Charge state of the fast gate in chloride channels: Insights from electrostatic calculations in a schematic model

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Fast gating is a unique property of chloride channels, where a permeating Cl\(^{-}\) ion acts as its own ligand in opening the channel. The glutamate residue implicated in fast gating normally carries a unit negative charge. Whether this charge needs to be protonated to enable permeation of a Cl\(^{-}\) ion is an important question that will affect how models of chloride channels are constructed. We investigate the energetic consequences of the charge state of this glutamate residue from continuum electrostatics using a schematic cylindrical channel model. Both analytical solutions of the Poisson equation for an infinite cylinder and numerical ones for a finite cylinder are employed in the calculations. © 2007 American Institute of Physics. [DOI: 10.1063/1.2804419]

I. INTRODUCTION

Ion channels play a key role in electrical signaling in cells and understanding their operation is a fundamental problem in neurology and physiology.\(^{1}\) While their functional roles are well characterized from numerous measurements of ionic currents through single channels, progress on their modeling has been slow due to lack of molecular structures. The recent breakthroughs in determining the crystal structures of first the bacterial potassium channels\(^{2}\) and then chloride channel proteins\(^{3,4}\) have thus given hope that we will finally be able to decipher the structure-function relationships in these classes of ion channels. A great deal of progress has already been made in modeling of potassium channels using Brownian and molecular dynamics simulations.\(^{5,6}\)

Chloride (CIC) channels play diverse roles in cell physiology from solute transport across epithelia to stabilizing the membrane potential and regulating the intracellular pH and cell volume.\(^{3}\) Mutations in the genes that transcribe the chloride channel proteins are linked with many genetic diseases and renal disorders.\(^{7,8}\) Despite their significance in physiology and neurology, much less is known about chloride channels compared to the mainstream cation channels. Lack of structural information, as well as their unusual gating and conductance properties, has been responsible for this slow progress.\(^{9-13}\)

A unique property of chloride channels is their fast gating in response to voltage changes, which has a great dependence on the extracellular Cl\(^{-}\) concentration but not on the internal one.\(^{10,11}\) Further, mutation of the negatively charged glutamate residue lining the channel wall to a neutral glutamine residue alters the fast gating of chloride channels. This has been interpreted as that a permeating Cl\(^{-}\) ion itself acts as a ligand in opening the channel. The recent crystal structures of bacterial CIC proteins\(^{3,4}\) support this interpretation. In the native structure, there are no Cl\(^{-}\) ions near the glutamate E148 residue but when it is mutated to a neutral residue, a Cl\(^{-}\) ion is observed at the position of the glutamate oxygen. These observations have been interpreted as that the appearance of a Cl\(^{-}\) ion in the selectivity filter of the channel displaces the glutamate side chain, opening the permeation pathway for anion conductance. Such a swinging of the glutamate side chain is one of the main hypotheses for fast gating at present.

In a more recent development, the bacterial CIC proteins were found to be Cl\(^{-}/H^+\) transporters,\(^{14,15}\) where outward permeation of Cl\(^{-}\) ions is coupled to influx of protons. That is, unlike in a channel where Cl\(^{-}\) ions would passively diffuse into the cell in the direction of the concentration gradient, they are pumped out utilizing the H\(^{+}\) concentration difference across the cell membrane. Some of the mammalian chloride channels (e.g., CIC-4 and CIC-5) also turned out to function like Cl\(^{-}/H^+\) transporters.\(^{16,17}\) This raises the intriguing possibility that the glutamate residue involved in fast gating may be protonated during the passage of a Cl\(^{-}\) ion, at least in some of the chloride channels. We note that despite similarities in key functional residues, such as the glutamate residue involved in fast gating, the overall topologies of CIC transporters and channels are quite different. Transporters are rather short and narrow (~20 Å length and 2 Å radius), whereas homology models of channels\(^{18}\) indicate that they are longer and somewhat wider (~50 Å length and ≥3 Å radius). Conduction rates of Cl\(^{-}\) ions in transporters are thousand times smaller compared to the CIC channels, which is presumably caused by the narrowness of the pore and lack of water in the transporter structure.\(^{3,4}\) These observations discourage application of continuum electrostatics to transporters but it could still provide useful insights for CIC channels.

Modeling of chloride channels are still in an early stage. In most studies to date, the crystal structures of bacterial CIC protein structures or their homologs are employed to calculate the potential energy profiles of Cl\(^{-}\) ions using continuum electrostatics.\(^{19,20}\) Monte Carlo methods,\(^{21}\) Brownian dynamics,\(^{18}\) or molecular dynamics simulations.\(^{22-24}\) While there is no consensus as yet, some of these studies suggest that the energy barrier near the E148 residue may be too high.
for permeation of Cl\textsuperscript{−} ions, and protonation of this residue may be required to achieve that end. This, of course, is what one expects from a Cl\textsuperscript{−}/H\textsuperscript{+} transporter.

Clearly, the charge state of this glutamate residue and its position in the open and closed states of the channel will play a significant role in modeling of chloride channels. Here we address this issue using a simplified cylindrical channel model that contains a Cl\textsuperscript{−} ion and a single fixed charge of magnitude \( e \) representing the charge on the side chain of the glutamate residue. The side chain is assumed to occupy an axial position in the closed state and swings to the channel wall in the open state. We solve the Poisson equation for various positions of this charge and determine the potential energy barrier acting on a Cl\textsuperscript{−} ion permeating along the pore axis. For the glutamate residue to act as a gate, there must be a substantial reduction in this potential energy barrier when the glutamate side chain swings away from the pore axis. Otherwise to enable permeation of a Cl\textsuperscript{−} ion, the glutamate residue must be protonated, which would abolish the potential energy barrier faced by the ion altogether.

Ion channels have often been represented by cylindrical conduits in the past. For example, solutions of the Poisson equation in infinite\textsuperscript{25} or finite cylinders\textsuperscript{26,27} have provided important insights on the role of the dielectric self-energy in ion channels. Dynamic descriptions of ion channels have been obtained by coupling the solutions of the Poisson equation to that of the Nernst-Planck equation, which allowed a self-consistent determination of the channel conductance under various physiological conditions.\textsuperscript{28,29} More realistic descriptions of channel currents have been obtained by coupling the solutions of the Poisson equation to the Langevin equation in Brownian dynamics simulations.\textsuperscript{30,31} Similar continuum electrostatic models of cylindrical channels have also been used in other fields, e.g., to describe the interaction of ionized particles with surface plasmons in cylindrical cavities\textsuperscript{32} and the interaction of charged species with the metallic single-walled carbon nanotubes.\textsuperscript{33}

II. ELECTROSTATIC CALCULATIONS

Chloride channels are formed by a membrane spanning protein with a long, narrow, water-filled pore forming the conduction pathway for Cl\textsuperscript{−} ions. The glutamate residue responsible for the fast gating is located near the middle of the channel. In general, the Poisson equation for such a geometry can only be solved numerically. However, for a long, narrow cylindrical pore, one can approximate it with an infinite cylinder, which is amenable to analytical solution.\textsuperscript{34} Analytical solutions have several advantages over the numerical ones such as rigorous results and computational ease. Also they do not present any problems when the charge is near the boundary. Therefore, we will first use the analytical solutions to study the potential energy on a Cl\textsuperscript{−} ion due to the glutamate residue and then obtain the numerical solutions in a specific case to show that similar results are obtained in a finite length channel.

\[ \nabla [\varepsilon(r) \nabla \Phi(r)] = - \frac{1}{\varepsilon_0} \rho_{fix}(r) \]  

(1)

for a fixed charge density \( \rho_{fix}(r) \) in a cylindrical boundary, we expand the potential \( \Phi(r) \) in a Fourier-Bessel series in cylindrical coordinates \( r=(\rho, \phi, z) \). For a point charge located at \( r'=(d,0,0) \), the potential can be expanded as

\[ \Phi_{p}(r) = \frac{1}{4\pi\varepsilon_0} \frac{q}{|r-r'|} \]

\[ = \frac{1}{4\pi\varepsilon_0} \frac{2q}{\pi\varepsilon} \int_{0}^{\infty} dk \cos(kz) \]

\[ \times \sum_{m=-\infty}^{\infty} e^{im\phi} I_m(k\rho_\perp) K_m(k\rho_\perp). \]  

(2)

Here \( I_m \) and \( K_m \) are the modified Bessel functions, and \( \rho_\perp \) denotes the smaller of \( (\rho,d) \) and \( \rho_\perp \) denotes the larger. The dielectric constant is determined by the medium the charge is in, that is, \( \varepsilon=\varepsilon_\perp \) for \( d<a \) and \( \varepsilon=\varepsilon_\parallel \) for \( d>a \). The potential due to the induced charges on the boundary satisfies the Laplace equation and can be similarly expanded.

A. Analytical solutions

Here we present the solutions of the Poisson equation for a charge \( q \) located at an arbitrary distance \( d \) from the axis of an infinite cylinder with radius \( a \). A schematic picture of the cylindrical channel indicating the positions of the glutamate charge and the Cl\textsuperscript{−} ion in an open configuration is shown in Fig. 1. Both water inside the cylinder and protein outside are treated as continuum with respective dielectric constants of \( \varepsilon_\perp \) and \( \varepsilon_\parallel \). The charge \( q \) represents the charge state of the glutamate residue, which is \( -e \) at normal \( \mathrm{pH} \) and 0 when it is protonated. Once the potential \( \Phi(r) \) due to the glutamate charge is determined, the potential energy of an ion of charge \( q \) at position \( r \) is simply given by \( U=q\Phi(r) \). To solve the Poisson equation

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\[ \Phi_2(r) = \frac{1}{4\pi\varepsilon_0} \int_0^\infty dk \cos(kz) \]
\[ \times \sum_{m=\infty}^\infty \ e^{imk} \left[ f_m(k) I_m(k\rho), \quad \rho < a \\ g_m(k) K_m(k\rho), \quad \rho > a, \right. \] \tag{3}

where the functions \( f_m(k) \) and \( g_m(k) \) are to be determined from the boundary conditions. Note that \( I_m \) diverges at infinity and \( K_m \) at zero; hence the above choice for potentials already includes these boundary conditions. The total potentials inside and outside the cylinder are given by appropriate combinations of Eqs. (2) and (3),

\[ \Phi_m = \Phi_p + \Phi_L (\rho < a), \quad \Phi_{out} = \Phi_p + \Phi_L (\rho > a). \tag{4} \]

These potentials must satisfy the usual boundary conditions at the cylinder surface, \( \rho = a \),

\[ \Phi_m = \Phi_{out}, \quad \frac{\partial \Phi_m}{\partial \rho} = \frac{\partial \Phi_{out}}{\partial \rho}, \tag{5} \]

which yield two equations in the two unknown functions \( f_m(k) \) and \( g_m(k) \). The first boundary condition simply gives

\[ I_m(ka) f_m(k) = K_m(ka) g_m(k). \tag{6} \]

To apply the second one, we need to specify whether the charge is inside or outside the cylinder, which are considered separately below.

### B. Charge inside the cylinder

For a point charge inside the cylinder \((d < a)\), we choose \( \rho_x = d, \quad \rho_y = \rho \), and \( \varepsilon = \varepsilon_w \) in Eq. (2). Applying the second boundary condition in Eq. (5) and using Eq. (6), we obtain for \( f_m(k) \)

\[ f_m(k) = -\frac{2q}{\pi\varepsilon_w} (\varepsilon_w - \varepsilon_p) I_m(kd) \left[ \frac{K_m K_m'}{\varepsilon_p I_m K_m - \varepsilon_p J_m K_m'} \right]_{x=ka}. \tag{7} \]

Here the prime on the modified Bessel functions denotes derivative with respect to the argument \( x = k\rho \). These can be obtained from the recurrence relations

\[ I'_m(x) = \frac{1}{2} [I_{m-1}(x) + I_{m+1}(x)], \tag{8} \]
\[ K'_m(x) = -\frac{1}{2} [K_{m-1}(x) + K_{m+1}(x)]. \]

Using Eq. (8) and the Wronskian, \( I_m(x)K_{m+1}(x) + I_{m+1}(x)K_m(x) = 1/x \), the function \( f_m(k) \) can be written as

\[ f_m(k) = \frac{2q}{\pi\varepsilon_w} (\varepsilon_w - \varepsilon_p) I_m(kd) \left[ \frac{xK_m K_m' + xK_m K_m'}{2\varepsilon_p + (\varepsilon_w - \varepsilon_p) xK_m (I_{m-1} + I_{m+1})} \right]_{x=ka}. \tag{9} \]

Substituting this expression for \( f_m(k) \) in Eq. (3), we obtain the potential due to the induced charges, \( \Phi_2(r) \) for \( \rho < a \). The total potential acting on a Cl\(^-\) ion near the pore axis \( (i.e., \rho = d) \) is obtained by combining \( \Phi_2(r) \) with the point particle solution \( \Phi_p(r) \) given in Eq. (2) with \( \rho_x = \rho \) and \( \rho_y = d \),

\[ \Phi_m(r) = \frac{1}{4\pi\varepsilon_0} \int_0^\infty dk \cos(kz) \sum_{m=-\infty}^\infty e^{imk} I_m(kp) \]
\[ \times \left[ K_m(kd) + (\varepsilon_w - \varepsilon_p) I_m(kd) \right] \]
\[ \times \left[ \frac{xK_m K_m' + xK_m K_m'}{2\varepsilon_p + (\varepsilon_w - \varepsilon_p) xK_m (I_{m-1} + I_{m+1})} \right]_{x=ka}. \tag{10} \]

When acting on an ion on the pore axis, \( r = (0, 0, z) \), \( I_m(0) = \delta_{n0} \), and Eq. (10) takes a simpler form,

\[ \Phi_m(z) = \frac{1}{4\pi\varepsilon_0} \int_0^\infty dk \cos(kz) \]
\[ \times \left[ K_0(kd) + (\varepsilon_w - \varepsilon_p) I_0(kd) \right] \]
\[ \times \left[ \frac{xK_0 K_1}{2\varepsilon_p + (\varepsilon_w - \varepsilon_p) xK_0 J_1} \right]_{x=ka}. \tag{11} \]

where we used \( I_{-1} = I_0 \) and \( K_{-1} = K_0 \). Here and in Eq. (10), the first term in the curly bracket is the point charge contribution from Eq. (2) and the second term represents the potential due to the induced charges at the boundary, which vanishes when there is no discontinuity in dielectric media, i.e., \( \varepsilon_w = \varepsilon_p \).

The electric field acting on an ion can be calculated from the gradient of the potential. Here we give the potential and the electric field due to a centrally located charge \( (i.e., d = 0) \), which will be needed when we consider off-axis permeation of a Cl\(^-\) ion,

\[ \Phi_m(r) = \frac{q}{4\pi\varepsilon_0 \varepsilon_w r} \left( \varepsilon_w - \varepsilon_p \right) \frac{2q}{4\pi\varepsilon_0 \varepsilon_w} \]
\[ \times \int_0^\infty dk \cos(kz) I_0(kp) \left[ \frac{xK_0 K_1}{2\varepsilon_p + (\varepsilon_w - \varepsilon_p) xK_0 J_1} \right]_{x=ka}. \tag{12} \]

\[ E_m(r) = \frac{q}{4\pi\varepsilon_0 \varepsilon_w r^2} \left( \varepsilon_w - \varepsilon_p \right) \frac{2q}{4\pi\varepsilon_0 \varepsilon_w} \]
\[ \times \int_0^\infty dk \left[ \cos(kz) I_0(kp) \hat{\rho} - \sin(kz) I_0(kp) \hat{z} \right] \]
\[ \times \left[ \frac{xK_0 K_1}{2\varepsilon_p + (\varepsilon_w - \varepsilon_p) xK_0 J_1} \right]_{x=ka}. \tag{13} \]

For convenience, we have written the direct Coulomb term in the usual form in the above expressions. Thus the second term represents the electric field due to the induced charges on the boundary.

### C. Charge outside the cylinder

The solution for a point charge outside the cylinder \((d > a)\) is obtained in a similar manner. In the point charge potential (2), we take \( \rho_x = \rho, \quad \rho_y = d, \quad \varepsilon = \varepsilon_p \) because the charge is now in the protein. With these provisions, we obtain for \( f_m(k) \)
where the fixed charge is canceled by the reaction field. Thus in addition to the interaction of the ion with the fixed charge, we need to consider the changes in its reaction potential energy. Due to cylindrical symmetry, the reaction potential of the ion depends only on the variable \( \rho \). The solution is similar to that of the charge inside the cylinder and can be obtained from Sec. II A. The reaction potential is given by the second term of Eq. (10) provided we set \( d=\rho, \phi=0, \) and \( z=0 \), which yields
\[
\Phi_\rho(\rho) = \frac{e_w - e_p}{4\pi \varepsilon_0} \frac{2q_i}{\pi} \sum_{m=-\infty}^{\infty} \int_0^\infty dk I_m^2(kp) \left[ \frac{xK_m(K_m+1)+K_m}{2\varepsilon_p+(e_w-e_p)xK_m(I_m+I_{m+1})} \right]_{x=ka}.
\]

The potential energy due to this field is given by \( U_R = \frac{1}{2}q_i\Phi_R \), where the factor of \( \frac{1}{2} \) arises from the fact that it is self-energy. The electric field acting on an ion off axis (\( \rho \neq 0 \)) follows from the derivative of the reaction potential with respect to \( \rho \),
\[
E_R(\rho) = -\frac{e_w - e_p}{4\pi \varepsilon_0} \frac{2q_i}{\pi} \sum_{m=-\infty}^{\infty} \int_0^\infty dk I_m^2(kp) \left[ I_m(kp) + I_{m+1}(kp) \right] \left[ \frac{xK_m(K_m+1)+K_m}{2\varepsilon_p+(e_w-e_p)xK_m(I_m+I_{m+1})} \right]_{x=ka} \hat{\rho}.
\]

Note that the field acts in the \( -\hat{\rho} \) direction, i.e., toward the axis, and it vanishes for \( \rho=0 \), which follows from \( I_m(0) = \delta_{m0} \).

### E. Numerical solutions for a finite cylinder

The pore of the CIC channels can be represented approximately by a cylindrical tube of length of 50 Å and radius of 3 Å, with rounded corners at the pore mouths (radius of curvature of 5 Å).\(^\text{10}\) We use the boundary element method to obtain the numerical solutions of the Poisson equation for this geometry.\(^\text{35,36}\) In this method, the channel boundary is divided into small segments of area \( s_j \), and the polarization charge density \( \sigma_j \) on each segment is calculated from Gauss’ law and the boundary conditions (5) as
\[
\sigma_j = 2\varepsilon_0 \frac{e_0 - e_1}{e_2 + e_1} E_{ex}(r_j) \cdot \hat{n},
\]
where \( E_{ex} \) is the external field at the segment center due to all the charges in the system except those on \( s_j \), and \( E_{ex} \cdot \hat{n} \) is determined from the normal derivative of the external potential at \( r_j \).
\[
\Phi_{ex}(r_j) = \frac{1}{4\pi \varepsilon_0} \sum_{j \neq k} \frac{\sigma_j s_j}{|r_j - r_k|} + \sum_k \frac{q_k}{\varepsilon_0 |r_k - r_j|}.
\]

Here \( j \) is summed over all the segments in the boundary and \( k \) over all the fixed charges in the system. Starting with \( \sigma_j = 0 \) as an initial guess, Eqs. (20) and (21) are iterated until the results converge to the desired accuracy. By taking the curvature of segments into account, this method has been made
very fast and accurate\textsuperscript{36} and works fine as long as the charges are not too close to the boundary (i.e., \(d > 1 \text{ Å}\)). For charge distances closer than 1 Å, one has to use smaller segments to maintain the accuracy. We could obtain accurate solutions for up to 0.5 Å charge distance to the boundary, but going beyond that required more memory than available to us. To obtain the full potential energy curve, we interpolated the solutions from inside and outside the boundary and exploited the fact that the potential is continuous across the boundary, Eq. (5).

III. POTENTIAL ENERGY RESULTS

A. Analytical results

The analytic expressions derived in Sec. II involve both summations and integrals, which are evaluated most conveniently using a mathematics software package such as \textsc{Mathematica}.\textsuperscript{37} There are three parameters that we need to specify to calculate the potential energy: The dielectric constants for water and protein, \(\varepsilon_w\) and \(\varepsilon_p\), and the channel radius \(a\). For the former, we will initially use the standard values of \(\varepsilon_w = 80\) and \(\varepsilon_p = 2\) and then discuss how variations from these values affect the results. The crystal structures of the ClC proteins and the homology models based on these structures exhibit a narrow pore suggesting use of a small radius of a Cl\textsuperscript{−} ion. Using these parameters and fixing the glutamate charge at the channel boundary (i.e., \(z = 0\) and \(d = 3 \text{ Å}\)), we calculate the potential energy of a Cl\textsuperscript{−} ion along the central axis using Eq. (11). The resulting potential energy (Fig. 2) sharply rises to 35\(kT\) at \(z = 0\) but its decay to zero is quite slow. This is due to the infinite length of the channel. In a finite size channel, the barrier height would be reduced in proportion to its length and the spread of the potential would be limited to the channel length. One way to compensate for this effect is to use a larger channel radius, which leads to a smaller barrier. The potential energy of a Cl\textsuperscript{−} ion for a channel with a radius \(a = 5 \text{ Å}\) is also shown in Fig. 2. The glutamate charge is again placed at the channel boundary, that is, \(d = 5 \text{ Å}\). The barrier is now reduced to 21\(kT\) at \(z = 0\), and importantly most of the change from the \(d = 3 \text{ Å}\) case occurs in the vicinity of the glutamate charge.

It is clear from Fig. 2 that the quantity that is of most interest for ion permeation is the barrier height at \(z = 0\). Therefore, we will focus on this quantity from now on. In Fig. 3, we generalize the previous result by showing how the barrier height decreases with the increasing channel radius. The curve exhibits a 1/\(a\) dependence as one would expect from Eq. (11). Note that this simplification occurs when the ion is on the pore axis (\(\rho = 0\)), and only the zeroth order Bessel function remains in the \(m\) sum. In the rest of this section, we will use \(a = 5 \text{ Å}\) for channel radius in order to compensate for the infinite length of the channel.

We next discuss the crucial question of how the movement of the glutamate side chain affects the potential energy barrier seen by a Cl\textsuperscript{−} ion. For simplicity, we first calculate the potential energy of the Cl\textsuperscript{−} ion at \(z = 0\) and \(\rho = 0\) while the glutamate charge is moved in the radial direction (Fig. 4). Here we have included the possibility that the glutamate charge may move further into the channel protein. The results for \(d < 5 \text{ Å}\) are calculated using Eq. (11), and those for \(d > 5 \text{ Å}\) are calculated from Eq. (17). In addition to the total potential energy, we also show the individual contributions from the direct and induced charge terms, which are given by the first and second terms in Eq. (11), respectively. The remarkable feature of this graph is that the induced term dominates the potential energy, and it hardly changes while the glutamate charge is moved inside the pore. From the crystal radius of a Cl\textsuperscript{−} ion (1.8 Å) and glutamate oxygen (1.4 Å), we do not expect the two charges to come closer than 3 Å. With

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{The potential energy of a Cl\textsuperscript{−} ion along the central axis of the channel for two different radii, \(a = 3\) and 5 Å. The glutamate residue with a charge \(-e\) is fixed at the channel boundary at \(z = 0\). The dielectric constants are \(\varepsilon_w = 80\) and \(\varepsilon_p = 2\). We use \(kT\) for energy unit with \(T = 298\ \text{K}\).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Change of the barrier height in the potential energy of a Cl\textsuperscript{−} ion with the channel radius. The glutamate charge is kept at the channel boundary as the radius is increased while the Cl\textsuperscript{−} ion remains on the axis.}
\end{figure}
this provision, the contribution of the direct term is seen to be limited to about $2kT$ while the induced term remains an order of magnitude larger than this value. These results indicate that swinging of the glutamate charge from the pore axis to the boundary will lead to only a small drop in the energy barrier. Energetically it is costlier for the glutamate charge to be buried inside the protein. Nevertheless, if we allow this possibility and consider the glutamate charge positions inside the protein ($d > 5\text{ Å}$), we see that the induced term starts falling down but even then it is quite a slow change. At $d = 10\text{ Å}$, which is the maximal range for a glutamate side chain to swing inside the protein, the barrier is about $14kT$. This is still a formidable barrier as it would suppress the channel current by a factor of millions.

While the results in Fig. 4 are very suggestive, we need one more calculation to make a clear case, namely, the energy barrier faced by a Cl$^-$ ion when the glutamate charge is on the pore axis. In this case, the Cl$^-$ ion will move to an off-axis position where the direct Coulomb repulsion from the glutamate charge cancels the repulsive force from the induced charges due to both the glutamate and the ion itself. Balancing the electric fields in Eqs. (13) and (19), we obtain $R = 3.1\text{ Å}$ for this point. It is instructive to calculate the potential energy of the Cl$^-$ ion not just at this point but for the whole radial range, which is presented in Fig. 5. From the symmetry of the potentials in Eqs. (11) and (12), the direct and induced contributions to the potential energy of the Cl$^-$ ion due to the glutamate are exactly the same as in Fig. 4. Because the Cl$^-$ ion is off-axis, there is now an additional potential energy term due to the reaction potential given by Eq. (18) (we have ignored this energy in earlier results because it is constant for a Cl$^-$ ion moving on the channel axis).

To make the comparison easier with the earlier results, we subtract this constant energy here as well, that is, we plot for the reaction potential energy, $U_R = \frac{1}{2} q_i [\Phi_R(\rho) - \Phi_R(0)]$. As seen in Fig. 5, the contribution from the reaction potential is extremely flat bottomed. The minimum of the total potential energy occurs at about $3.1\text{ Å}$, in agreement with the electric field calculation. The contribution of the reaction potential at this point is only $0.6kT$ and the total potential energy of the Cl$^-$ ion is $22kT$. From Fig. 4, it can be seen that the respective barrier when the glutamate charge is on the boundary is $21kT$. Thus the barrier faced by a Cl$^-$ ion is reduced by only $1kT$ when the glutamate charge is swung from the channel axis to the boundary, which is too small to make an impact on the gating of the channel.

Finally we discuss the sensitivity of the results to the chosen dielectric constants. It is well known that when water is confined in microscopic domains, its dielectric constant is reduced from the bulk value of 80. Similarly, proteins could have slightly larger dielectric constants than the value of 2 implied by the electronic polarizability.38,39 In Fig. 6, we show how the barrier height seen by a Cl$^-$ ion changes from the reference value of $21kT$ (the value at $d = 5\text{ Å}$ in Fig. 4) when either the water or protein dielectric constant is varied from its standard value. The top figure shows that the barrier height increases when $\varepsilon_w$ is reduced from 80 as one would expect from charge screening arguments. However, the change is much slower than the $1/\varepsilon_w$ dependence this implies, highlighting the complicated role played by the boundary. The converse is observed in the bottom figure. The barrier height decreases when $\varepsilon_p$ is increased from 2. The origin of this effect is clearly more subtle than indicated by the
A closer look at Eq. 11 reveals that the \( p \) term in the denominator actually plays a dominant role in the integration. Overall, the changes in \( w \) and \( p \) exhibit similar but opposite trends, and one would expect them to mostly cancel each other in a more realistic calculation. Thus our results are not very sensitive to the choice of the dielectric constants. Effects of the water and protein dielectric constants on ion permeation were discussed before in potassium channel and other channel models, and similar conclusions were reached with regard to the influence of their variations from the standard values of \( w=80 \) and \( p=2 \).

**B. Numerical results**

Here we present the numerical results for the finite length (50 Å) channel with radius of 3 Å and compare them with the analytical results. Our aim is to provide a justification for the choice made for the radius of the infinite channel as well as to demonstrate that the gist of our argument is preserved regardless of the channel being finite or infinite. The main reason for choosing the radius of 5 Å in the infinite case was to compensate for the infinite length of the channel. In Fig. 7 we compare the potential energy obtained for this channel (from Fig. 2) with that of the finite length one. Because of the difficulty of placing charges near the boundary in numerical solutions, the glutamate charge is placed 0.5 Å away from the boundary \( \rho=2.5 \) Å and \( z=0 \) in the latter. This is the closest distance of the charge to the boundary where accurate numerical solutions can be obtained.

Next we repeat the calculation in Fig. 4 for the finite channel, which shows how the potential energy barrier seen by a Cl\(^-\) ion changes as the glutamate charge is moved from the channel axis to the boundary and then beyond into the protein (Fig. 8). Comparison of Figs. 4 and 8 shows that essentially the same picture is obtained in the finite channel, namely, the main contribution to the potential energy comes from the induced charges on the boundary and it hardly changes as the glutamate is moved away from the axis. Thus our main conclusion about the ineffectiveness of the gating mechanism that involves only swinging of the glutamate charge from the channel axis (closed state) to the protein wall (open state) remains intact.
which will abolish the large energy barrier faced by the Cl− ion when the gluta-
mate residue will help protonate it, but Cl− ion should be more or less flat with barriers at most when the channel is open, the potential energy surface faced by a Cl− ion will be less than in the closed state. Thus protonation of the glutamate solves two problems simultaneously—enabling the swinging of the glutamate side chain and abolishing the energy barrier faced by a Cl− ion. It is the combination of these two effects that makes it an attractive hypothesis for fast gating. So far there have been several experimental studies of pH dependence of fast gating in CIC channels,1,42–44 which support the linking of fast gating to protonation of the glutamate residue. However, the uniqueness of this mechanism has not been established yet.15,45

We stress that the simplified channel model used in this work has allowed us to focus on the fundamentals of fast gating without getting bogged down in the complexities of the actual channel structure. Naturally the insights gathered from this study need to be checked using more realistic channel structures.

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