Effects of electrical polarization on the opening rate constant of a voltage-gated ion channel

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Ion channel gating kinetics can be described using Kramers’ diffusion theory of reaction rates between several closed and open states, where transition rates between states depend exponentially on the membrane potential \( V \). It has been suggested that transition rates have a more complex dependence on \( V \) at voltage extremes, but this has never been quantified. We measured the rate constant of the last closed to open transition in a voltage-gated ion channel and show that it does not depend exponentially on membrane potential at values of \( V \) greater than \( \approx 150 \text{ mV} \). To explain this behavior, we estimate the effects of electrical polarization of the water contained in crevices within the channel protein, using an electrostatic model of the approximate three-dimensional geometry and the nonlinear effects of charges on the polarization of water.

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I. INTRODUCTION

Voltage-gated ion channels are responsible for a number of transport-related functions of the cell membrane. Among the most important are electrical signaling and control of ion concentrations. These proteins have a large number of discrete conformational states with most of them being nonconducting (closed) and generally one ion-conducting (open) state.

The conformational changes associated with the opening transition are regulated through the interaction of gating charges (charged domains in the channel protein with limited mobility) and the membrane potential. As the membrane potential is made more positive (inside of the cell with respect to the outside), this transition usually leads to an increased occupancy of the open state.

The structure of ion channels is heterogeneous and complex [1]. Opening and closing of the ion-conduction pathway occurs at the so called pore domain; this domain is in turn coupled to a voltage sensor domain (VSD), which is largely responsible for the voltage dependence of gating. This VSD makes extensive and essential interactions with both the pore domain and membrane lipids. It also contains crevices where water may be highly organized (Fig. 2). Out of the six \( \alpha \) helices that form the channel, the voltage sensor proper is formed by the fourth (S4) helix, which moves along these crevices. The presence of these crevices or gating canals seems to produce an effective thinning of the protein region with low dielectric constant, giving rise to a focused electric field at a narrow septum [2,3].

The rates of transition between closed or open states have been shown to be exponentially dependent on membrane potential, with a steepness that reflects the amount of gating charge movement associated with the individual transition [4]. Up to date, most experimental evidence is consistent with this exponential voltage dependence, although the range of voltages explored has not been very large [5,6].

It has been suggested that electrical polarization may play a role in determining the voltage dependence of transitions [7]; although polarization of the protein is unlikely to be saturated by the electric fields generated at the VSD, the water in crevasses should be highly organized and the focused electric field may be of such magnitude to further saturate the water polarization.

Accepted theories of the dielectric behavior of bulk water show that the dielectric constant can be significantly reduced at field strengths between 0.1 and 1 V/nm [8] and it has been shown that these fields and the reduced dielectric constant of water can be of considerable magnitude in the pores of the bacterial ion channels such as porin [9]. Additionally, the highly charged S4 segment should contribute a large local potential and field, which will further decrease water polarization. Polarization effects on the rate constants of activation of ion channels should become apparent at membrane potentials more positive than the ones that have been used up to date.

Polarization effects might be apparent also in other parameters such as the protonation state of titratable groups, but currently, measurements of these effects are precluded in proteins in the complex environment of the membrane. In the present work we observe an unexpected behavior of the single closed to open voltage-dependent rate constant at elevated positive voltages and describe how the nonlinear polarization of water in crevices present in the structure of the voltage sensing domain could be responsible for the observed saturation of the opening rate constant. This work provides evidence that electrical polarization effects are indeed important in determining the voltage dependence of transitions in membrane proteins.

II. EXPERIMENTAL PROCEDURES

Ionic currents from the fast inactivation-removed Shaker potassium-selective channel were recorded from membrane patches of Xenopus laevis oocytes, harvested by standard methods [10]. Oocytes were injected with 30 ng of mRNA \textit{in vitro} synthesized from the plasmid Shaker-pGEMA, kindly provided by F. J. Sigworth (Yale University). Currents were filtered at 10 kHz and recorded at 50 kHz in the cell-attached configuration of the patch-clamp technique, using an EPC-7 amplifier and borosilicate glass pipettes with resistance of 2–10 M \( \Omega \) [11].
In order to provide a description of the effects of polarization on rate constants, it is necessary to isolate and measure a single rate constant. Since channel gating involves several rate processes with different time scales, it is difficult to measure rate constants in isolation.

In the Shaker channels considered in this work, it is well established that the single open state can be reached through a closed state that is part of a main activation pathway [12]. This main pathway is determined by the motion of the voltage sensor in Fig. 3, from the closed state $C_2$ to the open state crossing the $C^*$ energy barrier. Moreover, it is also well established that the rate constant associated with the transition is strongly voltage dependent. It has also been shown that the open state can lead to closed states not in the activation pathway, that are probably associated with conformational fluctuations outside the VSD [5,6]. These states should have low occupancies because the rate constants leading to them are much smaller than the rate constants in the activation pathway. Importantly, these rates are weakly voltage dependent.

To measure reliably the rate constant of the closed-open transition, it is necessary to minimize the effects of these closed states. To this end we made use of a double pulse protocol [13], where the first depolarizing pulse fully activates the channels, a second, variable-duration, very negative pulse closes the channels, and a third positive pulse re-opens the channels. The time constant of the reactivating current gives the rate of the last closed to open transition, provided the negative pulse is sufficiently short. As the repolarization interval is made shorter, the time constant of re-opening currents becomes faster, approaching an asymptotic value that corresponds to the rate constant of this last transition.

Since the rate constant of the reopening process is very different, the effect of states not in the activation pathway is minimized by the three pulse protocol. The very short duration of the second, negative pulse ensures closure to the single state $C_2$, because the rate of entrance to other closed states is slower than the duration of the pulse. The third pulse will reopen the channels and they will have a chance of entering the closed states occurring after opening, but again the duration of this third pulse is shorter than the rate of entering these states, minimizing their contribution. Here we use this protocol, with the added change that the voltage of the third (depolarizing) pulse was varied between $-20$ and $260$ mV. Voltages larger than these usually produced electric breakdown of the membrane or membrane-pipette seal.

Because the time resolution of our recordings is limited by the filter rising time, which at $10$ kHz is $33 \mu$s [14], we assess the reliability of our measurements, specially for the shortest repolarization intervals, by comparing the relaxation corresponding to the reopening of channels with the response of the recording system. We measured the step response of the amplifier and from it calculated the impulse response. Figure 1 compares the impulse response convolved with a square pulse of $50-\mu$s duration with the exponential relaxation of current obtained at $160$ mV after a repolarization of $50 \mu$s. Even for this shortest interval, the current is slower than the response of the recording apparatus and an exponential relaxation can be unambiguously fitted to the data, to estimate the rate, which we will call $\alpha(V)$.

FIG. 1. Comparison of the expected response of the recording apparatus with the current relaxation. The dotted line is the convolution of a $50-\mu$s pulse with the impulse response. The continuous line is the actual current recording and the gray line is an exponential fitted to the upper half of the relaxation.

III. THEORETICAL MODEL

To describe the voltage dependence of the opening rate constant, we first formulate the corresponding exit time problem for the movement of the voltage sensor responsible for the transition. This formulation is based on the description of a rate constant as a problem of diffusion over an energy barrier as proposed by Kramers [15] and applied to ion channel gating in [16]. To this end we consider a model for the voltage sensor and its environment proposed in [17] (Fig. 2), which in turn is based on the available structural information [1] as

FIG. 2. (Color online) Schematic representation of the voltage sensor domain (VSD) showing the regions with different dielectric constant $\varepsilon$ and water penetration in crevices. The VSD from the Kv1.2 channel in the activated state is represented by the blue (light grey) surface and ribbon and the water-filled region is shaded dark grey. The white region corresponds to the membrane and protein parts of the rest of the channel, which have low dielectric constant. The pore domain is not shown. The crosses represent the approximate position of the water-exposed charges in the S4 helix. The septum region is denoted by $S$. 

\(\frac{d<}{d=}\)
The height of the potential barrier is $A$ and the wells of this potential function represent three stable states of the channel; the transition between the last two, $C_2 \rightarrow O$, is the transition described by $\alpha(V)$ that we measure. This form of the potential energy function is similar to that used in [16].

The voltage-sensor position satisfies the stochastic differential equation [16]:

$$
\gamma \frac{dy}{dt} = -U(y) + k_B T F(t).
$$

The constant $\gamma$ corresponds to the Stokes viscous damping of the voltage sensor and $F(t)$ is the appropriate Brownian forcing. We approximate the viscous damping of the voltage sensor by the Stokes damping of a cylinder of length $y = 10 \times 10^{-10}$ m and radius $a = 3 \times 10^{-10}$ m, which are appropriate dimensions for the voltage sensor [1], immersed in water which has viscosity $\eta = 1.002 \times 10^{-3}$ Pa s, in the form

$$
\gamma = \frac{4\pi \eta Y}{\ln\left(\frac{32}{\gamma r}\right) - 1}.
$$

The solution of the exit problem (1) yields the Kramers formula [15,16] for the rate constant $\alpha(U)$ in the form

$$
\alpha = \frac{1}{2\pi y} \left| \int U''(\phi_{\text{max}}) \int U''(\phi_{\text{min}}) e^{-\left[U(\phi_{\text{max}}) - U(\phi_{\text{min}})\right]/k_B T} \right|.
$$

Assuming $y_{\text{max}} - y_{\text{min}} = L/3$ we obtain for the potential $U(y) = G(y) - z\phi(y)$:

$$
\alpha = \frac{18\pi A}{Y^2 \gamma} \exp\left(-\frac{2A}{k_B T} + \frac{1}{k_B T} \left[\phi(y_{\text{max}}) - \phi(y_{\text{min}})\right]\right),
$$

where $y_{\text{max}}$ and $y_{\text{min}}$ are the positions of the maximum and minimum of the function $G(y)$ relevant to the transition (positions $C'$ and $O$). Equation (2) gives the dependence of the opening rate in terms of the potential. If the geometric structure of the channel is not taken into account, the potential is just a linear function of $y$ and the usual result is recovered. To use the solution for $\alpha$, we need to calculate the potential in the situation described in Fig. 3. We thus need an accurate calculation of the electric potential $\phi(y)$, which takes into account the geometry of the cavities around the voltage sensor. Before we solve in detail the electrostatic problem, we can obtain a simple estimate of the effects which produce the field at the point $p$ in the voltage sensor, using a one-dimensional model. Assuming that the main potential drop is along the vertical direction $y$, then the potential $\phi(y)$ satisfies the linearized Poisson-Boltzmann equation in the form

$$
\frac{d}{dy} \left( \varepsilon(y) \frac{d\phi(y)}{dy} \right) + \varepsilon(y) \kappa^2 \phi(y) = \frac{4\pi \rho(y)}{\varepsilon_a}, \quad \phi(0) = 0,
$$

where $\varepsilon(y)$ is the position-dependent permittivity and $\kappa^2$ is the Debye screening factor. The charge density is concentrated on the voltage sensor. Since $\varepsilon(y)$ changes rapidly at $y = p$, the continuity of the dielectric displacement gives

$$
E_p = \frac{\varepsilon_w}{\varepsilon_p} E_w,
$$

where $E_p$ is the field at $p$ in the voltage sensor and $E_w$ is the field in the cavity water next to $p$. It is a well-established fact...
that for relatively strong electric fields \( E = -\frac{\partial \phi}{\partial y} \), the \( \varepsilon_w \) is a decreasing function given by the Booth theory \([8,9]\).

It has been shown that in narrow pores and field strength of 0.8 V nm\(^{-1}\), the permittivity of water decreases from 80\(\varepsilon_0\) to half its value \([9]\). The permittivity is given by the equation

\[
\varepsilon_w = n^2 + \frac{a}{E_w} L(bE_w), \quad L(x) = \coth(x) - \frac{1}{x},
\]

where \( L \) is the Langevin function \([9]\).

If this is used in (3) and the electric field \( E_w \) is estimated by \( V/d \) we obtain saturation of \( E_p \), since as \( V \) increases, the product \( \varepsilon_w(\frac{V}{d})\frac{Y}{Y} \) saturates. Nevertheless, this happens at voltages much larger than the experimentally attained values in the present work.

However, it is also an established fact that when water is confined and close to charged surfaces, as it would be in the crevices of the VSD, its permittivity decreases even more dramatically to \( \varepsilon_w \approx 2\varepsilon_0 \). This phenomenon is related to the electrostriction of water molecules that happens around the strong fields generated by ions in confined spaces \([18,19]\).

Recently modifications of the Langevin formula have been proposed to account for this situation \([20]\). Finally, this theory has been used in specific situations \([21,22]\). The effect of charges in the water close to a charged wall changes the Langevin function by an extra saturation term which depends not only on the local field, but on the local potential as well. It is this effect which provides a further decrease in the permittivity. We thus use the formula for \( \varepsilon_w \) in the form

\[
\varepsilon_w = \varepsilon_0 + \frac{n_n n_w p_o F(p_o E^\beta)}{E H(\phi, E)},
\]

where

\[
H(\phi, E) = 2n_o \cosh(e_o \beta \phi) + \frac{n_w}{p_o E^\beta} \sinh(p_o E^\beta),
\]

\[
\beta = 1/\kappa BT,
\]

\[
F(U) = L(U) \frac{\sinh(U)}{U},
\]

where \( L \) is the Langevin function in (5). The quantities \( e_o \) and \( p_o \) refer to the unit charge and dipole moment, respectively.

The values \( n_n \) and \( n_w \) refer to the density of ions and water dipoles, respectively, and also take into account a specific statistical configuration of the lattice array formed by ions and dipoles which is used to calculate (6). Notice that when no ions are present, we recover the Booth theory. Also, the presence of charges, such as the ones in the voltage sensor, bring a strong decreasing effect on the value of \( \varepsilon_w \) as the cosh term becomes large. Thus, using the appropriate values for the concentrations and to avoid in the numerics the apparent singularity at \( E = 0 \), we can approximate the water permittivity in the form

\[
\varepsilon_w = 78 \left( 1 - \frac{0.00033}{1 + 0.00037|E|^2} \right) \exp(-0.02|\phi|) + 2.
\]

When this formula for \( \varepsilon_w \) is used in (4), the saturation is obtained in the experimental range.

To calculate the field at point \( p \), the full electrostatic three-dimensional axisymmetric problem is solved numerically. We solve

\[
\nabla \cdot (\varepsilon(y,r,\phi,|\nabla \phi|)|\nabla \phi|) + \varepsilon \kappa^2 \phi = \frac{4\pi \rho}{\varepsilon_o}, \quad \phi(0) = 0,
\]

\[
\phi(d) = V
\]

with periodic boundary conditions in \( r \) and \( \phi(d,r) = V \), \( \phi(0,r) = 0 \). The charge density \( \rho \) is specified in the outer portion of the voltage sensor of Fig. 3 with value

\[
\rho = \frac{3\varepsilon_o}{\pi a^2 Y} = 1.1 \times 10^{-21} \text{coul/Å}^3.
\]

This value corresponds to the expected charge density at a depolarized potential, when each voltage sensor has moved an effective charge \( 3\varepsilon_o \) from the inside to the outside \([23]\).

We defined \( \rho \) in the last 7 Å of the voltage sensor helix. This choice of density is also consistent with the voltage sensor motion from \( C' \) to \( O \), which from the equation for \( G(y) \) is \( L/12 \) and corresponds to an effective charge of 0.25\(\varepsilon_o \), similar to the charge determined from the voltage dependence of \( \alpha \).

The permittivity \( \varepsilon_w \) is given by equation (7) in the water contained in crevices. Elsewhere its value is 80. In the protein region, \( \varepsilon_p = 2 \). To solve Eq. (8) numerically, we modified the finite volume formulation of [24] to take into account the nonlinear permittivity, using for \( \varepsilon \phi(y) \) the finite difference approximation in cylindrical coordinates. The matrix problem to be solved iteratively now has a matrix which depends on the solution. Due to the decaying nature of the permittivity, the Gauss-Seidel iteration is also a contraction with a fixed point which gives the desired solution with no difficulties since the finite volume approximation averages the singularities at the corners. The code to solve Eq. (8) was written in MATLAB and is based on [24].

A typical potential distribution is shown in Fig. 4, for two voltages. The figure also displays the potential profile and electric field along the \( y \) axis at \( r = 0 \), which corresponds to the voltage sensor. Note the compression of the equipotential lines in the region of the septum and the apex of the voltage sensor, where the point \( p \) is located. The high field intensities are located in these two regions. This result is in accordance with previous calculations of the distribution of potential in the VSD \([2,3]\). The numerical solution of Eq. (8) for several voltages gives the electric field at the local point \( p \) in the voltage sensor as a function of voltage. The results are displayed in Fig. 6(a) and the saturation effect is clearly shown.

To correlate the numerical calculation of \( \phi(y) \) in Fig. 4 with a calculation of \( \alpha(V) \), we recall that in Eq. (2) we expressed the potential difference in the form

\[
\frac{\varepsilon}{K_B T} [\phi(y_{\text{max}}) - \phi(y_{\text{min}})] = \frac{\varepsilon}{K_B T} E_p \frac{L}{3},
\]

where \( E_p \) is the value of the electric field at \( p \) given in Fig. 4. It is to be noted that, strictly speaking, the field and the position of the voltage sensor need to be calculated self-consistently. However, we checked that in the range of positions of interest, that is, when the voltage sensor has the charges exposed to the extracellular side, the field is approximately constant with respect to the position of the voltage sensor.
The final expression for the exit time takes the form

$$
\alpha(V) = \frac{18\pi A}{V^2} \exp\left(\frac{-2A}{k_BT}\right) \exp\left(\frac{z}{k_BT}E_p\right), \quad (9)
$$

which gives the rate constant in terms of the microscopic parameters. The parameters to be estimated are the height $A$ of the potential barrier and the charge $z$ moved during the motion of the voltage sensor. These are determined by fitting the value of $\alpha(V)$ at $V = 0$ mV, which is 5276 s$^{-1}$, giving

$$
|A| = 8.9k_BT
$$

and $z = 0.35e_p$.

**IV. RESULTS**

Reopening currents after a negative voltage pulse become faster as the repolarization interval is made shorter, as shown in Fig. 5(a). The reopening time constant $\alpha$ can be estimated from an exponential fit to the current relaxation. This estimate approaches an asymptotic value [Fig. 5(b)] for short repolarizations. This behavior is not an artifact due to the repolarization pulse since it is present at all short repolarizations (data not shown). The reopening rate constant was previously measured for Shaker and, for small voltages between 0 and 100 mV, our values of this rate are comparable to those obtained previously [5,6].

In order to estimate $\alpha(V)$, we take the rates obtained at a repolarization interval of 371.5 $\mu$s. Reopenings after longer intervals are not considered, since they contain a convolution of several rate constants due to channels that visit closed states farther away from the open state, and can no longer be used to estimate a single rate constant.

The voltage dependence of $\alpha$ for an extended range of voltages is shown in Fig. 5(c). In the range of moderate voltages, between 0 and 120 mV, $\alpha(V)$ grows exponentially, with a steepness equivalent to an associated charge of 0.35$e_p$. The exponential voltage dependence is to be expected from an activated process with an energy barrier that is modulated by.
The voltage dependence of the voltage sensor is very different at low or high field strengths. As the applied potential increases, the field at the point in the voltage sensor caused by the nonlinear polarization of water confined by the charged walls which surround the charged voltage sensor shows a saturating behavior. We propose that this saturation of the rate constant is due to a corresponding saturation of the electric field in the presence of ions and charged walls. The effect is then a combination of the geometric focusing of the field lines together with the nonlinear polarizability of the confined water which leads to saturation of the electric field on the sensor.

Our geometric model is based on the known structure of the voltage sensing domain of these channels and takes into account details such as the approximate dimensions of the water cavities and the fact that the voltage sensor has explicit charges. These charges in fact can generate potentials of several tens of millivolts, even when no external voltage is applied. This model is similar to others that have been recently proposed to study mechanisms of voltage sensing [27].

In recent years there has been increased interest in the role of nonlinear properties of water in the function of proteins. We provide evidence and define significant contributions from water polarizability to ion channel kinetics, as was proposed several years ago [7]. It is also noteworthy that a continuum model provides the desired description.

V. CONCLUSIONS

The experiments presented here were carried out using a wider range of voltages than previously assessed. As a result, we show that the rate of the closed to open voltage-dependent transition in Shaker channels can deviate markedly from the expected exponential behavior, saturating even at the relatively low voltages of 150–250 mV.

We propose that this saturation of the rate constant is due to a reduction of the actual field at the voltage sensor, which in turn is a consequence of both the geometric configuration of the sensor and the fact that the dielectric properties of water contained in its crevices are strongly dependent on voltage and electric field in the presence of ions and charged walls. The effect is then a combination of the geometric focusing of the field lines together with the nonlinear polarizability of the confined water which leads to saturation of the electric field on the sensor.

We now use Eq. (9) to compare the experimental data and the model. The comparison is shown in Fig. 6(b). The basis of this result is the strong decay in the polarizability of the water confined by the charged walls which surround the charged voltage sensor. The resulting reduction in the value of the water dielectric constant $\varepsilon_w$, in turn saturates the electric field in the S4 voltage sensor.

The model is able to reproduce closely the experimental results and explains the saturation of the rate constant at high voltages.

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EFFECTS OF ELECTRICAL POLARIZATION ON THE...
