salts. Ionic liquids usually have organic cations (e.g., imidazolium, pyridinium, pyrrolidinium, phosphonium, and ammonium). Anions could be inorganic, including Cl^- , PF_6^- , BF_4^- , and organic such as trifluoromethylsulfonate, bis[(trifluoromethyl)sulfonyl]imide, trifluoroethanoate, etc. Here, we report that ionic liquid tetrakis(hydroxymethyl)phosphonium chloride [$\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$] solution can induce gating for the αHL channel. Further, the opening and closing of the αHL pore is regulated by the pH of the solution.

EXPERIMENTAL SECTION

Materials

Except for bis-tetrakis(hydroxymethyl)phosphonium sulfate [$\text{P}(\text{CH}_2\text{OH})_4\text{-SO}_4$], which was obtained from TCI Chemicals Inc (Portland, OR), all other reagents, including $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$, were obtained from Sigma-Aldrich (St. Louis, MO).

All electrolyte solutions, i.e., 1 M $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$, 1 M $\text{P}(\text{CH}_2\text{OH})_4\text{-SO}_4$, 1 M BMIM-Cl, 1 M NaCl, and 1 M Bis-Tris methane were prepared in HPLC-grade water unless otherwise stated, and buffered with either 10 mM citric acid/sodium citrate buffer (pH range = 3.0 – 6.2), 10 mM NaH_2PO_4 (pH range: 5.8 – 7.2), or 10 mM HEPES (pH range: 7.0 – 8.0). A stock solution of 4 mM β -cyclodextrin (βCD) was also prepared in HPLC-grade water (ChromAR, Mallinckrodt chemicals), and was used as the analyte of this study.

Wild-type αHL monomer was first synthesized by coupled *in vitro* transcription and translation (IVTT) using the *E. Coli* T7 S30 Extract System for Circular DNA from Promega (Madison, WI). Subsequently, they were assembled into homoheptamers by adding rabbit red cell membranes and incubating for 1 h.³¹ The heptamers were purified by SDS-polyacrylamide gel electrophoresis and stored in aliquots at -80°C .

Methods

A bilayer of 1,2-diphytanoylphosphatidylcholine (Avanti Polar Lipids; Alabaster, AL) was formed on an aperture (150 μm) in a Teflon septum (25 μm thick; Goodfellow, Malvern, PA) that divided a planar bilayer chamber into two compartments, *cis* and *trans*. The formation of the bilayer was achieved by using the Montal-Mueller method,³² and monitored by using a function generator (BK precision 4012A; Yorba Linda, CA). The experiments were performed at $22 \pm 1^\circ\text{C}$ in various electrolyte solutions as described in the “Materials” section. The αHL protein (with the final concentration of $0.2\text{--}2.0\text{ ng}\cdot\text{mL}^{-1}$) was added to the *cis* compartment, which connects to “ground”, while βCD was added from the *trans* compartment. Once the channel inserts into the bilayer, the mushroom cap of the αHL pore positions towards the *cis* compartment, while the β -barrel of the αHL is located at the *trans* of the chamber device. The applied potential was -40 mV , unless otherwise noted. Currents were recorded with a patch clamp amplifier (Axopatch 200B, Molecular Devices; Sunnyvale, CA). They were low-pass filtered with a built-in four-pole Bessel filter at 5 kHz and sampled at 25 kHz by a computer equipped with a Digidata 1440 A/D converter (Molecular Devices). To shield against ambient electrical noise, a metal box was used to

serve as a Faraday cage, inside which the bilayer recording amplifying headstage, stirring system, chamber, and chamber holder were enclosed.

Flow nanopore sensing device

To facilitate adjusting the pH of the solution in the bilayer chamber compartments, a flow set-up was constructed. As shown in Figure 1, a four channel peristaltic pump, PP (Dynamax, Rainin, 0.1 in. i.d. pump tubing, 0.8 rotations per minute; rpm), was used to perform buffer exchange, where a polyvinyl chloride (PVC) pump tubing (0.75 mm internal diameter, id) was connected to each channel of the PP. Two tubes, each connected to the *cis* and *trans* inlets of the reaction chamber (RC), supplies the desired electrolyte from the reservoir to the RC, and the other two tubes connected to the outlet of *cis* and *trans* compartments withdraws the electrolyte to be replaced from the RC to waste (Figure 1). The buffer exchange process is carried out in such a way that the liquid level inside the reaction chamber is constant. A low flow rate of 0.2 mL/min was maintained in order to prevent any disruption to the bilayer and the inserted protein channel. Pump tubings were further connected to polytetrafluoroethylene (PTFE) tubes (0.86-mm i.d., 1.68-mm o.d., 20 SW).

pH adjustments and on-line monitoring of the pH value of the solution were performed at 22 ± 1 °C by using a pH microprobe electrode, (P/N MI-414-4 cm pH combination Electrode, Microelectrodes Inc.) which was connected to a pH meter (Symphony SB70P, VWR) for all the experiments. As to adjusting the pH of the solution in the on/off-line experiments, pre-determined amount of a 6 M NaOH/6 M HCl was added to both the *cis* and *trans* compartments. Again, the pH of the solution was confirmed with the pH meter.

The ^{31}P NMR spectra were recorded for 1 M $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ at pH 3.0, then at pH 6.0, which was obtained by the addition of a pre-determined amount of 6 M NaOH to the solution, and back at pH 3.0 by adding 6 M HCl to the same solution. The readings were taken after incubation at room temperature for 24 hours and also on-line without any incubation. Further, readings were taken with and without the buffer.

Data analysis was achieved using Clampfit 10.0 software (Molecular Devices) and Origin 6.0 (Microcal, Northampton, MA). Conductance values were obtained from the amplitude histograms after the peaks were fit to Gaussian functions. The values of τ_{on} (the inter-event interval) and τ_{off} (the residence time) for βCD were obtained from dwell time histograms by fitting the distributions to single exponential functions by the Levenberg-Marquardt procedure.³³ Kinetic constants were calculated by using $k_{\text{off}} = 1/\tau_{\text{off}}$, $k_{\text{on}} = 1/(C\tau_{\text{on}})$, and $K_d = k_{\text{off}}/k_{\text{on}}$, where C is the concentration of βCD . All the results were reported as mean values $\pm$ standard deviation.

RESULTS AND DISCUSSION

Effect of pH on the interaction between βCD and the αHL pore in the $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ solution

The initial experiments were carried out at -40 mV in 1 M $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ solutions at pHs ranging from pH 3.0 to 6.9. The results showed that, with the increase in the pH of the solution, both the channel current and the residence time of βCD decreased (Figure 2). The pH dependence of the channel instantaneous current could be best fit with the Hill equation,³⁴ which yields a pK value of 5.38 and the hill slope of 1.8, indicating cooperative behavior. When the pH of the solution was greater than ~ 5.6 , all βCD events disappeared, and a larger current noise was observed. One likely interpretation is that the gating of the αHL pore might occur, thus preventing the interaction between βCD and the pore when the pH of the solution is greater than pH 5.6. To support this hypothesis, the effects of the pH of the $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ solutions on their conductivities and the αHL channel in the absence of

β CD were investigated. The experimental results showed that the conductivities of the bulk $P(CH_2OH)_4$ -Cl solutions were not significantly different with the change in the pH of the solution (Figure 3). The same phenomenon as described in the case of having β CD in the solution (i.e., the pH of the solution significantly affected the α HL channel) was observed in the solution without β CD except that there were only occasional spikes (~ 1 – 4 events/min) in the current trace when the pH of the solution was less than pH ~ 5.6 (Figure S1, Supporting Information). Taken together, the combined results suggest that in the absence or presence of β CD, the gating of the α HL pore really occurs in $P(CH_2OH)_4$ -Cl solutions when the solution pH is greater than pH 5.6, which may find potential application as a molecular switch to regulate molecular transport. To demonstrate this application, the interaction of peptide YYYYYY with the α HL pore was investigated in the $P(CH_2OH)_4$ -Cl solutions at pH 3.0 and pH 6.0, where the peptide was added to the *cis* compartment. As expected, current modulation events were observed at pH 3.0 but not at pH 6.0 (data not shown), providing evidence that the transfer of the peptide through the α HL pore in the $P(CH_2OH)_4$ -Cl solution was permitted at pH 3.0, but inhibited at pH 6.0.

The blockage of the mutant α HL channel by phosphate compounds (ligands) has been reported previously,¹⁷ and shown to be voltage dependent. However, in $P(CH_2OH)_4$ -Cl solutions, although the applied potential affected the channel current, it would not cause the channel to open or close (Figure S2). Misakian and Kasianowicz showed that changing the pH of the NaCl solution altered the open α HL channel's I–V relationship.³⁵ However, the pH effect on the pore's I–V relationship was much more significant in the $P(CH_2OH)_4$ -Cl solution (Figure S2). Similar to the observation that the applied voltage affected the PEG/ α HL channel reaction kinetics in the inorganic salt solution,³⁶ we found that the rate of entry of β CD into the pore (k_{on}) and the mean residence time (τ_{off}) of β CD in the $P(CH_2OH)_4$ -Cl solution at pH 3.0 were also significantly influenced by the applied potential (Figure S2). It has also been shown that divalent and trivalent cations could block the α HL channel,³⁷ β CD molecules partially block the anthrax PA63 ion channel,³⁸ and that cations alter the lifetime of PEGs in the α HL channel.³⁶ Further, Kasianowicz et al. reported that aqueous protons and deuterium ions could bind to the α HL channel and reduce the open channel conductance,³⁹ and stochastic gating of the α HL channel might occur more frequently at a lower pH.⁴⁰ However, it should be noted that unlike the permanent channel block in the $P(CH_2OH)_4$ -Cl solution, the background current modulations of the α HL pore in the inorganic salt solution were rarely observed even at pH 3.0. Further, the α HL channel would usually re-open in a few seconds or less once it closed.⁴¹

Effect of pH on the α HL channel in other electrolytes

To investigate whether the α HL channel gating phenomenon was induced by the $P(CH_2OH)_4$ -Cl solution or was only due to the pH effect, four other solutions were examined, including $P(CH_2OH)_4$ -SO₄, BMIM-Cl, NaCl, and Bis-Tris methane (Zwitter ionic buffer with pK_a of 6.46), in the presence and absence of β CD. Our experiments showed that in the BMIM-Cl, NaCl and Bis-tris methane solutions, both the channel current and the residence time of β CD decreased when the pH of the solution increased, however, the current noise was not changed significantly and the β CD events could still be observed (Figure 4 and Supporting Information, Figure S1). This indicates that the α HL pore does not lose its permeability in these electrolyte solutions under various pH values. Consequently, pH effects cannot be solely responsible for any observed gating in the $P(CH_2OH)_4$ -Cl solution. When 1 M $P(CH_2OH)_4$ -SO₄ solution was used, the gating behavior was again observed (data not shown, $n = 3$). Note that the pH effect on the event residence time was also observed by other researchers, which may be attributed to the charge selectivity of the protein pore.^{42–44} The data analysis of the three representative cases (i.e., $P(CH_2OH)_4$ -Cl, BMIM-Cl, and NaCl) showed that the effect of pH on the α HL channel was significantly

different in the $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ solution and other electrolytes (Figure 5). With a decrease in the pH, the dissociation constant (K_d) of βCD in the αHL pore did not vary much in the $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ solution, but decreased significantly in both the NaCl and BMIM-Cl solutions. One of the major reasons for that is because pH affected the dissociate rate constant (k_{off}) more significantly than the association rate constant (k_{on}) in the cases of NaCl and BMIM-Cl , but not in the $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ solution. For example, when the pH of the solution decreased from pH 8.0 to pH 3.0, k_{off} decreased by 16 fold (from $1167 \pm 197 \text{ s}^{-1}$ to $74.0 \pm 2.9 \text{ s}^{-1}$), and 159 fold (from $923 \pm 42 \text{ s}^{-1}$ to $5.8 \pm 0.1 \text{ s}^{-1}$), respectively, while k_{on} decreased by 2.9 fold (from $24.2 \pm 3.8 \times 10^4 \text{ M}\cdot\text{s}^{-1}$ to $84.5 \pm 1.4 \times 10^3 \text{ M}\cdot\text{s}^{-1}$), and 17.9 fold ($23.4 \pm 3.0 \times 10^4 \text{ M}\cdot\text{s}^{-1}$ to $13.1 \pm 0.2 \times 10^3 \text{ M}\cdot\text{s}^{-1}$) fold, respectively, in the NaCl and BMIM-Cl solutions. In contrast, k_{off} decreased by 2.4 fold (from $76.6 \pm 9.1 \text{ s}^{-1}$ to $31.5 \pm 1.2 \text{ s}^{-1}$) fold, while k_{on} decreased by 2.3 fold (from $77.7 \pm 1.8 \times 10^3 \text{ M}\cdot\text{s}^{-1}$ to $33.6 \pm 0.8 \times 10^3 \text{ M}\cdot\text{s}^{-1}$) in the $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ solution as pH decreased from pH 5.0 to 3.0. Like the case of $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$, the conductivities of both the NaCl and BMIM-Cl bulk solutions did not vary much with the change in the pH of the solution (Figure 3). Taken together, the experimental results suggest that $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ is responsible for the channel gating, whereas pH acts as the molecular activator.

Reversible gating of the αHL channel

$\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ is a monoprotic acid, which has an apparent pK_a of 5.5.⁴⁵ As mentioned previously, we found that at pH 5.6, the αHL channel was closed but it was in an open state at pH 5.48. The literature suggests that $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ reacts with NaOH as follows⁴⁶:

To support the proposed mechanism as depicted in the above equation, NMR studies were carried out at different pH values. Data clearly suggests that there is a change in the phosphonium moiety at lower and higher pH values. As shown in Figure S3 (Supporting Information), at pH 3.0, it behaves as a cationic species, whereas it becomes a neutral species above pH 5.6. The results also indicate that the reaction appeared to be reversible with a high rate constant (no incubation required). Consequently, $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ was subjected to an on-line reversibility study.

In preliminary reversibility studies, the pH was varied from lower to higher values, and then from higher to lower pH values. In the first case, the experiment was carried out at -40 mV in $1 \text{ M } \text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ solution at pH 3.0 and in the presence of $80 \mu\text{M } \beta\text{CD}$. A pre-determined amount of $6 \text{ M } \text{NaOH}$ was added sequentially to both the *cis* and *trans* compartments until channel gating was observed. In the second case, a similar approach was adopted by starting from pH 6.0 with sequential addition of $6 \text{ M } \text{HCl}$ until the channel opened. Although a desired channel gating was observed with a gradual increase in the pH of the solution ($n=5$) (Figure 6), the reverse direction could not be achieved due to the bilayer instability upon direct addition of $6 \text{ M } \text{HCl}$ ($n=10$).

To overcome the destructive effect of HCl on the lipid bilayer, a flow set-up was integrated to the nano-pore stochastic sensing device as described in the Experimental section. This allowed efficient exchange of buffers without breaking the bilayer and disturbing the channel. In this study, the experiment began with $1 \text{ M } \text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ (pH 6.0), in the presence of $80 \mu\text{M } \beta\text{CD}$. After insertion of the αHL pore into the lipid bilayer and single channel recording for ~ 2 minutes at -40 mV , the electrolyte solutions in both the *cis* and *trans* compartments were replaced with $1 \text{ M } \text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ solution (pH 3.0) via the peristaltic pump at a flow rate of 0.2 ml/min . To maintain a constant concentration of the

analyte throughout the experiment, the solution that flowed into the *trans* compartment was pre mixed with β CD. Note that the flow rates, as well as the tube length and diameter were optimized for maintaining a constant, unchanging liquid level, which is critical for the bilayer stability and single channel recording. It takes ~12 minutes to replace the electrolyte solution from the RC using the flow set-up, judged by the open channel current as well as the pH value measured online using a pH microprobe. Once the channel opens, the trace was recorded for another ~5 minutes. And then, a pre-determined amount of 6 M NaOH was added directly to both the *cis* and *trans* compartments to change the pH of the solution back to pH 6.0. Although the flow setup was capable of refilling the compartment with the pH 6.0 electrolyte solution again, we preferred manual addition of the NaOH solution to raise the pH, as this approach was faster and did not affect the existing bilayer and the protein pore. The online monitoring of pH effect on the α HL channel in 1 M $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ solution was performed with both buffered [(citric acid and sodium citrate) $n=3$] and unbuffered ($n=10$) solution. The results presented in Figure 7 confirms that the gating is indeed reversible.

Investigating the gating mechanism in the α HL pore

As described in the previous sections, the gating phenomenon was observed in the $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ solution with at pH values higher than ~5.6. To support the hypothesis that the gating may be attributed to $\text{P}(\text{CH}_2\text{OH})_3$, four sets of experiments were performed in 1M NaCl solution with/without $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}/\text{P}(\text{CH}_2\text{OH})_3$ in the presence of β CD. Specifically, the first set of experiments involved using a 1 M NaCl (pH 3.0) supporting electrolyte with subsequent addition of $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ to the *trans* compartment via 10 mM sequential increments, up to 60 mM, and then to the *cis* compartment in a similar fashion. The second set of experiments was the same as the first one with the exception that $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ was first added to the *cis* compartment. The third and fourth sets of experiments were similar to the first and second sets, respectively, except that the experiments were carried out at pH 6.0. The results indicated that at pH 3.0, the β CD signal (the event mean dwell time and amplitude) didn't change significantly upon addition of $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ even up to 60 mM to both the *cis* and *trans* compartments ($n=3$). At pH 6.0, the addition of $\text{P}(\text{CH}_2\text{OH})_3$ to the *trans* compartment up to 60 mM didn't negate the β CD events, but reduced the frequency of the β CD events by $33.8 \pm 0.9 \%$, while increased the event residence time by $18.1 \pm 1.0 \%$; further, the channel current decreased gradually with the recording time (note that a $16.2 \pm 1.0\%$ current decrease was observed in 20 minutes). In sharp contrast, at pH 6.0, a complete gated channel was observed when 30 mM $\text{P}(\text{CH}_2\text{OH})_3$ was present in the *cis* compartment. The voltage effect on these gated channels also was studied. The experiments showed that the α HL channel could not be restored to the open stage over a voltage range from -20 to -300 mV. This suggests a voltage independent gating. Similar experiments were also performed in 1M BMIM-Cl solution instead of the 1 M NaCl solution ($n=3$), and similar results were observed. This provides further evidence that gating is due to the binding of tris(hydroxymethyl)phosphine to the α HL pore, which occurs in solutions with pH ~ 5.6.

Conclusions

By adding $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ to the α HL pore and adjusting the pH of the electrolyte solution, the α HL channel can be controlled (i.e., opened or closed). The gating mechanism was found to involve the reversible transformation of a specific phosphonium ion to a neutral organo-phosphine. A variety of other salts and their solutions are currently being investigated to examine whether the gating phenomena in the α HL pore is limited to the phosphonium ionic liquid in this study. It is well known that voltage-gated and ligand-gated ion channels offer tremendous opportunities for drug discovery and targeted drug delivery.⁴⁷⁻⁴⁹ pH-responsive drug delivery systems have also attracted great interest especially because of their potential use in tumor targeting due to the acidic extracellular pH

environment of human tumors.⁵⁰ In this study, we have found that the presence of a phosphonium ionic liquid in the solution allows pH to regulate ionic flow through the α HL channel. This finding can be employed to develop a novel molecular switch to regulate molecular transport. Such a chemical induced pH-mediated nano-valve mechanism should find potential applications as a ‘smart’ drug delivery method.⁵¹ For example, controlled or targeted drug release based on local pH levels could be potentially achieved by incorporating ion channels in drug and chemical encapsulated liposomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Schematic representation of the flow nanopore stochastic sensing system, where the reservoir labeled “P.Cl” contains 1 M $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ solution, and the one labeled “P.Cl + βCD ” contains a mixture of 1 M $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ and 80 μM βCD .

Figure 2.

The effect of pH on the interaction between β CD and the α -hemolysin pore in 1 M phosphonium IL solution. a) Representative single channel current recording traces; and b) the plot of the channel current vs. pH of the solution. Dashed lines in Figure 2a represent the levels of zero current. The theoretical curves fitted to the data in Figure 2b use the form $i(\text{pH}) = i_{\text{min}} + \Delta i / [1 + 10^{n(\text{pH}-\text{pK})}]$, where the dashed one was obtained by setting the Hill coefficient equal to 1.0, while the solid curve was obtained by setting all parameters free. A better fit is seen for $n = 1.8$. The experiments were performed at -40 mV.

Figure 3. Conductivities of 1 M solutions of (■) NaCl; (●) P(CH₂OH)₄-Cl; and (▲) BMIM-Cl at various pH values.

Figure 4.

pH dependence of the β CD's binding to the α HL channel in 1 M BMIM-Cl solution at -40 mV. Dashed lines represent the levels of zero current. Similar phenomena were observed in 1 M NaCl and 1 M Bis-Tris methane solutions (data not shown) where both the channel current and the mean residence time of the β CD events decreased as pH of the solution increased.

Figure 5. pH dependence of β CD/ α HL ion channel reaction kinetics in 1 M solutions of (■) P(CH₂OH)₄-Cl; (●) BMIM-Cl; and (▲) NaCl at various pH values. a) k_{off} ; b) k_{on} ; and c) K_d . The experiments were performed at -40 mV.

Figure 6.

The effect of pH on the interaction between β CD and the α HL pore, where a pre-determined amount of 6 M NaOH solution was sequentially added to both the *cis* and *trans* compartments to increase the pH of the electrolyte solution. Initially, the compartments contained 1 M $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ solution with the pH adjusted to 3.0.

Figure 7.

On-line monitoring of the pH effect on the α HL channel in the presence of 80 μ M β CD, showing that the gating of the α HL channel by the $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ solution is reversible by adjusting the pH of the solution. To reduce the pH of the solution from 6.0 to 4.0, the electrolyte solutions in both the *cis* and *trans* compartments were replaced with 1 M $\text{P}(\text{CH}_2\text{OH})_4\text{-Cl}$ solution (pH 3.0) via the peristaltic pump for 12 minutes, while a pre-determined amount of 6 M NaOH was added directly to both the *cis* and *trans* compartments to bring the pH of the solution back to pH 6.0.