SOME UNEXPECTED CONSEQUENCES OF A SIMPLE PHYSICAL MECHANISM FOR VOLTAGE-DEPENDENT GATING IN BIOLOGICAL MEMBRANES

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ABSTRACT We consider a model for voltage-dependent gating of channels in which the gating charges are on the channel wall and move only a small distance. When this movement occurs across the closed gate, the charges move through the entire transmembrane potential, which is energetically equivalent to their moving across the entire membrane. The channel exists in two open states, O_1 and O_2, and two closed states, C_1 and C_2; each open and closed configuration is divided into two states because of the two possible positions of the gating charges. An unusual property of this model is that the electrical work in going from an open to a closed configuration (for example, in going from O_1 to C_2) is path dependent, and net work can result from going reversibly around a complete cycle. The model channel, like many biological channels, shows bursting activity. This flickering on and off of the channel enables the gate to sense the electric field and decide if it should be in the open or closed configuration. We prove here some general theorems concerning the electrical work associated with the movements of the walls of channels and the movements of charges on these walls.

INTRODUCTION

Voltage-dependent opening and closing of channels in the plasma membrane is the basis for electrical excitability in nerve and muscle (Katz, 1966). In addition, voltage-dependent channels play other important roles both in excitable and nonexcitable cells (Hagiwara and Byerly, 1981). Despite the vast and growing literature on the kinetic characteristics of these channels, however, the basic mechanism(s) by which transmembrane voltage controls the state of the channel remains unknown. The vexing problem is to account plausibly and physically for the steepenss of the conductance-voltage relationship over certain voltage ranges (Hodgkin and Huxley, 1952); this formally requires the transfer of several charges from one side of the membrane to the other. Although such a transfer may actually occur for some voltage-dependent channels induced in lipid bilayer membranes by relatively small (~1,000 mol wt) molecules of bacterial origin (Baumann and Mueller, 1974; Heyer et al., 1976), it is doubtful that this occurs for the voltage-dependent channels known to be formed by large proteins in plasma membranes. The large energy barrier imposed by the lipid bilayer on transmembrane movement of protein charges associated with bulky, hydrophilic groups would seem to preclude this mechanism of voltage dependence.

What is desired is a gating mechanism that is formally equivalent to charge movement across the entire bilayer but involves only small charge movements. Small movements within the bilayer, however, only move charge through a fraction of the total transmembrane potential. To obtain by this means the equivalent of a single charge moving across the entire bilayer requires movement of many charges through small distances; such a mechanism has been proposed by Armstrong (1981). We take here a different tack and consider systems in which the gating charges are on the walls of the channel itself, rather than lying within the bilayer proper. (Energetically, the walls of the channel are a much more favorable location for the gating charges than is the interior of the bilayer, because of the high dielectric constant of the aqueous channel relative to that of the lipid bilayer.) Gating charges on the channel walls that move a short distance across a closed gate move through the entire transmembrane potential,[1] and this is energetically equivalent to their moving across the entire membrane. The analysis of such systems reveals unexpected consequences and properties of some interest, and

[1]We assume that the Debye length within the channel is small compared with the length of the channel, so that it is a good approximation to place the entire voltage drop across the gate. This assumption is appropriate to a channel containing either a high salt concentration or a high fixed charge density.

suggests that single-channel data can be fruitfully interpreted in terms of channels having gating charges on their walls.

THE MODEL

Consider the situation depicted in Fig. 1. A membrane with a cylindrical channel traversing it separates symmetrical salt solutions and is voltage clamped at some value, V. The channel is opened and closed by a thin dielectric circular shutter, which is composed of the material from the surrounding bilayer. Fixed to the walls of the channel just above and below the shutter are circular rings of positive and negative charge of equal magnitude; i.e., the charges form a dipole ring. The dipole moment can orient either with or against the electric field. The magnitude of the dipole moment (μ) is $q\delta$, where q is the magnitude of the positive or negative charges and δ is their separation distance (i.e., the shutter thickness). There are four channel states: two open and two closed. (The open and closed configurations are each divided into two states by the two possible orientations of the dipole moment.) Note that for cases of interest the dipole is physically coupled to the shutter, and therefore the rate constants for the transitions $O_1 \rightleftarrows C_1$ need not be equal to the corresponding rate constants for the transitions $O_2 \rightleftarrows C_2$. Similarly, the equilibrium constant for the former reaction will, in general, not be equal to the equilibrium constant for the latter reaction; that is, in Eq. 1 below, $K_1 \neq 1/K_2$.

At $V = 0$, the system may be in equilibrium. An equilibrium constant is associated with each transition depicted in Fig. 1, and each constant is simply related to the difference in free energy between the two states of that transition. Thermodynamics tells us that there can be no net free energy change in going around the cycle in Fig. 1 (at $V = 0$), and consequently the free-energy differences of the four transitions are not totally independent. In terms of the equilibrium constants, this constraint is expressed by the relation

$$K_1 \cdot K_2 \cdot K_{12}^{(o)} \cdot K_{21}^{(c)} = 1. \quad (1)$$

Alternatively, it can be written in terms of the rate constants as

$$k_1 \cdot k_2 \cdot k_{12}^{(o)} \cdot k_{21}^{(c)} = k_{-1} \cdot k_{-2} \cdot k_{-12}^{(o)} \cdot k_{-21}^{(c)}. \quad (2)$$

For an ensemble of channels at equilibrium, the relative number in each state is expressed through the equilibrium constants; for a single channel, the same expressions give the relative time spent in each state.

The above considerations are rather elementary and generally familiar. The situation is more complex, however, if a voltage is applied across the membrane (i.e., $V \neq 0$). The system is no longer in equilibrium, and a priori one cannot expect equilibrium considerations to apply. Nevertheless, equilibrium analyses are often appropriate for nonequilibrium processes. Helmholtz's quasi-thermodynamic analysis of the Peltier and Thompson heats in thermoelectricity are classic examples of this (see Denbigh, 1951). In the case of voltage-dependent channels, equilibrium analyses are generally applied, and Eqs. 1 and 2 are assumed to hold even at $V \neq 0$ (e.g., Armstrong and Gilly, 1979; Armstrong, 1981). (The Ks and ks are, of course, functions of voltage.) Usually, this approach is suitable and the dissipative processes that parallel the gating phenomenon can be safely ignored. In the present instance, however, equilibrium analysis is inappropriate and, as we shall see, Eqs. 1 and 2 are not valid. In fact, one cannot even write equilibrium constants for the transitions.

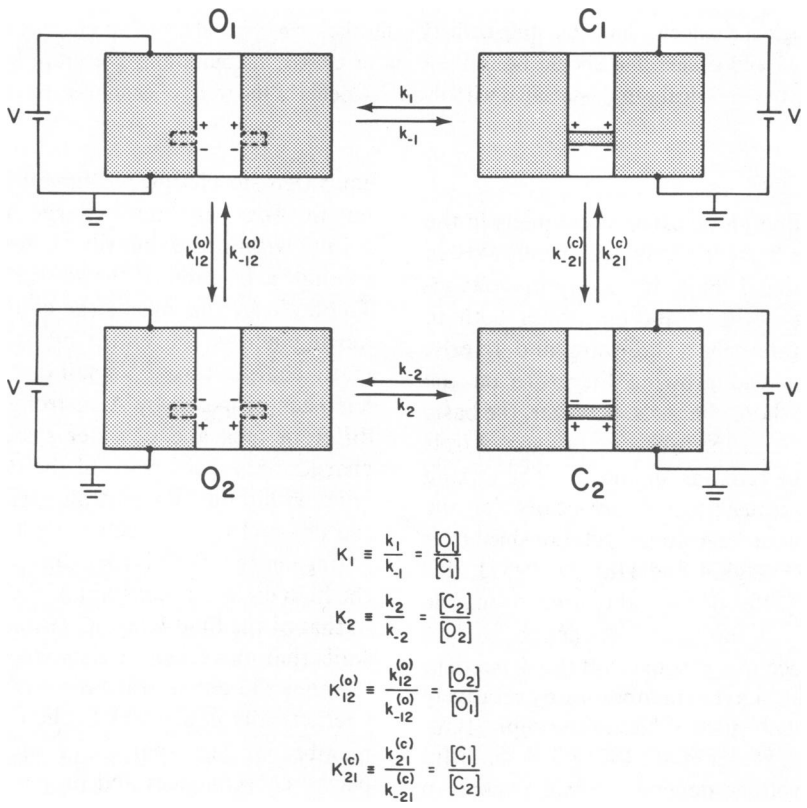

FIGURE 1 The shutter model for a voltage-dependent channel. The channel can be in one of four states: two open states (O_1 and O_2) and two closed states (C_1 and C_2). The gating charges, indicated by the plus signs and the minus signs, are in the form of a dipole ring with a ring of charge on either side of the thin gate. Transitions between O_1 and O_2 or between C_1 and C_2 involve the reorientation ("flipping") of the dipole; transitions between O_1 and C_1 or between O_2 and C_2 involve opening or closing of the thin gate (shutter). The membrane is clamped at the voltage V. Rate constants (k) and their ratios (K) are defined in the figure. The interpretation of the Ks as equilibrium constants is only possible at $V = 0$. At all other voltages, equilibrium is impossible and the equations relating the Ks to the concentrations do not apply.

Qualitative Considerations

Let us first analyze the situation in Fig. 1 intuitively. Consider, for example, starting in open state O_1, and taking some path that ends in closed state C_2. We know that if $V = 0$, the work in going reversibly between these states is independent of path; i.e., there is a unique free energy associated with O_1 and C_2 and hence a unique difference in that energy. If $V \neq 0$, however, this is not the case. Compare the path $O_1 \rightarrow O_2 \rightarrow C_2$ with the path $O_1 \rightarrow C_1 \rightarrow C_2$. The flipping of the dipole in the transition $O_1 \rightarrow O_2$ involves negligible electrical work, if the thickness (δ) of the gate (shutter) is small compared with the total membrane thickness (d), because only a small fraction (δ/d) of V appears across the gate. The transition $C_1 \rightarrow C_2$, on the other hand, involves electrical work $2qV$, since both charges of the dipole move in a direction favored by the electric field through the entire potential difference, V. Next, we compare the transitions $O_2 \rightarrow C_2$ and $O_1 \rightarrow C_1$ that involve the closing of the gate with the dipole fixed in either of its two orientations. We shall show in the Appendix that the electrical work (if any) associated with these two transitions is the same, independent of the orientation of the dipole. This follows from the fact that the *stationary* dipole is always exactly neutralized by counterions. Thus, the total electrical work in going from O_1 to C_2 is path dependent: the path $O_1 \rightarrow C_1 \rightarrow C_2$ involves excess electrical work, $\simeq 2qV$, compared with the path $O_1 \rightarrow O_2 \rightarrow C_2$.

A related kind of path dependence appears when we consider gating currents. In the path $O_1 \rightarrow C_1 \rightarrow C_2$, the dipole flips with the gate closed. This requires a gating current (whose integral over time is $2q$) to flow through the battery. In the path $O_1 \rightarrow O_2 \rightarrow C_2$, however, the dipole flips with the gate open, and much of the required movement of counterions can occur through the open gate. Thus, the open gate shorts out a substantial part of the gating current that would otherwise appear in the external circuit flowing through the battery.

The underlying cause of these path-dependent effects is that the state of the system is not completely specified by the state of the channel; the state of the battery clamping the voltage at V must also be included. For instance, $2q$ units of charge are added to or withdrawn from the battery (depending on the sign of V) in going reversibly[2] around the cycle $O_1 \rightarrow O_2 \rightarrow C_2 \rightarrow C_1 \rightarrow O_1$. Although the channel has returned to O_1 and is therefore back in its original state, the battery is not; hence the system has not returned to its original state. It is clear from considering the above cycle that Eq. 2 does not hold, and equilibrium constants do not exist for the transitions.

The nonequilibrium situation that we have just described comes about because the channel is a conducting medium, which we model as a classical conductor with conductivity α. It is natural to ask how the results of our analysis depend on α. In particular, we might expect that the limit $\alpha \rightarrow 0$ leads to recovery of an equilibrium situation. In fact, this is not the case. Even when α is arbitrarily small (still considering the conductor as classical, i.e., ignoring thermal effects), the steady distribution of potential in the channel is drastically different when the gate is closed than when the gate is open. What does happen as $\alpha \rightarrow 0$ is that the time required to achieve the steady state approaches ∞. Thus the reversible work around a closed path is independent of α, but the transitions have to be slower and slower to be considered reversible as $\alpha \rightarrow 0$.

Because the preceding conclusions are somewhat novel and contrary to generally held beliefs, a formal proof is desirable. The conclusions follow from our assertion that the opening and closing of the gate in the model (i.e., $O_1 \longleftrightarrow C_1$ and $O_2 \longleftrightarrow C_2$) require the same amount of electrical work independent of the orientation of the dipole; consequently, it is this assertion that requires proof. This necessitates determining the energy changes in both the channel and the battery associated with the transitions. In principle, this can be accomplished by reversibly opening and closing the gate and summing the energy changes for each incremental movement. Two difficulties, however, are encountered in the calculations. The first is in determining the field lines in the channel and surrounding bilayer for a given position of the gate. The second is in determining the excess charge that flows into or out of the battery with each incremental movement of the gate. We found no exact way of making either of these determinations. Therefore, instead of performing an explicit calculation on this model, we prove (in the Appendix) a general theorem about the energetics of conformational changes in the shape of axisymmetric channels. In particular, we show that the electrical work (if any) associated with radial deformations of the channel is independent of the distribution of fixed charges on the walls, provided that the fixed-charge distribution remains constant during the deformations. (We also show that the electrical work associated with such channel deformations is proportional to V^2, and hence independent of the sign of V.) Since the dipole in our model is stationary while the gate moves, the theorem applies. Thus the electrical work involved in opening or closing the gate is independent of the orientation of the dipole and also independent of the sign of V.

The theorem that we have just stated applies to transitions in which the gating charges do not move in the transmembrane direction. In the Appendix we also consider more general conformational changes that include rearrangement of the gating charges as well as radial deformations of the walls of the channel. We find useful expressions for the electrical work in two limiting cases: reversible (slow) transitions and the narrow-channel limit.

Quantitative Behavior of the Model

In this section we study the behavior of the model channel in greater detail. In particular we investigate the voltage dependence, the single-channel (flickering) behavior, and the apparent violation of detailed balance. To do this we consider a specific example in which the rate constants have been chosen in a particularly simple way. The simplifying assumptions that we introduce are as follows.

Dipole-flipping Reaction. When the gate is open, we assume that the two orientations of the dipole are in rapid equilibrium with an equilibrium constant of 1. (Recall that the dipole-flipping reaction is essentially voltage independent when the gate is open.) When the gate is closed we assume that the rate constants for the dipole-flipping reaction take the form $k_0\xi$ and $k_0\xi^{-1}$, where

$$\xi = \exp\left(\frac{qV}{kT}\right) \tag{3}$$

and where k_0 is a constant with units of reciprocal time.

Gating Reactions. As emphasized in the previous section (and proved mathematically in the Appendix), we know that the electrical work of opening or closing the gate is independent of the orientation of the dipole. In general, however, there may be nonelectrical work associated with gating that does depend on the dipole orientation. Moreover, there may be kinetic coupling between the orientation of the dipole and the gating reaction. Here we assume that the coupling is entirely kinetic. Specifically, we assume that the ratio of the rate constants for the opening reaction (i.e., the equilibrium constant at $V = 0$) is $1/2$, independent of the orientation of the dipole, but that rates of opening and closing are faster by a factor a^2 when the dipole is down than when the dipole is up.

These assumptions are summarized in Fig. 2 *A*. Because of the rapid-equilibrium assumption for the dipole-flipping reaction when the gate is open, the box scheme of Fig. 2 *A* is equivalent to the triangular scheme of Fig. 2 *B*. In these schemes, the only voltage dependence appears in the

[2]We reversibly open and close the shutter and reversibly rotate the dipole. While this is going on, there is, of course, continual dissipation of energy because of the charge flowing through the channel. For the purposes of this intuitive discussion, we neglect this irreversible process in talking about the state of the battery. In the Appendix, we shall present a rigorous analysis that does not require this artificial separation between the reversible and dissipative currents.

reaction $C_1 \longleftrightarrow C_2$ through the factor

$$\xi = \exp\left(\frac{qV}{kT}\right). \tag{3}$$

When $V = 0$, $\xi = 1$, and the rate constants satisfy the constraint that the product of the equilibrium constants around a closed loop must equal 1. At all other voltages, this condition is violated and there must be a net flux around the loop.

The steady-state behavior of the system shown in Fig. 2 *B* may be determined as follows. Let [O], [C_1], and [C_2] be the fraction of time spent by the channel in each of the three states. (We may also interpret these quantities as the fraction of channels in a large population that may be found in the different states at any particular instant.) Let f be the net flux around the loop (positive counterclockwise). Then

$$\frac{f}{k_0} = a^{-1}([C_1] - [O]) = a([O] - [C_2])$$
$$= \xi^{-1}[C_2] - \xi[C_1]. \tag{4}$$

Of course, we also have

$$[O] + [C_1] + [C_2] = 1. \tag{5}$$

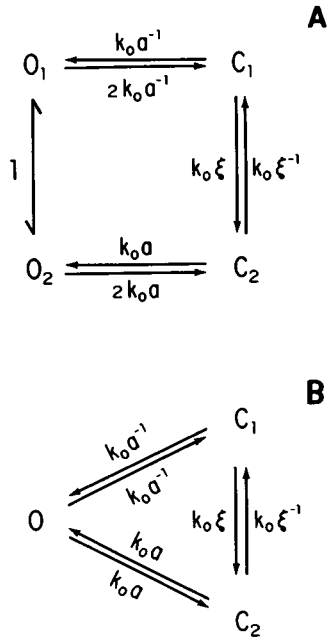

FIGURE 2 (*A*) A particular choice of the rate constants in Fig. 1. is shown assuming that there is only kinetic coupling between the orientation of the dipole and the opening and closing of the gate; that is, in the notation of Fig. 1, $k_1/k_{-1} = k_{-2}/k_2$. (We have arbitrarily set this ratio equal to 1/2.) The extent of kinetic coupling is determined by the dimensionless parameter a. We also assumed that the rate constants for the transitions between O_1 and O_2 are rapid and equal in magnitude. The voltage dependence of the transitions between C_1 and C_2 is controlled by the parameter $\xi = \exp(qV/kT)$. The constant k_0 (with units of reciprocal time) determines the time scale of the transitions. (*B*) Triangular scheme equivalent to the rectangular scheme in *A* is shown. Because of the assumption of rapid equilibrium between O_1 and O_2, these two states can be combined into a single state O, where $[O] = [O_1] + [O_2]$. (This is why the factors of two do not appear in the effective rate constants for the transitions $O \rightarrow C_1$ and $O \rightarrow C_2$.)

The system of Eqs. 4–5 contains four equations that may be solved uniquely for the four unknowns [O], [C_1], [C_2], and f/k_0. The results are

$$[O] = \frac{\xi^2 a + a^{-1} + \xi}{(1 + 2\xi^2)a + (2 + \xi^2)a^{-1} + 3\xi} \tag{6}$$

$$[C_1] = \frac{a + a^{-1} + \xi}{(1 + 2\xi^2)a + (2 + \xi^2)a^{-1} + 3\xi} \tag{7}$$

$$[C_2] = \frac{\xi^2 a + \xi^2 a^{-1} + \xi}{(1 + 2\xi^2)a + (2 + \xi^2)a^{-1} + 3\xi} \tag{8}$$

$$\frac{f}{k_0} = \frac{1 - \xi^2}{(1 + 2\xi^2)a + (2 + \xi^2)a^{-1} + 3\xi}. \tag{9}$$

Note that the net flux is 0 when $\xi = 1$ ($V = 0$). This is the only situtation in which we have true equilibrium as opposed to a steady state.

The steady-state voltage dependence of the channel conductance is determined by Eq. 6, which gives the fraction of time that the channel is open as a function of the voltage V (which appears through ξ). The result is plotted in Fig. 3 for the special case $a = 100$. For negative voltages there is an exponential range in which $[O] \approx \exp(2qV/kT)$. To see this analytically, consider the limit $a \rightarrow \infty$ (instead of the present case of $a = 100$). In that case

$$[O] = \frac{\xi^2}{1 + 2\xi^2}. \tag{10}$$

Then, if $2\xi^2 << 1$ (i.e., $V << 0$), we have $[O] = \xi^2$, which gives the exponential voltage dependence claimed above.

Next we consider the single-channel (flickering) behavior of the model. To do this we arbitrarily set $a = 4$, and we pick for convenience the voltage $\xi = a^{-1}$. The resulting state diagram of the model is shown in the inset to Fig. 4, in which the unit of time is k_0^{-1}. The simulated flickering behavior in this particular case is shown in Fig. 4, where we have distinguished the two closed states as though (as sometimes happens) one of them has a small but measurable conductance.

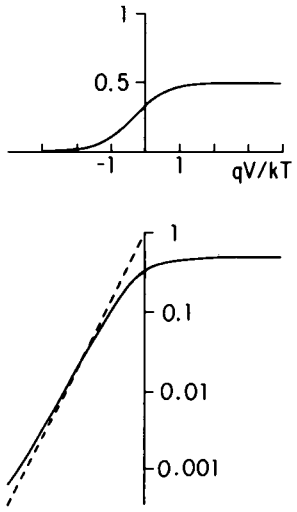

FIGURE 3 The steady-state voltage dependence for the model channel given by the scheme in Fig. 2 with $a = 100$ is shown. *Ordinate:* [O] = fraction of open channels (linear scale, above; logarithmic scale, below). *Abscissa:* normalized voltage in units of kT/q (same scale for both graphs). Note that there is a range of negative voltages where the voltage dependence approaches the relationship: $[O] = \exp(2qV/kT)$ (the dashed line [---] in the lower graph).

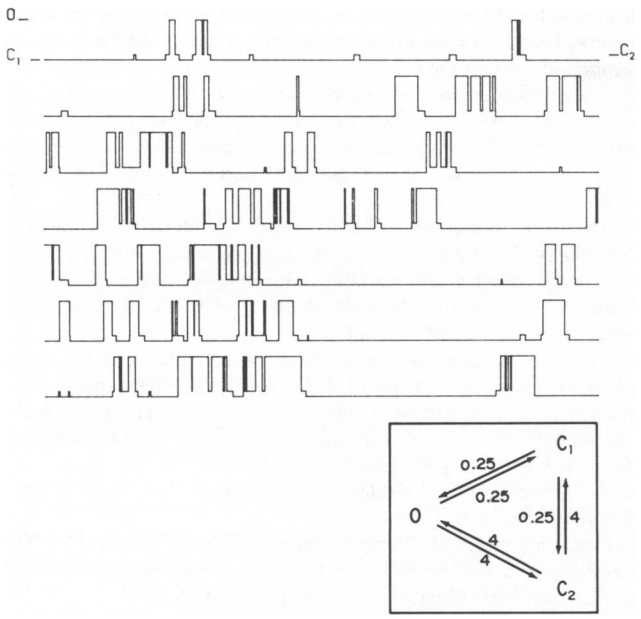

FIGURE 4 Single-channel (flickering) behavior for the model channel given by the scheme in Fig. 2 is shown. The simulated record is taken for the particular case of $a = 4$ at the voltage $\xi = a^{-1}$. (The state diagram for the model under these conditions is shown in the inset, in which the units of time are k_0^{-1}.) We have distinguished between the two closed states by allowing one of them (C_2) to have a small, measurable conductance. The method of simulation was, briefly, as follows: For each state of the channel, the waiting time between transitions was chosen as a random variable with exponential distribution $(k_1 + k_2)\exp[-(k_1 + k_2)t]$, where k_1 and k_2 are the rate constants for the two pathways by which the channel can leave the state in question. The path that the channel actually takes to leave the state was also chosen as a random variable (independent of the waiting time) with the distribution: Pr {channel takes path i} = $(k_i)/(k_1 + k_2)$, $i = 1,2$.

In Fig. 4, the bursts, during which the channel opens and closes repeatedly, may be attributed to the relatively fast reaction $C_2 \longleftrightarrow O$. During the long interburst intervals, the channel is stuck in the relatively stable state C_1. The expected fraction of the time spent in each of the three states may be calculated by direct substitution (of $\xi^{-1} = a = 4$) in Eqs. 6–8. The results are

$$[O] = 0.1301 \tag{11}$$

$$[C_1] = 0.7805 \tag{12}$$

$$[C_2] = 0.0894. \tag{13}$$

Given these quantities, the expected number of transitions per unit time (time in units of k_0^{-1}) for each of the six possible transitions can be found by multiplying the rate constant for the transition (see inset of Fig. 4) by the appropriate occupation probability ([O], $[C_1]$, or $[C_2]$) of the state that precedes the transition. The results are shown in Table I.

Note that the excess of forward to reverse transitions is the same for all three reactions, as required by the steady-state hypothesis. The fact that there is an excess, however, shows that the system is not at equilibrium, because the principle of detailed balance requires that at equilibrium each forward transition proceeds at the same rate as its corresponding reverse transition. Moreover, the ratio of forward to reverse transitions is necessarily greater for the slower reactions. Thus the transition $O \rightarrow C_1$ occurs six times less frequently than the transition $C_1 \rightarrow O$ (on the average), whereas the transition $C_2 \rightarrow O_2$ occurs only 1.5 times less frequently than the transition $O \rightarrow C_2$. Maintenance of the system in a nonequilibrium steady state requires a source of energy. In this case, the energy source is the battery that sustains the transmembrane potential V.

TABLE I

EXPECTED NUMBER OF TRANSITIONS PER UNIT TIME* FOR THE SIX POSSIBLE TRANSITIONS IN FIG. 4

	Forward	Reverse	Forward − Reverse	$\frac{\text{Forward}}{\text{Reverse}}$
$C_2 \rightarrow C_1$	0.3576	0.1951	0.1625	1.8
$C_1 \rightarrow O$	0.1951	0.0325	0.1626	6.0
$O \rightarrow C_2$	0.5204	0.3576	0.1628	1.5

*Time is measured in units of k_0^{-1}.

DISCUSSION

This paper considers some consequences for voltage-dependent channels when the gating charges are located on the walls of the channel itself and their movement occurs across the gate. The presence of gating charges within or near the polar, high dielectric-constant medium of the channel is physically reasonable and preempts the energetic problems associated with their being in the low dielectric-constant medium of the lipid bilayer. Their movement across the gate, moreover, resolves another conceptual difficulty with voltage-dependent channels. That is, it offers a plausible physical mechanism for the steep conductance-voltage relation seen with such channels, since movements of charge through small distances when crossing the gate result in large electrostatic energy differences. One feature of this arrangement of gating charges and gate is that the field that the charges experience is a function of the state (open or closed) of the channel. A notable consequence of this, which we have stressed in this paper, is that the equilibrium considerations usually applied to gating phenomena are not applicable to such systems. In particular, the relative time spent in each state by the channel cannot be determined from equilibrium constants or Boltzmannian relations, and the standard interconnection among forward and backward rate constants in a cyclic path, as in Eq. 2, does not hold.

Before we comment further on this point, we wish to mention another important feature of our model, unrelated to the above issue. For simplicity, let us assume that k_{-2} and $k_{12}^{(o)}$ are very small compared with the other rate constants, and therefore that the transitions $C_2 \rightarrow O_2$ and $O_1 \rightarrow O_2$ are so infrequent that they can be ignored. Then the cyclic scheme of Fig. 1 reduces to the following linear sequence of reactions[3]

$$C_2 \underset{k_{-21}^{(o)}}{\overset{k_{21}^{(c)}}{\rightleftharpoons}} C_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} O_1.$$

[3]In a linear scheme, perfectly proper equilibrium constants can be written for each transition. Note that the cyclic scheme of Fig. 1 becomes a linear scheme with four states if any one of the four arms of the cycle is broken.

The model channel will generally show bursting activity (see Quantitative Behavior of the Model); that is, once it opens it will flicker off and on

$$C_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} O_1.$$

Flickering is apparently a ubiquitous feature of biological channels (e.g., Conti and Neher, 1980; Colquhoun and Sakmann, 1981; Stevens et al., 1982) and our model attributes a function to it: it is a way for the gate to sense the electric field and see if it should be in the open or closed state.

Returning to the point that Eq. 2 is not satisfied in our model, we note that this may be particularly relevant to inactivation kinetic schemes proposed for the sodium channel (e.g., Armstrong, 1981). In those, it is generally assumed that Eq. 2 must apply and also that in going around a given closed path the integral of the gating currents must be 0. This will not be the case, however, if the gating charges are associated with the gate and are on the walls of the channel.

In a recent paper on multiple conductance states of acetylcholine receptor channels (Hamill and Sakmann, 1981), transitions from the sublevel to the closed level were observed, but no transitions from the closed level to the sublevel were seen. One explanation of this apparent violation of the principle of detailed balance could be that the effect we have described in our model system operates in this channel.

The considerations we have raised are also relevant to those situations in which an ion can block or plug a channel (e.g., Miller, 1982). In those cases, it is customary to interpret the voltage dependence of the block in terms of the fraction of the field through which the blocking ion must move, and hence to arrive at an electrical distance within the channel for the blocking site. If, however, the field is more or less uniform throughout the channel in the unplugged state but appears only across the block site when the channel is plugged, the situation is complicated. We do not pursue this point at this time.

The model we have considered has two closed states and two open states, but more of each could be had by adding more dipoles to the gating mechanism. Some of these possible states might in practice be excluded because of the low probability of certain transitions. The model appears rich enough to include all of the types of single-channel behavior so far observed.

APPENDIX

The purpose of this Appendix is to study the energetics of conformational changes of voltage-dependent channels with gating charges on the walls of the channel. We consider two types of conformational changes: radial motion of the walls of the channel and longitudinal redistribution of the gating charges. (These changes may occur simultaneously and at arbitrary rates.) The energetic terms that we evaluate are the rate of change of electric-field energy in the dielectric and the channel, the rate of joule heating in the channel, and the rate of electrical work performed by the battery. From these we calculate the rate at which external work is required to produce the conformational changes in question. Note that this calculation ignores any purely chemical work (that is, voltage-independent work) associated with the conformational changes. We assume that such chemical work terms can be derived from a free energy and hence that they do not contribute to the net work around any closed path.

The main result of this Appendix is that pure radial deformations of the walls (with the gating charges fixed) require an amount of external work that is independent of the (static) distribution of the gating charges. Moreover, we show that the external work involved in such conformational changes is proportional to V^2.

We also consider the special cases of reversible conformational changes in an arbitrary channel and of arbitrary conformational changes in a channel that is narrow in comparison with its length. In the latter case, we derive more explicit expressions for the external work and also for the gating currents generated by the transitions of the channel. In particular, we show that the integral of the gating current around a closed path is not equal to 0.

Consider a membrane that occupies the slab $z_1 \leq z \leq z_2$ (Fig. 5). The membrane contains a single axisymmetric channel whose moveable walls are described (in cylindrical coordinates) by the equation

$$r = r_0(z,t) \tag{A1}$$

and for simplicity, we assume that the two ends of the channel are fixed

$$r_0(z_1,t) = r_1$$
$$r_0(z_2,t) = r_2. \tag{A2}$$

Some important geometrical quantities derived from $r_0(z,t)$ are the area per unit length of the walls of the channel

$$a(z,t) = 2\pi r_0(z,t)\left\{1 + \left[\frac{\partial r_0}{\partial z}(z,t)\right]^2\right\}^{1/2} \tag{A3}$$

and the unit outward normal

$$\hat{\mathbf{n}}(z,t) = \left(\hat{\mathbf{r}} - \frac{\partial r_0}{\partial z}\hat{\mathbf{z}}\right)\Big/\left(1 + \left(\frac{\partial r_0}{\partial z}\right)^2\right)^{1/2} \tag{A4}$$

We shall use the notation $\partial/\partial n$ for the normal derivative $\hat{\mathbf{n}} \cdot \nabla$.

The membrane (M) is modeled as a classical dielectric with dielectric constant ϵ_M. The channel (C) is modeled as a classical conductor with

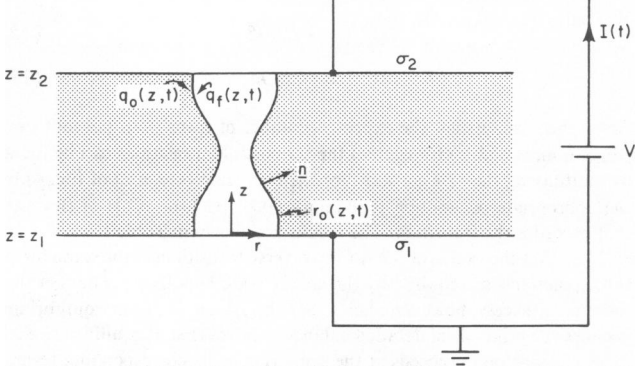

FIGURE 5 An axisymmetric channel with moveable walls in a membrane that occupies the slab $z_1<z<z_2$ is shown. The membrane (and channel) is clamped at voltage V. The meanings of the symbols shown in the figure are given in the text.

conductivity α and dielectric constant ϵ_C. At the membrane-channel interface (MC) we may distinguish three types of surface charge. (*a*) The polarization surface charge that arises from polarization of the membrane or the channel. This charge will never appear explicitly in the following considerations. (*b*) The bound surface charge (e.g., $-COO^-$ and $-NH_3^+$ groups on proteins) that is attached to the walls of the channel. (*c*) The free surface charge (counterions) contributed by the solution in the channel.

We remark that this terminology is not completely standard. Usually "free" charge simply means all charge other than the polarization charge, but in the following we shall call this instead the nonpolarization charge. In our terminology the free surface charges enter and leave the membrane-channel interface (i.e., the walls of the channel) via the solution, whereas the bound charges never leave the interface, although they may be rearranged on the walls of the channel.

We assume that the walls of the channel are lined with bound surface charge in the amount $q_0(z,t)$ per unit length and free surface charge in the amount $q_f(z,t)$ per unit length. Thus the total (nonpolarization) surface charge on the walls of the channel is given by

$$q(z,t) = q_0(z,t) + q_f(z,t) \tag{A5}$$

per unit length. The quantities q, q_0, and q_f may be divided by $a(z,t)$ to find the corresponding charges per unit area.

Next, we consider the electrostatic potential $\phi(r,z,t)$. We assume that a battery is applied to the system in such a way that

$$\phi(r,z_2,t) = V \tag{A6}$$

$$\phi(r,z_1,t) = 0. \tag{A7}$$

That is, there are perfectly conducting electrode plates along $z = z_1$ and $z = z_2$. (This is a reasonable approximation to a large volume of solution.) The (nonpolarization) charges on these plates will be designated $\sigma_1(r,t)$ and $\sigma_2(r,t)$ per unit area.

In the following, it will be very convenient to have an expression for the value of ϕ on the membrane-channel interface. Thus we define

$$\Phi_{MC}(z,t) = \phi[r_0(z,t),z,t]\,. \tag{A8}$$

The equations that determine $\phi(r,z,t)$ at any particular time t are as follows. First, note that the (nonpolarization) charge density ρ is 0 by definition in the interior of the dielectric membrane. Then, since $\mathbf{E} = -\nabla\phi$ and $\epsilon_M \nabla \cdot \mathbf{E} = \rho = 0$, we have $\nabla^2\phi = 0$ in the interior of the membrane.

In the channel we may also take $\rho = 0$ for the following reason. The channel is filled with a liquid conductor, which may be in motion to accommodate conformational changes in the walls of the channel. Let $\mathbf{u}$ be the fluid velocity and assume $\nabla \cdot \mathbf{u} = 0$; i.e., the aqueous solution in the channel is incompressible. Now the current density in the channel is given by $\mathbf{J} = \alpha\mathbf{E} + \rho\mathbf{u}$ and the equation of charge conservation reads as follows:

$$\begin{aligned} 0 &= \frac{\partial\rho}{\partial t} + \nabla \cdot \mathbf{J} \\ &= \frac{\partial\rho}{\partial t} + \nabla \cdot (\rho\mathbf{u}) + \alpha\nabla \cdot \mathbf{E} \\ &= \frac{\partial\rho}{\partial t} + \mathbf{u} \cdot \nabla\rho + \frac{\alpha}{\epsilon_C}\rho \\ &= \frac{D\rho}{Dt} + \frac{\alpha}{\epsilon_C}\rho \end{aligned} \tag{A9}$$

where D/Dt is the material derivative $(\partial/\partial t + \mathbf{u} \cdot \nabla)$, which is the time derivative seen by an observer who follows a particular material point. It follows from Eq. A9 that any charge density ρ that is placed in the interior of a classical conductor (even a classical conductor in motion) decays away exponentially in time to 0 at each material point. We shall assume that this decay has already taken place before we begin considering movements of the channel walls and the fixed charges on them, and that therefore $\rho = 0$ in the interior of the channel at all times. In summary

$$\nabla^2\phi = 0, \quad r > r_0(z,t) \tag{A10}$$

$$\nabla^2\phi = 0, \quad r < r_0(z,t). \tag{A11}$$

We still have to determine the boundary conditions at the membrane-channel interface. First, the potential ϕ is continuous across the membrane-channel interface. Next, recall that $\hat{\mathbf{n}}$ points out of the channel into the membrane, that the jump in $\mathbf{D} \cdot \hat{\mathbf{n}}$ is the (nonpolarization) surface charge, and that $\mathbf{D} = \epsilon\mathbf{E} = -\epsilon\nabla\phi$. It follows that

$$-\epsilon_M \frac{\partial\phi}{\partial n}\bigg|_M + \epsilon_C \frac{\partial\phi}{\partial n}\bigg|_C = \frac{q(z,t)}{a(z,t)} \tag{A12}$$

along $r = r_0(z,t)$. Eqs. A6–A7 and A10–A12 suffice to determine ϕ uniquely, given q.

We still need an equation for $\partial q/\partial t$. This equation is derived as follows. Consider an arbitrary interval (z_a,z_b) of the channel. By conservation of the free charge and for radial motion of the walls[4]

$$\frac{d}{dt}\int_{z_a}^{z_b} q_f\,dz = -\int_{z_a}^{z_b} \alpha \frac{\partial\phi}{\partial n}\bigg|_C a\,dz. \tag{A13}$$

Because z_a and z_b are fixed and arbitrary, this implies

$$\frac{\partial q_f}{\partial t} = -\alpha \frac{\partial\phi}{\partial n}\bigg|_C a. \tag{A14}$$

Because $q_f = q - q_0$, this equation may also be written

$$\frac{\partial q}{\partial t} = \frac{\partial q_0}{\partial t} - \alpha \frac{\partial\phi}{\partial n}\bigg|_C a. \tag{A15}$$

This is the required equation for $\partial q/\partial t$.

Note that the bound charge density $q_0(z,t)$ enters into our equations only through the term $\partial q_0/\partial t$ in Eq. A15. This has the important consequence that, when $\partial q_0/\partial t \equiv 0$, the stationary charge density $q_0(z)$ has no effect on ϕ or q. In this special case, ϕ and q have the same values as when $q_0 \equiv 0$. Similarly, when $q_0(z,t)$ is slowly varying, the values of ϕ and q will be close to their values when $q_0 \equiv 0$. These remarks will be very important in the following.

We are now ready consider the energetics of the system. The potential energy U is given by

$$\begin{aligned} U &= \frac{1}{2}\int_{M+C} \mathbf{D} \cdot \mathbf{E}\,dv \\ &= \frac{1}{2}\int_M \epsilon_M |\nabla\phi|^2\,dv + \frac{1}{2}\int_C \epsilon_C |\nabla\phi|^2\,dv. \end{aligned} \tag{A16}$$

To simplify this expression, we use Green's theorem in the form

$$\int_\Omega |\nabla\phi|^2\,dv = \int_S \phi \frac{\partial\phi}{\partial n}\,dA\,. \tag{A17}$$

Here Ω is a region with surface S, $\mathbf{n}$ is the outward normal on S, and $\nabla^2\phi = 0$ in Ω.

[4]Eq. A13 is confined to radial movement because nonradial motion can give rise to surface convection currents of free charge across $z = z_a$ or $z = z_b$; such currents are not included in Eq. A13.

The result is

$$U = \frac{1}{2}\int_{MC}\left(-\epsilon_M \phi \left.\frac{\partial\phi}{\partial n}\right|_M + \epsilon_C \phi \left.\frac{\partial\phi}{\partial n}\right|_C\right) dA + \frac{1}{2} V \int_0^{r_2} \epsilon_C \frac{\partial\phi}{\partial z}(r,z_2,t)\, 2\pi\, r dr + \frac{1}{2} V \int_{r_2}^{\infty} \epsilon_M \frac{\partial\phi}{\partial z}(r,z_2,t)\, 2\pi\, r dr. \quad \text{(A18)}$$

There is no contribution from $z = z_1$, because $\phi = 0$ on $z = z_1$. We can rewrite Eq. A18 in terms of surface charges. To do this, use the boundary condition (Eq. A12) and also the condition $\epsilon\, \partial\phi/\partial z = \sigma_2$, which holds on both parts of the upper electrode. On the membrane-channel interface, recall that $dA = a dz$. With these substitutions, Eq. A18 becomes

$$U = \frac{1}{2}\int_{z_1}^{z_2} \Phi_{MC}(z,t)\, q(z,t)\, dz + \frac{1}{2} V \int_0^{\infty} \sigma_2(r,t)\, 2\pi\, r dr. \quad \text{(A19)}$$

(Eq. A19 could have been written down immediately, but we have included its derivation from Eq. A16 for completeness.) For future reference, we note the time derivative of this expression

$$\dot{U} = \frac{1}{2}\int_{z_1}^{z_2} \frac{\partial}{\partial t}(\Phi_{MC} q)\, dz + \frac{1}{2} V \frac{\partial}{\partial t}\int_0^{\infty} \sigma_2\, 2\pi\, r dr. \quad \text{(A20)}$$

It will be convenient to express the last term of Eq. A20 in terms of currents. Let $I(t)$ be the current flowing in the battery and let $I_2(t)$ be the current entering the channel through the upper electrode. That is

$$I_2(t) = \alpha \int_0^{r_2} \frac{\partial\phi}{\partial z}(r, z_2, t)\, 2\pi\, r dr. \quad \text{(A21)}$$

Then

$$\frac{\partial}{\partial t}\int_0^{\infty} \sigma_2\, 2\pi\, r dr = I(t) - I_2(t) \quad \text{(A22)}$$

and we have

$$\dot{U} = \frac{1}{2}\int_{z_1}^{z_2} \frac{\partial}{\partial t}(\Phi_{MC} q)\, dz + \frac{1}{2} V [I(t) - I_2(t)]. \quad \text{(A23)}$$

The next step in the analysis of the energetics is to calculate the rate of joule heating in the channel. This is given by

$$\dot{H} = \int_C (\mathbf{J}\cdot\mathbf{E})\, dv = \alpha \int_C |\nabla\phi|^2\, dv. \quad \text{(A24)}$$

Using Green's theorem as before, this becomes

$$\dot{H} = \alpha \int_{z_1}^{z_2} \Phi_{MC}(z,t) \left.\frac{\partial\phi}{\partial n}\right|_C a(z,t)\, dz + \alpha V \int_0^{r_2} \frac{\partial\phi}{\partial z}(r, z_2, t)\, 2\pi\, r dr. \quad \text{(A25)}$$

To rewrite $\dot{H}$ in terms of surface charges, we use Eq. A14 and the definition of I_2 (Eq. A21) to obtain

$$\dot{H} = -\int_{z_1}^{z_2} \Phi_{MC} \frac{\partial q_f}{\partial t}\, dz + V I_2(t). \quad \text{(A26)}$$

Next, we evaluate the rate at which work is done by the battery. As always

$$\dot{W}_b = V I(t). \quad \text{(A27)}$$

Finally, we consider the external work that has to be done in order to change q_0 or r_0. Let this rate of work be denoted by $\dot{W}_e$. By conservation of energy, we see that

$$\dot{W}_e = \dot{U} + \dot{H} - \dot{W}_b = \int_{z_1}^{z_2}\left[\frac{1}{2}\frac{\partial}{\partial t}(\Phi_{MC} q) - \Phi_{MC}\frac{\partial q_f}{\partial t}\right] dz - \frac{1}{2} V [I(t) - I_2(t)] \quad \text{(A28)}$$

or, substituting Eq. A5 into this, we have

$$\dot{W}_e = \int_{z_1}^{z_2}\left[\frac{1}{2}\frac{\partial}{\partial t}(\Phi_{MC} q) - \Phi_{MC}\frac{\partial q}{\partial t}\right] dz - \frac{1}{2} V [I(t) - I_2(t)] + \int_{z_1}^{z_2} \Phi_{MC}\frac{\partial q_0}{\partial t}\, dz. \quad \text{(A29)}$$

Eq. A29 is exact for any movements of q_0 and for radial movements of the channel walls. Let us now consider some special cases.

Case 1

q_0 Is Stationary. That is, $\partial q_0/\partial t = 0$. (This is the situation that pertains to the opening and closing of the shutter in our model.) In this case, Eq. A29 reduces to

$$\dot{W}_e = \int_{z_1}^{z_2}\left[\frac{1}{2}\frac{\partial}{\partial t}(\Phi_{MC} q) - \Phi_{MC}\frac{\partial q}{\partial t}\right] dz - \frac{1}{2} V [I(t) - I_2(t)] \quad \text{(A29a)}$$

if q_0 is stationary. Moreover, we have shown (following Eq. A15) that when q_0 is stationary, ϕ and q have the same values as when $q_0 \equiv 0$. But because the values of ϕ and q also determine the values of Φ_{MC}, I, and I_2, it follows from Eq. A29 that $\dot{W}_e$ is independent of $q_0(z)$ when $\partial q_0/\partial t \equiv 0$.

This is our main result, since it implies that the work of opening or closing the gate (shutter) in our specific model is independent of the orientation of the dipole. Thus, we have proved our intuitive result that the electrical work in going from O_1 to C_2 is path dependent and that net work can result from going reversibly around a complete cycle in the model. As a further remark, we note that the quantities ϕ, q, Φ_{MC}, I, and I_2 are all proportional to V when $\partial q_0/\partial t \equiv 0$. (This follows from the linearity of the equations.) Thus $\dot{W}_e$ is proportional to V^2, and the work required to open or close the gate is also independent of the sign of V.

Case 2

Reversible Movements of Walls and q_0. When $r_0(z,t)$ and $q_0(z,t)$ are slowly varying, it is easy to verify (see remark following Eq. A15) that q, Φ_{MC}, $I(t)$, and $I_2(t)$ are proportional to V and independent of $q_0(z,t)$. Therefore Eq. A29 reduces to an expression of the form

$$\dot{W}_e = \left(\frac{V}{V^*}\right)^2 \dot{W}_e^* + \frac{V}{V^*}\int_{z_1}^{z_2} \Phi_{MC}^* \frac{\partial q_0}{\partial t}\, dz. \quad \text{(A29b)}$$

Here V^* is an arbitrary reference voltage, $\dot{W}_e^*$ is the rate of work associated with the wall motion in question (i.e., with the radial motion $r_0[z,t]$) when $V = V^*$ and $q_0 \equiv 0$, and Φ_{MC}^* is the value of Φ_{MC} when $V = V^*$ and $q_0 \equiv 0$. By definition, $\dot{W}_e^*$ and Φ_{MC}^* are independent of $q_0(z,t)$.

Thus, in the case of reversible changes, we can split the rate of external work into two terms. The first term is quadratic in the voltage, it depends

on the motion of the walls, and it is independent of the distribution of fixed charges on the walls. The second term is linear in the voltage and depends both on the configuration of the walls and also on the rate of rearrangement of the fixed charges on the walls.

Case 3

The Narrow Channel. The limit of a narrow channel is very instructive because many of the general expressions derived above can be evaluated more explicitly. We shall present here only an informal analysis of the narrow-channel limit, but the results can also be derived by a formal asymptotic expansion.

We consider the limit obtained by setting $r_0(z,t) = \lambda \bar{r}_0(z,t)$ and $\alpha = \lambda^{-2}\bar{\alpha}$ and then letting $\lambda \to 0$ with $\bar{r}_0$ and $\bar{\alpha}$ fixed. (In this limit the channel has a finite conductance.) We assume that $q_0(z,t)$ is given, independent of λ. Since the channel collapses to a line when $\lambda = 0$, it is clear from elementary electrostatics that $q(z,t) \equiv 0$ in the limit; if not, Φ_{MC} will be infinite. Moreover, since $q = 0$, the narrow channel does not disturb the distribution of potential at any finite distance from the channel. It follows from this that $\sigma_2(r,t) = \sigma_2(\infty,t)$ everywhere except possibly at the isolated point $r = 0$, where σ_2 still remains finite even when $\lambda = 0$. Of course, $\sigma_2(\infty,t)$ is independent of t. Thus $I(t) = I_2(t)$. Combining these facts, we see that Eq. A29 reduces to

$$\dot{W}_e = \int_{z_1}^{z_2} \Phi_{MC} \frac{\partial q_0}{\partial t}\, dz. \tag{A29c}$$

(narrow-channel limit). This looks like a special case of Eq. A29b, but we have not assumed here that the changes in q_0 or r_0 are slow. It remains to evaluate $\Phi_{MC}(z,t)$. To do this we consider the current in the channel. In the narrow-channel limit we have $\phi = \Phi_{MC}$ in the channel, so the current is given by

$$i(z,t) = -\bar{\alpha} \frac{\partial \Phi_{MC}}{\partial z} \pi \bar{r}_0^2(z,t). \tag{A30}$$

The continuity equation for this current is

$$\frac{\partial q_f}{\partial t} + \frac{\partial i}{\partial z} = 0. \tag{A31}$$

But $q_f = q - q_0 = -q_0$, since $q = 0$. Therefore $\partial i/\partial z = \partial q_0/\partial t$, and Φ_{MC} satisfies the differential equation

$$-\frac{\partial}{\partial z}\left(\bar{\alpha}\pi \bar{r}_0^2(z,t) \frac{\partial \Phi_{MC}}{\partial z}\right) = \frac{\partial q_0}{\partial t} \tag{A32}$$

with boundary conditions

$$\Phi_{MC}(z_1) = 0$$
$$\Phi_{MC}(z_2) = V. \tag{A33}$$

These equations suffice to determine Φ_{MC} at each time t, provided that we are given $\bar{r}_0(z,t)$ and $\partial q_0/\partial t\,(z,t)$.

A particularly useful aspect of the narrow-channel approximation is that we can derive an explicit formula for the current entering or leaving the channel. These currents are the same, since we impose the constraint that

$$\int_{z_1}^{z_2} \frac{\partial q_0}{\partial t}\, dz = 0. \tag{A34}$$

Let

$$-I(t) = i(z_1,t) = i(z_2,t). \tag{A35}$$

Then $I(t)$ is (as above) the current flowing through the battery with the usual sign convention. To derive a formula for $I(t)$, integrate Eq. A32 from z_1 to z, divide by $\bar{\alpha}\pi\bar{r}_0^2$, and then integrate from z_1 to z_2. The result can be put in the form

$$I(t) = \frac{1}{R_0(t)}\left[V - \int_{z_1}^{z_2} R(z,t) \frac{\partial q_0}{\partial t}(z,t)\, dz\right], \tag{A36}$$

where $R(z,t)$ is the resistance of the part of the channel from z_1 to z

$$R(z,t) = \int_{z_1}^{z} \frac{dz'}{\bar{\alpha}\pi \bar{r}_0^2(z',t)} \tag{A37}$$

and where $R_0(t)$ is the resistance of the entire channel

$$R_0(t) = R(z_2,t). \tag{A38}$$

Clearly, the term V/R_0 is the ohmic current, and the term involving $\partial q_0/\partial t$ is the gating current. Note, however, that the gating current is associated not with the motion of the walls of the channel but with redistribution of the gating charges.

In our shutter model when the gate is almost closed, most of the resistance of the channel appears across the gate, so $R \simeq 0$ at the bottom of the gate and $R \simeq R_0$ at the top of the gate. Thus, when the gate is closed, the integral of the gating current associated with flipping the dipole is equal to twice the dipole charge. When the gate is open, however, the change in R across the gate is only $(\delta/d)R_0$, so the integral of the gating current is δ/d times twice the dipole charge. If $\delta << d$, the gating current associated with flipping the dipole is much smaller if the flip occurs when the gate is open than when it is closed.

Finally, for ease of interpretation, we combine the reversible approximation with the narrow-channel limit. Then $\partial q_0/\partial t$ may be neglected in Eq. A32 (but not in Eq. A29c), and Φ_{MC} satisfies the equations

$$\frac{\partial}{\partial z}\left[\pi \bar{r}_0^2(z,t) \frac{\partial \Phi_{MC}}{\partial z}\right] = 0 \tag{A39}$$

$$\Phi_{MC}(z_1) = 0$$
$$\Phi_{MC}(z_2) = V. \tag{A40}$$

Thus, Φ_{MC} is determined by the configuration $\bar{r}_0$ of the channel at each time t. If we separate changes in $\bar{r}_0$ from changes in q_0, we may now say (see Eq. A29c) that changes in $\bar{r}_0$ require no electrical work, whereas changes in q_0 require an amount of work that is proportional to V and that also depends (through Φ_{MC}) on the configuration $\bar{r}_0$ at which the change in q_0 occurs.

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