Stochastic theory of singly occupied ion channels

II. Effects of access resistance and potential gradients extending into the bath

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ABSTRACT In a previous paper (Jakobsson, E., and S. W. Chiu. 1987. *Biophys. J.* 52:33–46), we presented the stochastic theory of the singly occupied ion channel as applied to sodium permeation of gramicidin channels, with the assumption of perfect equilibration between the bathing solutions and the ends of the ion channel. In the present paper we couple the previous theory to electrodiffusion of ions from the bulk of the bathing solution to the channel mouth. Our electrodiffusion calculations incorporate estimates of the potential gradients near the channel mouth due to image forces and due to the fraction of the applied potential that falls beyond the ends of the channel. To keep the diffusion calculation one-dimensional, we make the assumption that the electrical potentials in the bath exhibit hemispherical symmetry. As in the previous paper, the flux equations are fit to data on sodium permeation of normal gramicidin A, and gramicidins modified by the fluorination of the valine at the No.1 position (Barrett Russell, E. W., L. B. Weiss, F. I. Navetta, R. E. Koeppe II, and O. S. Anderson. 1986. *Biophys. J.* 49:673–686). The conclusions of our previous paper with respect to the effect of fluorination on the mobility, surface potential well depth, and central barrier, are confirmed. However the absolute values of these quantities are somewhat changed when diffusive resistance to the mouth is taken into account, as in the present paper. Future possibilities for more accurate calculations by other methods are outlined.

INTRODUCTION

To contribute to currents as measured in membrane biophysics, ions must overcome three resistances in series: (*a*) a resistance to moving from the bulk solution to the mouth of the channel; (*b*) a resistance to moving across the channel; and (*c*) a resistance to moving from the far end of the channel to the bulk solution on the other side. In a previous paper (Jakobsson and Chiu, 1987*a*), we have applied a theory due to Levitt (1986) for the special case of a singly occupied ion channel with negligible access resistance to the analysis of data on sodium permeation of gramicidin A channels (Barrett Russell et al., 1986). We pointed out that negligible access resistance does not mean negligible access limitation of current. This is because in any rate process the rate may be limited by either the thermodynamic driving force (potential) or the resistance against which the driving force works. Thus the sublinear *I-V* curves seen at low sodium concentration both experimentally (Andersen and Procopio, 1980; Andersen, 1983*a–c*; Eisenman and Sandblom, 1984), and also in our calculations that assumed negligible access resistance, are properly ascribed to access limitation; the only question is to what extent is the access limitation thermodynamic (the chemical potential) and to what extent is it kinetic (the resistance), as presented by Andersen (1983*a–c*).

The theory for describing diffusion up to the mouth of the channel is essentially that for describing diffusion-controlled chemical reactions (Hille, 1984, p. 186). For situations of arbitrary geometrical complexity, an accurate calculation is necessarily complicated and computer-intensive. It involves mapping the electrostatic potential field in the vicinity of the reactants (for example, a protein and an ion) and sampling times required to move the reactants together by computing many Brownian trajectories through that vicinity (Sharp et al., 1987*a,b*; McCammon et al., 1986). Even for simplified geometries, such as a perfectly cylindrical ion channel in a perfectly planar membrane, the electrostatic potential field in the bath outside the channel is sufficiently complicated that an analytical solution for the electrodiffusion to the mouth of the channel is of questionable feasibility (Jordan et al., 1988). If symmetries can be assumed, analytical solutions result (Shoup and Szabo, 1982). As a first approximation to representing an ion's approach to a channel mouth, we will make the simplifying assumption that the electrical potentials near the channel mouth have a hemispherical symmetry (Läuger, 1976; see Fig. 1). We follow Läuger also in using the concept of a "capture radius." The "capture radius" is the radius of a small hemisphere whose center coincides with the center of the channel mouth. The surface of this hemisphere forms the boundary between the bulk solution and the region in which only one permeant ion can be at a time. The geometry of the situation suggests that a natural value for the capture radius is the difference between the channel radius and the radius of the bare ion (Andersen, cited in

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Läuger, 1976), although it has been argued that the "effective" capture radius may be larger than this (Läuger, 1976). The assumption of hemispherical symmetry permits us to pose the calculational problem as a one-dimensional rather than higher-dimensional problem.

The whole approach of using bulk electrodiffusion theory to describe regions near the channel mouth so small that only a few ions may reside there has been criticized by Hladky (1984). We will consider in the Discussion section of this paper the extent to which these concerns impinge on the validity of our calculations.

To calculate the electrical potentials in the bath, we will adapt the results of Jordan (1982) for the extension of "image" forces into the bath. (Professor Jordan has kindly provided us with image force results in tabular form.) The Jordan results are calculated with the assumption of zero ionic strength in the bath. To correct those results for ionic strength, in the calculations for bath potentials we multiply the distance from the channel mouth by an exponential factor whose argument is the distance divided by the Debye length, that is, the Debye-Hückel shielding factor (Friedman, 1985, p. 141). Our assumption of hemispherical symmetry implies that we neglect any effects due to the curvature of the surface of the phospholipid membrane (Huang, 1986; Jordan, 1987*a*; Helfrich and Jakobsson, 1988).

Once assumptions about the model system are made, the final theoretical question is how to couple the equations for diffusion to the channel mouth to the equations for movement across the channel. We believe an appropriate boundary condition is to consider the mouth of the channel to be an absorbing boundary for ions inside the channel, and further that the boundary condition presented by Levitt (1986) is equivalent to an absorbing boundary. The argument for that equivalence is that passage times calculated by the Levitt boundary conditions (Jakobsson and Chiu, 1987*a*, Eq. 6) are the same as those calculated for a particle moving between absorbing boundaries (Gardiner, 1983, Eq. 4.35). Before his 1986 paper, Levitt had coupled the approach to the channel with movement across the channel by a hybrid electrodiffusion-absolute rate theory approach (Levitt, 1982). The expression for the flux that we get by applying Levitt's 1986 boundary condition (this paper, Eq. 9) is different from the expression in the Levitt 1982 paper (Eq. 14). The 1982 result has been shown to be consistent with mixed boundary conditions, in which an ion in the channel sees a reflecting boundary at the mouth from which it entered and an absorbing boundary at the other end (Cooper et al., 1987). These mixed boundary conditions seem to us unphysical, since there doesn't seem to be any way for an ion in the channel to "remember" from which end of the channel it entered. Therefore we believe that the Levitt 1986 treatment of the bath–channel boundary is the more correct one and have adapted it in this paper to couple diffusion to the mouth with transport across the channel.

METHODS

A. Calculation of ion flux through a single-occupancy channel including access resistance

All mathematical notation in this paper is designed to be compatible with the Levitt 1986 paper and our previous paper on singly occupied channels (Jakobsson and Chiu, 1987*a*). The equations for flux and occupancy of univalent cations are

$$J = \frac{(C_L e^{\psi_L} - C_{-a} e^{\psi_{-a}})}{H_L} \tag{1}$$

$$J = \frac{P_0 (C_{-a} e^{\psi_{-a}} - C_a e^{\psi_a})}{H_{ch}} \tag{2}$$

(analogous to Levitt, 1986, Eq. 5)

$$J = \frac{(C_a e^{\psi_a} - C_R e^{\psi_R})}{H_R} \tag{3}$$

$$P_0 = \left\{1 + A \int_{-(a+L/2)}^{a+L/2} dx e^{-\psi(x)} \left[C_{-a} e^{\psi_{-a}} - (C_{-a} e^{\psi_{-a}} - C_a e^{\psi_a}) \frac{H(x)}{H_{ch}}\right]\right\}^{-1}, \tag{4}$$

where H_L, H_{ch}, H_R, and $H(x)$ in Eqs. 1–4 are given by

$$H_L = \int_{\infty}^{a} dr_L e^{\psi(r_L)}/(2\pi D_B r_L^2) \tag{5}$$

$$H_{ch} = \int_{-(a+L/2)}^{a+L/2} dx e^{\psi(x)}/(D_{ch} A) \tag{6}$$

$$H_R = \int_{\infty}^{a} dr_R e^{\psi(r_R)}/(2\pi D_B r_R^2) \tag{7}$$

$$H(x) = \int_{-(a+L/2)}^{x} dx' e^{\psi(x')}/(D_{ch} A). \tag{8}$$

The geometry of the situation is shown in Fig. 1. Specific definitions of the symbols, in order of their appearance in Eqs. 1–8, are: J is flux, ions/s; C_L is permeant concentration in bulk solution on left side, ions/m^3; ψ_L is dimensionless electrical potential in bath on left far from channel, in units of $FV/(RT)$; C_{-a} is permeant concentration on left side at radial distance a from center of channel mouth where a = capture radius, m (assumed equal to physical channel radius minus ion radius); ψ_{-a} is dimensionless electrical potential in left-hand bath at the capture radius a; H_L is access resistance in left bath, s/m^3; P_0 is fraction of time channel is empty of ions; C_a is permeant concentration on right side at radial distance a from center of channel mouth; ψ_a is dimensionless electrical potential in right-hand bath at the capture radius a; H_{ch} is

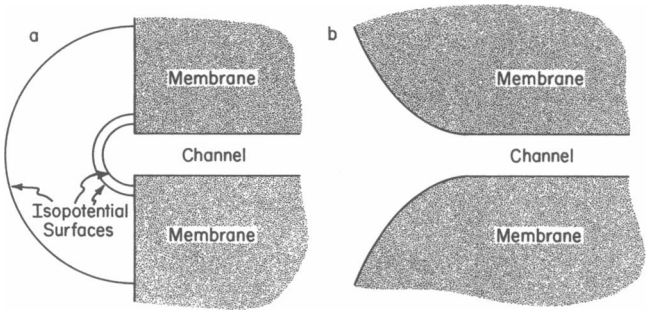

FIGURE 1 (*a*) Sketch of symmetry assumed in bath near mouth of channel in order to simplify mathematics of flux calculations. (*b*) Sketch of more realistic geometry near mouth of channel, showing effect of channel length being less than the normal lipid membrane thickness. This sketch is not to scale, but general shape of surface is that produced by preliminary calculations done in our lab, utilizing liquid crystal theory (Helfrich and Jakobsson, 1988).

resistance for translocation across channel; C_R is permeant concentration in bulk solution on right side; ψ_R is dimensionless electrical potential in bath on right side far from channel; H_R is access resistance in right bath; A is cross-sectional area of channel, m^2; L is channel length, m; x is position in channel relative to the center, m; $\psi(x)$ is dimensionless electrical potential at x; $H(x)$ is resistance for translocation from left-hand channel mouth to x; r_L is distance in left bath from center of channel mouth on left side; D_B is diffusion coefficient in bath, m^2/s; D_{ch} is diffusion coefficient in channel, m^2/s; and r_R is distance in right bath from center of channel mouth on right side.

If we select potential functions, mobilities, and channel dimensions, then Eqs. 1–4 constitute four equations in the four unknowns: (*a*) flux, (*b*) fraction of time the channel is empty, (*c*) bath permeant concentration at the left-hand capture radius, and (*d*) bath permeant concentration at the right-hand capture radius. For fitting to experimental data, it is of particular interest to solve these equations for the flux. The expression for the flux turns out to be a quadratic

$$J = (-\alpha \pm \sqrt{\alpha^2 - 4\alpha\beta\gamma})/(2\beta), \tag{9}$$

where

$$\alpha = 1 + (A/H_{ch}) \int_{-(a+L/2)}^{a+L/2} dx e^{-\psi(x)} \{C_R e^{\psi_R} H(x) + C_L e\psi_L [H_{ch} - H(x)]\} \tag{10}$$

$$\beta = (A/H_{ch}) \int_{-(a+L/2)}^{a+L/2} dx e^{-\psi(x)} \{H_R H(x) - H_L [H_{ch} - H(x)]\} \tag{11}$$

$$\gamma = (C_L e^{\psi_L} - C_R e^{\psi_R})/(H_{ch} + H_L + H_R). \tag{12}$$

The positive root of the quadratic is the physical one, which can be verified by inspection, since it is the positive root for which $J \to 0$ at the Nernst potential. The purpose of writing the flux in this form is to eliminate a number of non-measurable quantities from our original expressions. Eq. 9 is analogous to Eq. B6 of Andersen (1983*c*), which is the corresponding equation one gets if one uses a rate theory rather than a diffusion formalism for the permeation process.

B. Bases for choosing numerical values of parameters for calculations

The data base for fitting the theory will be data published by Barrett Russell et al. (1986) for sodium permeation through gramicidin channels, and by Hainsworth and Hladky (1987) for cesium permeation through gramicidin channels at very low concentrations of cesium. For cesium, the single-occupancy assumption is only valid for low permeant bath concentrations. For sodium, we will make the assumption that only one sodium ion can enter the channel at any time, even at high concentrations. One basis for this assumption is the tracer studies of Procopio and Andersen (1979) in which they could find no evidence for ion–ion interaction within the channels at sodium concentrations all the way up to 5 M. (For further elaboration of the Procopio-Andersen analysis, see Andersen, 1988.) It also is a general finding of several experimental groups that curves of low-voltage gramicidin sodium conductance versus concentration conform very closely to a Michaelis-Menten relationship, which is expected to be characteristic of single-ion occupancy. (The Michaelis-Menten relationship is an exact consequence of single-ion occupancy when access resistance is neglected [Levitt, 1986; Jakobsson and Chiu, 1987*a*] and is probably a fairly good approximation when access resistance is considered.) We certainly do not believe that a gramicidin channel never contains two sodium ions, but rather that double occupancy is a sufficiently unusual situation that it may be fairly neglected in our statistical treatment of sodium fluxes. The permeation of ions other than sodium through gramicidin is quite different. The above-mentioned tracer study by Procopio and Andersen (1979) showed clear ion–ion interaction for cesium ions in the gramicidin channel, as did the study by Schagina et al. (1978) for rubidium in gramicidin. Also, the conductance versus concentration curves for cesium and other permeant ions in gramicidin (except for sodium) are double-valued, i.e., the conductance decreases at large concentrations. This is a natural consequence of multiple occupancy in the channel when the flux is interpreted either by the traditional transition state theory or by diffusion theory. (See Jakobsson and Chiu, 1987*a*, for preliminary diffusion theory results, and see Eisenman and Sandblom [1983] and Hladky and Haydon [1984] for data on a variety of ionic species interpreted by transition state theory.) Thus it appears from flux data that sodium is markedly different from other permeant cations in the difficulty of a second ion entering the channel. The reason underlying this is unknown and beyond the scope of this paper; any explanation would have to involve molecular mechanics and molecular dynamics calculations. A recent review of the status of such calculations is given by Jordan (1987*b*).

Some of the parameters in the equations may be reasonably deduced from the physical characteristics of the system. The structure of the channel itself is a left-handed beta helix, with the peptide backbone forming the lumen of the channel (Urry, 1971; Koeppe and Kimura, 1984). Based on this generally accepted structure, it is reasonable to approximate the interior of channel as a 2-Å radius, 26-Å long cylinder. Based on crystal radii, it is reasonable to assign a radius of 1 Å to the sodium ion, and 1.7 Å to the cesium ion (Hille, 1984, p. 164). If the capture radius is given by the channel radius minus the ionic radius (Andersen, cited in Läuger, 1976), then the capture radius for sodium will be 1 Å, and for cesium will be 0.3 Å. We will use these values in our computations. Another determinant of the access resistance will be the ionic mobility in the bath. We use the value of $1.33 \times 10^{-9} m^2/s$ for the bath diffusion coefficient of sodium (Hille, 1984, p. 157) and $2.06 \times 10^{-9} m^2/s$ for the bath diffusion coefficient of cesium.

In our calculations for the central barrier due to "image" forces, we will follow the practice of our earlier paper (Jakobsson and Chiu, 1987*a*)

and simply scale up empirically the central barrier as calculated by Jordan (1982, 1983) for a 26-Å long, 2.5-Å radius channel, using the scaling factor as a variable parameter to which to fit the data. The portion of the barrier that extends out into the bath is corrected for electrolyte concentration as described in the Introduction.

The other major electrostatic quantity relevant to our calculations is the form of the potential function resulting from a potential gradient applied across the membrane. Only part of that potential is across the channel itself and part of the potential difference is in the bath external to the membrane. The Jordan (1982) calculations present, for the idealized right circular channel, what fraction of the applied potential falls across the channel and what fraction is in the bath. For a channel 26-Å long and 4-Å diam, almost exactly 90% of the potential is across the channel and 10% in the bath (5% on each side). We use these numbers in our calculations. We assume that the fraction inside the channel is linear. For the fraction in the bath, the potential from the published Jordan results is corrected for electrolyte concentration in the manner described in the Introduction.

There is another feature of the channel potential profile that has not yet been treated with any nearly quantitative success theoretically. That is the reduction of the free energy for univalent cations as they enter the channel mouth, i.e., the tendency of ions to partition from the bath into the channel. We know from saturation curves of conductance vs. permeant concentration in the bath that this reduction must exist, since the mean ionic concentration in the channel can be several molar at a time when the ionic concentration in the bath is of the order of a hundred millimolar or so. We suspect that the full theory for calculating this partition energy for ions into the channel is very demanding, involving such things as the orientation of the channel polar groups toward the ion as it approaches the channel, and competition between the ion and phospholipid head groups for solvation waters in the vicinity of the channel mouth. That theory is worth doing but beyond the scope of this paper. We will use the magnitude of the partition energy as an adjustable parameter in our calculations. We will assume that the entire partition energy drop takes place between 1 Å out into the bath and 1 Å into the channel and also use as an adjustable parameter what fraction of the energy drop takes place in the bath and what fraction takes place in the channel.

The above assumptions about the form of the potential profile are shown graphically in Fig. 2 *a*. There are shown each of the three components that are added together to comprise the total potential profile that is assumed to determine the ion translocation, together with indication of the numerical parameters that are varied to fit the data. In outline, this potential profile is similar to that postulated by previous workers (see Levitt, 1978*b*, Fig. 1).

Another parameter that goes into the numerical calculations is the diffusion coefficient of the ion–water complex in the channel. In our earlier paper (Jakobsson and Chiu, 1987*a*), we treated this quantity as a parameter to vary empirically, since we knew of no physically based theory to predict its value. Simultaneous to our calculations but unknown to us, Skerra and Brickmann (1987) were calculating this quantity for various univalent cations in an idealized gramicidin-like channel by the technique of molecular dynamics, using simulations in which the ions were driven through the channel by a strong applied electric field. In the present calculations, rather than simply using the number calculated by Skerra and Brickmann, we will again vary this parameter to ascertain the best fit to the data and a range of uncertainty for this quantity. (Skerra and Brickmann did not estimate a range of

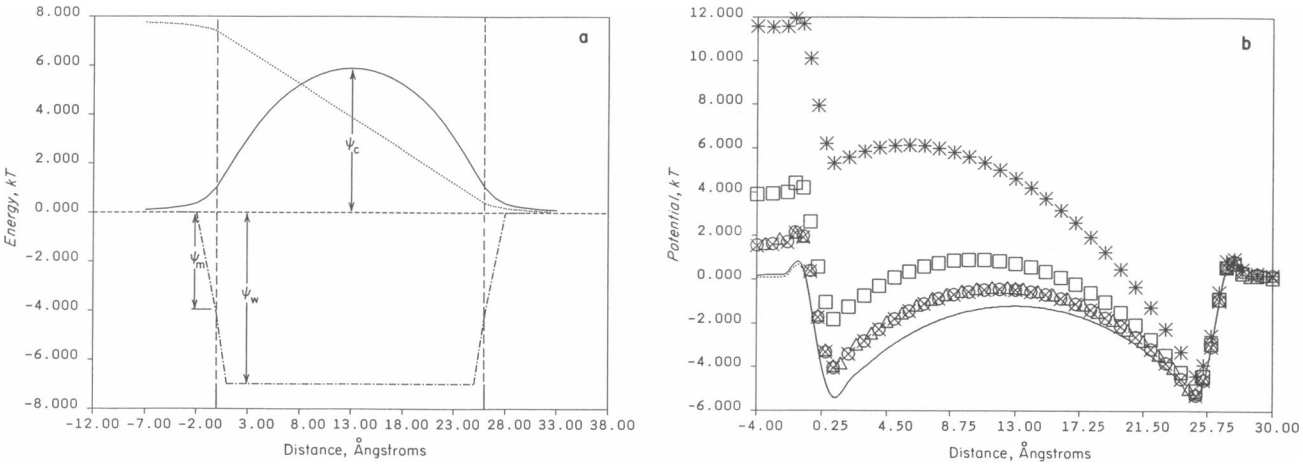

FIGURE 2 (*a*) Components that contribute to the potential profile across the channel for sodium permeation, according to the assumptions of this paper. One component is the central barrier, having the shape shown, whose height is the adjustable parameter ψ_c. The second component is the partition energy for the ion to leave the bulk solution and enter the channel. This energy is assumed to consist of two parts, ψ_m from the capture radius to the channel mouth and ψ_w from the channel mouth to a position 1 Å in from the channel mouth. The third component is the applied potential, 90% of which is assumed to take place across the channel and 10% of which is assumed to take place in the external baths, as described in the text. Vertical dashed lines indicate the physical ends of the channel. Curves are scaled for the parameters that gave best fit to the data for sodium permeation through the unmodified gramicidin channel. Indicated transmembrane potential is 200 mV. (*b*) Potential profiles for sodium permeation through unmodified gramicidin A channel for parameters ψ_c, ψ_m, and ψ_w giving optimum fit to experimental data. Vertical axis is dimensionless potential, in units of $(FV)/(RT)$, relative to potential in right-hand bath. Horizontal axis is distance in angstroms, with origin at left-hand channel mouth. Symbols represent profiles at the following potential concentrations and transmembrane potential differences: —, 50 mM, 0 mV; · · · ·, 1,000 mM, 0 mV; O, 50 mM, 37.5 mV; ×, 200 mM, 37.5 mV; △, 1,000 mM, 37.5 mV; □, 1,000 mM, 100 mV; *, 1,000 mM, 300 mV. Particularly noteworthy features are reduction of entrance barrier at high-voltage side due to fraction of applied voltage extending into the bath and near complete elimination of central barrier for ions moving from high to low voltage, with reverse being true for ions moving up a voltage gradient.

uncertainty for their results. In separate calculations [Jakobsson and Chiu, 1988], we have made such an estimate by looking at the variance of passage times predicted by stochastic dynamics for the conditions simulated by Skerra and Brickmann.) The end result of this process will be a range of possible values for the diffusion coefficient calculated by us on phenomenological grounds, which can be compared to the range of values consistent with the molecular dynamics computations of Skerra and Brickmann.

The criterion for fitting of the adjustable parameters in the theory to the published experimental data was to minimize the sum of the squares of the fractional difference between calculated and experimental sodium conductances. To get the overall optimum parameters, our computer program started with an initial guess for the values of the adjustable parameters and then continued to adjust ψ_c, ψ_m, ψ_w, and D by the method of steepest descent until no further significant improvement in fit could be achieved. We also did "sensitivity" calculations, in which one of the adjustable parameters was fixed and the other three parameters varied, in order to test the sensitivity of the goodness of fit to each of the adjustable parameters. The computer time for each optimization was very variable, being a very strong function of how good the initial guess was. The total computer time for generating the global optimizations and the points on the curves of Fig. 3 was ~65 h of central processor time on a Digital Equipment Corp. (Marlboro, MA) MicroVax II.

The procedure for fitting to the cesium data of Hainsworth and Hladky (1987) was slightly different, since the nature of the data was different. Because of the very low permeant concentrations, measurement of single channel conductances was not feasible. Thus the data consisted of relative conductances at different voltages, deduced from the currents for many channels simultaneously. Therefore the quantities that were fit were normalized rather than absolute currents.

RESULTS

The potential profiles produced by the above set of assumptions and methods have a number of noteworthy features as a function of applied potential and bath

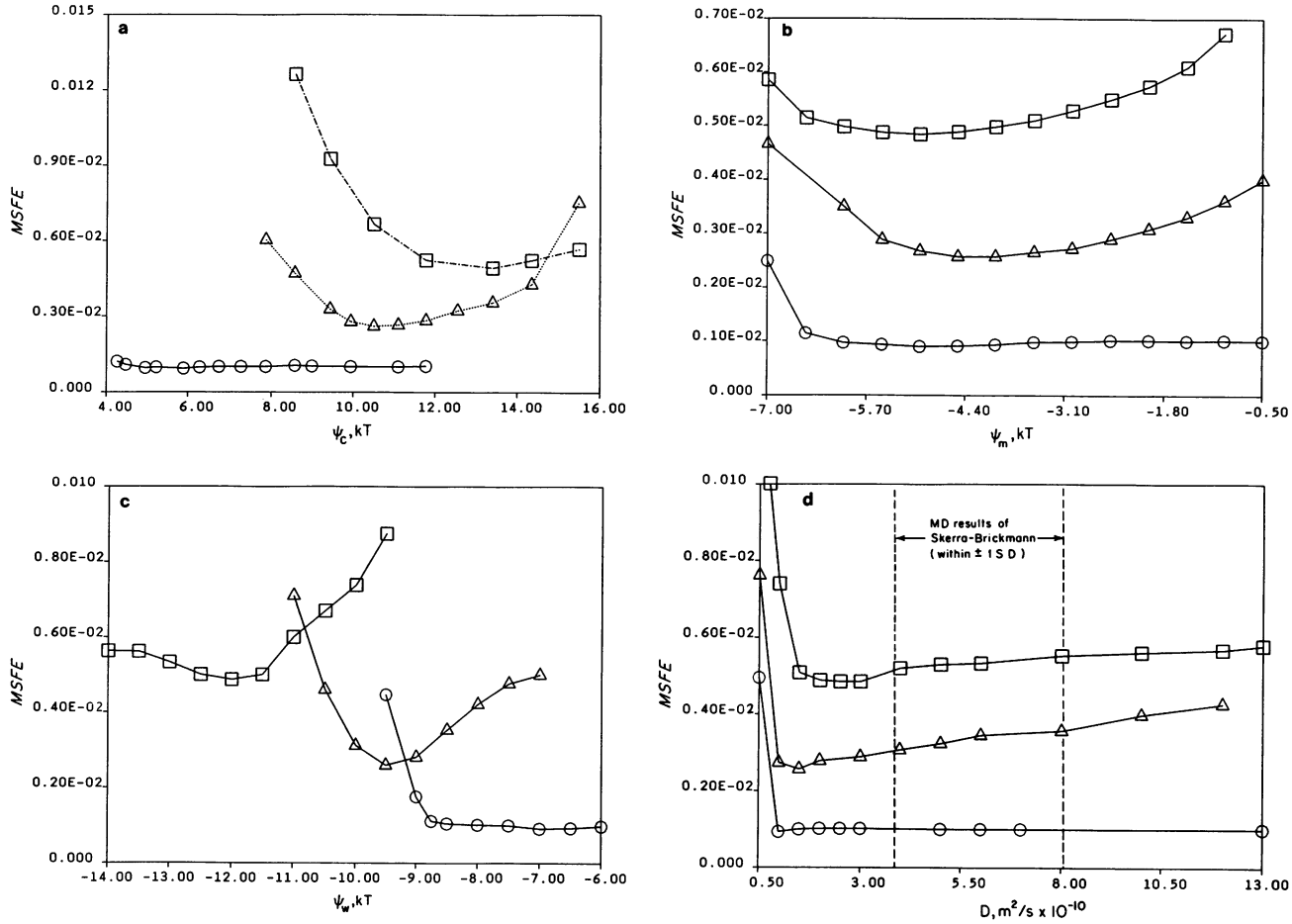

FIGURE 3 (*a–c*) Sensitivity analysis of the fit to the sodium permeation data of the potential parameters ψ_c, ψ_m, and ψ_w, respectively; (*d*) sensitivity analysis of diffusion coefficient. In each case the vertical axis is the mean square fractional deviation of the computed points from the experimental points when the quantity along the horizontal axis is fixed at the indicated value and the other three adjustable parameters are varied to give the optimum fit to the data by a search program that uses the steepest-descent method. In the case of the diffusion coefficient the range of values inferred from the 1987 molecular dynamics calculations of Skerra and Brickmann (to within one standard deviation) is superimposed on the sensitivity graph for purposes of comparison. Symbols are: O, normal gramicidin; Δ, TFV gramicidin; □, HFV gramicidin.

electrolyte concentration. These features can be seen in Fig. 2. This figure shows the potential profiles for sodium ion translocation from one side of the membrane to the other, at a variety of transmembrane potentials and bath sodium chloride concentrations, for values of the adjustable parameters that give an optimum fit to the data for unmodified gramicidin A. In contrast to our earlier paper in which access resistance was neglected (Jakobsson and Chiu, 1987*a*), these profiles do show a potential barrier to ion entry. Consistent with that paper, however, the barrier is outside the one-ion region.

Since the access resistance is a function of the potential as it extends into the bath (Eqs. 5 and 7), it is seen that the access resistance will be a function of electrolyte concentration and applied potential. The effects are as follows: (*a*) Since the image forces extend farther out into the bath for low electrolyte concentrations, the part of the access resistance due to the image forces is higher in low bath electrolyte concentration. (*b*) The fraction of the applied potential that extends into the bath tends to reduce the access resistance to cations on the high-voltage side of the membrane and increase the access resistance to cations on the low-voltage side of the membrane. (*c*) The effect in *b* above is more pronounced in low bath electrolyte concentration. As can be seen from the figure, the effect of electrolyte concentration is relatively minor for this system, but the applied potential extending into the bath does significantly modify the access resistance for cations to enter the channel, reducing the entrance barrier on the high-voltage side and increasing the barrier on the low-voltage side.

Qualitatively, these calculations which include access resistance tell us the same story about the effects on sodium permeation of fluorinating the valine at the number 1 position in the channel as did the earlier calculations (Jakobsson and Chiu, 1987*a*) that neglected the access resistance. This story is told graphically in Fig. 3, which shows how sensitive the quality of fit to the data is to assumed values of the adjustable parameters determining the potential profile ψ_c, ψ_m, ψ_w (see Fig. 2 *a* for a definition of these parameters), and the diffusion coefficient in the channel D. The data base for these calculations is the full set of single channel sodium conductance data presented by Barrett Russell et al. (1986) at various concentrations and voltages. In this analysis one of the adjustable parameters is fixed at a series of values and the others optimized by a search program that uses the steepest descent method. The quantity that is minimized and plotted on the vertical axes of Fig. 3 is the mean of the squared fractional error per data point. From the curves for ψ_w it can be inferred that fluorinating the valine increases the depth of the potential minimum near the channel mouth. The curves for ψ_c are, on their face, somewhat less clear because of how flat the error curve is for the unmodified gramicidin. However, we believe in fact that the correct ψ_c is probably smaller for the unmodified gramicidin than for the tetrafluorovaline (TFV) and hexafluorovaline (HFV) varieties for another reason. That is, to get the best fits to the normal gramicidin data for the very high values of ψ_c it was necessary to take the value of D to a value significantly higher than the value for bulk water. Specifically, for $\psi_c = 11.1$ and $11.8kT$, the values of D that gave the best fit were 2.0 and $3.0 \times 10^{-9} m^2/s$, respectively. The value of the diffusion coefficient for sodium ions in bulk water, as cited in the previous section, is 1.33×10^{-9} m^2/s. While not absolutely ruling out larger values for the diffusion coefficient in the channel, they seem intuitively unlikely on grounds of how relatively constricted the space inside the channel is. We also know of no molecular calculations that suggest that the diffusion coefficient in the channel is larger than that for the bath. Therefore we believe that ψ_c for the unmodified gramicidin is probably somewhat lower than that for the TFV and HFV varieties. These results are summarized in Table 1, in which the central barrier and well depth derived from the optimum values of ψ_c, ψ_w, and ψ_m are shown and compared with the corresponding results from the earlier calculations. There may also be an effect on the diffusion coefficient from fluorinating the valines, but since such a wide range of diffusion coefficients gives approximately the same degree of goodness of fit to the data, such an effect cannot be inferred from our results. It is clear however that the newly calculated diffusion coefficients inside the channel are larger than the previous ones. This seems reasonable since the more complete calculation adds resistance in series with the channel resistance. Thus to pass the same amount of current the channel resistance would tend to be smaller.

Table 1 also shows the best fit parameters for the low concentration (0.1 and 1 mM) cesium flux through

TABLE 1 Best-fit diffusion parameters

Amino acid at position No. 1	D	Well depth	Barrier height
	$m^2/s \times 10^{11}$	kT	kT
Valine	9.4 (4.8)	5.4 (5.3)	4.2 (3.6)
Trifluorovaline	14.9 (4.1)	6.4 (6.5)	7.8 (6.5)
Hexafluorovaline	25.5 (3.0)	8.2 (7.5)	9.2 (6.9)
Valine (cesium as permeant ion)	40.8	8.62	4.61

Diffusion coefficient, surface well depth, and central barrier height corresponding to best fit to data for assumptions embodied in the calculations here, compared with corresponding quantities (in parentheses) from calculations that neglected the access resistance (Jakobsson and Chiu, 1987*a*). From the sensitivity analysis for the sodium data (Fig. 3) we judge that the differences in the diffusion coefficients for the different channel types may not be significant, but that the differences in well depth and barrier height probably are.

normal gramicidin as presented by Hainsworth and Hladky (1987). These results show that cesium may have a somewhat higher mobility in the channel than does sodium (perhaps paralleling its higher mobility in electrolyte solution), that it may bind more tightly at the channel mouth than sodium in valine gramicidin, and that the central barrier height is very similar to that for sodium. The goodness of fit to the low concentration cesium permeation data directly used for establishing the optimum parameters is given in Fig. 4. The fit is seen to be quite good. We also attempted to fit the 1 M cesium data from Barrett-Russell et al. (1987) to the one-ion model and found no good fit, neither with the low concentration optimized parameters nor with any other set. We ascribe this failure to the fact that there is double occupancy of cesium at such high concentrations, and that the double-occupancy current–voltage curves are essentially different from the single-occupancy curves. We intend to do in the future a more comprehensive treatment of cesium in which we combine the single occupancy analytical treatment of the low concentration situation with a double occupancy Brownian dynamics treatment (Jakobsson and Chiu, 1987*b*) of the high bath concentration situation.

The goodness of fit to the sodium permeation data (directly represented in Fig. 5) is somewhat better than in our earlier calculations (Jakobsson and Chiu, 1987*a*), which is to be expected since these calculations are a more complete representation of the permeation process.

One significant way that the present calculations give a different physical picture of what is happening than do our earlier calculations is that the concentration of permeant ions at the channel mouth is different from the bulk concentration in the bath. There are two major

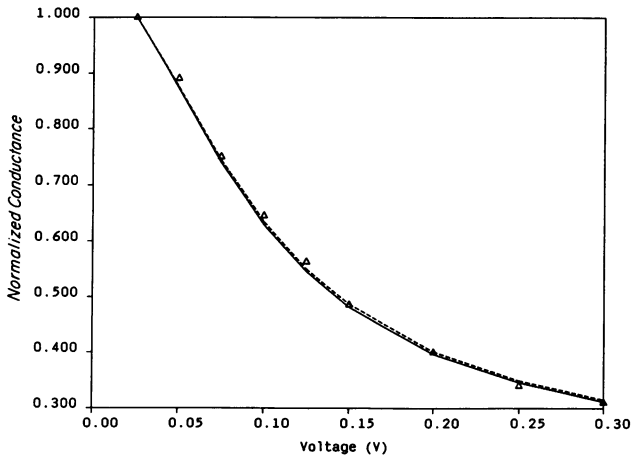

FIGURE 4 Normalized current–voltage curve for cesium at low concentrations (0.1 mM, *solid line* and 1 mM, *dashed line*) calculated with parameters optimized for cesium permeation of gramicidin. Symbols are from published data (Hainsworth and Hladky, 1987).

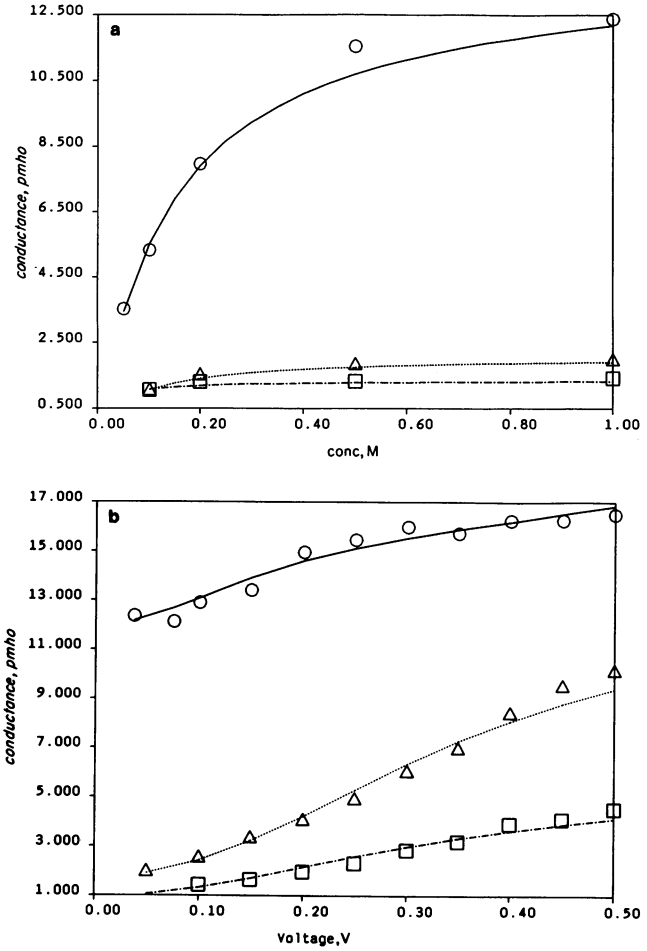

FIGURE 5 Direct comparison between experimental data for sodium permeation (Barrett Russell et al. [1986]) and computed data with optimized parameters. (*a*) Conductance vs. concentration at small transmembrane potential and (*b*) conductance vs. voltage at 1 M permeant concentration. Symbols are: O, normal gramicidin; △, TFV gramicidin; □, HFV gramicidin.

effects that cause the concentration at the channel mouth to deviate from the bulk concentration. One is the voltage gradient in the bath, which will cause cations to be distributed preferentially towards more negative potentials. The second is the effect of accumulation and depletion in response to flow, which will cause ions to accumulate on the "downstream" side of the channel, and be depleted on the "upstream" side of the channel. The mathematical representation of these effects can be readily seen by rearranging Eqs. 1 and 3 to solve for the concentrations at the channel mouths, C_{-a} and C_a, respectively. These rearrangements yield

$$C_{-a} = C_L e^{(\psi_L - \psi_{-a})} - JH_L e^{-\psi_{-a}} \tag{13}$$

$$C_a = C_R e^{(\psi_R - \psi_{-a})} + JH_R e^{-\psi_{-a}}. \tag{14}$$

The form of the right-hand sides of Eqs. 13 and 14 show clearly the two effects that determine the concentration at the channel mouth. The first term on the right-hand side is bath concentration multiplied by the Boltzmann factor, which is purely determined by the potential. The second term gives the accumulation-depletion effect, which in this case depends on the potential as well as the flow, through the dependence of the access resistance on the potential. Eqs. 13 and 14 correspond to Eqs. B2 and B3 in Andersen (1983*c*) but have an important difference. In the Andersen equations, the first term on the right-hand side is not multiplied by the Boltzmann factor. Since the electrical potential energy at the capture radius may differ from that in the bulk solution by several kT, this is a significant difference.

The reader may observe at this point that in our determination of the potential we have not taken into account the potential caused by separation of bath cations and anions in the applied field near the channel mouth. We neglect this effect not because it is insignificant, but because continuum theory cannot account for this effect on a level as microscopic as the dimensions of the space around the channel mouth. This point, which is essentially equivalent to the objections raised by Hladky (1984) to the use of continuum theory for the calculation of access resistance, will be considered more fully in the Summary and Discussion section.

With the caveat that the accuracy of the calculations is compromised by this fundamental limitation of the continuum theory, we present in Fig. 6 the permeant concentrations in the bath at the upstream and downstream capture radii as a function of applied potential for permeation through normal gramicidin with a couple of different bulk bath concentrations. The concentrations at zero applied voltage differ from the bulk just by the Boltzmann factor due to the entrance barrier. Away from zero applied potential two opposing phenomena come into play. The part of the applied potential that extends into the bath tends to increase the concentration of sodium ions at the channel mouth on the high-voltage side, while the accumulation–depletion phenomenon associated with the flux tends to reduce the concentrations on the high-voltage side. These same phenomena have just the reverse effect on the low-voltage side. From Fig. 6, we see that for the assumptions in our calculations, the effect due to the applied potential is the dominant one, so that the concentration at the capture radius goes up with voltage on the high-voltage side and down with voltage on the low-voltage side. Thus even when there is no bath-to-bath concentration gradient, sodium flux through the channel proper has the character of a combined voltage and concentration gradient, with both gradients in the same direction. The inclusion of the Boltzmann factor in the first term on the right-hand side of Eqs. 13 and 14 is essential to this result.

Fig. 7 shows the change in shape of the current–voltage curves of the one-occupancy model as the bath permeant concentrations are varied. These curves are calculated using the model parameters optimized for the Barrett Russell et al. (1986) sodium data. The distinctive change in shape of the I-V curve from sublinear to superlinear as the concentration is increased is characteristic of the

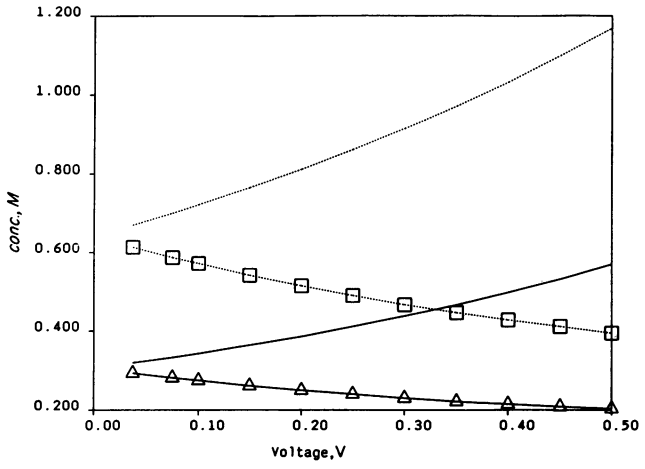

FIGURE 6 Permeant concentrations at the capture radii as a function of applied voltage for two different bath sodium concentrations for normal gramicidin. Symbols are: ····, 1 M concentration, high-voltage side; □, 1 M concentration, low-voltage side; —, 0.5 M concentration, high-voltage side; △, 0.5 M concentration, low-voltage side.

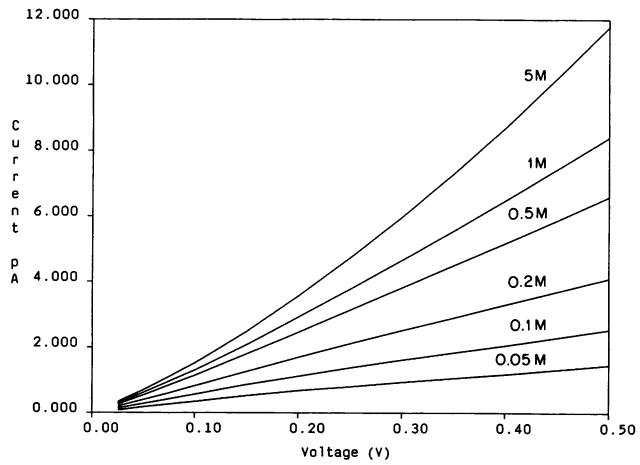

FIGURE 7 Current–voltage curves at various bath concentrations of permeant ions for the parameters optimized for sodium permeation through normal gramicidin channels. The change in shape of the curves with bath concentration is similar to that seen experimentally in Andersen and Procopio (1980) and Eisenman and Sandblom (1984).

single-ion occupancy model presented and of gramicidin sodium conductance data such as that of Andersen and Procopio (1980) and Eisenman and Sandblom (1984).

SUMMARY AND DISCUSSION

In this paper, we have attempted to present a reasonably complete physical theory down to, but not including, the atomic level of detail for the permeation of ions through a channel when only one ion can be in the channel at once and when access resistance to the channel mouth is not negligible. We have fit the theory to a body of permeation data for which it seems appropriate; that is, sodium permeation through gramicidin channels. In so doing, we have produced on phenomenological grounds estimates for the reasonable range of values of quantities that have been calculated on physical grounds, such as the height of the central barrier to permeation and the diffusion coefficient of the sodium–water complex through the channel. We have also produced an estimate for a quantity for which no successful physical calculation yet exists, and that is the partition energy for sodium ions to move between the channel and the bath. Finally we have attempted to use the theory to portray some aspects of the microscopic physical picture of what happens during permeation, such as the extent to which ions accumulate and are depleted at the channel mouths due to the combined effects of potential gradients near the surface and flux through the channel. In the balance of this section we will state the inferences we draw from the results of our calculations, first stating what the results seem to say on their face and then discussing what limitations we see in the calculations due to various assumptions and to the constitutive rather than bulk level of detail embodied in our methods.

Fig. 3 is the sensitivity analysis showing the goodness of fit to the data of our set of assumptions. In general, our conclusions presented earlier based on a more approximate calculation (Jakobsson and Chiu, 1987*a*) about the effects on the potential profile of fluorinating the methyl groups of the number 1 valines are confirmed. That is, it appears that this chemical modification increases the height of the central barrier, increases the depth of the potential well near the channel mouth, but has no clear effect on the diffusion coefficient. Interestingly, Andersen et al. (1987) inferred the same qualitative effects on the potential profile of this substitution from an approximate electrostatic calculation of the effects of introducing an orientable dipole in the center of the channel, although the effect on the surface well from those calculations was much smaller in magnitude than what we infer from the transport theory. The assumptions of our calculation are a bit crude in that we simply change the height of the central barrier without changing the shape in order to account for fluorinating the valine methyl groups, even though the presumed physical basis for the additional potential associated with the fluorination (electron induction by the fluorines and consequent polarization of the side chain) is quite different from the image forces that give rise to the central barrier in the normal gramicidin. To refine the potential profile significantly it would be necessary to do a truly molecular calculation involving not only electrostatics but also free energy as the ion translocates across the modified channel. Such a calculation has begun (Shankar et al., 1988) but is beyond the essentially phenomenological scope of the present paper.

In this paper, the diffusion coefficients that fit the sodium data are larger in magnitude than in the earlier analysis that neglected access resistance. It is clear from Fig. 3 that the minimum diffusion coefficient that will fit the data well for all three types of channels is of the order of 2.5×10^{-10} m^2/s, but that indeed it may be several times larger. It should also be kept in mind that small signal conductances for sodium in gramicidin more than double in magnitude those in the data we are fitting have been reported (Dani and Levitt, 1981). Thus the range of plausible values for the diffusion coefficient deduced from this theory may easily extend to the 6×10^{-10} m^2/s implied by the trajectory presented by Skerra and Brickmann (1987) from their molecular dynamic calculations of the rate of drift of the sodium–water complex through gramicidin under the influence of a strong electric field. The Skerra–Brickmann value for the diffusion coefficient is also uncertain, with a standard deviation of $\pm 2.1 \times 10^{-10}$ m^2/s Jakobsson and Chiu, 1988). (Essentially this result is the width of the gaussian distribution associated with a particle of diffusion coefficient 6×10^{-10} m^2/s drifting for 120 ps.) Thus there is a large overlap between the ranges of diffusion coefficient reasonably inferred from our phenomenological calculations and the molecular calculations of Skerra and Brickmann. The two sets of results are consistent with each other, although regrettably the mutual consistency is at present due to the imprecision as well as to the accuracy of the results. It should be noted that the above discussion and our calculational methods assume that the diffusion coefficient has one constant value throughout the channel and a different constant value in the bath, with an abrupt transition between these values at the capture radius. This assumption, made for the sake of calculational tractability, is certainly not precisely true. The diffusion coefficient is some more complicated function of ion position (Vertenstein and Ronis, 1986). It is possible that the details of the transition near the channel mouth between the higher bath mobility and the lower channel mobility may be

highly significant for channel function. This issue warrants further detailed molecular calculations.

In an important way we have come up against an essential limitation of the continuum description of the single-ion channel. One phenomenon which the continuum theory is inadequate in describing is the interaction between potential gradients and ion distributions in the bath near the channel mouth. We noted in the Results section that we had neglected the potential associated with the separation of cations and anions in the applied potential near the channel mouth. In the continuum formalism the way to do this is to combine the Nernst-Planck electrodiffusion equation with Poisson's equation:

$$\nabla^2 V = -\rho/(\epsilon\epsilon_0).$$

This has been done for diffusion within a membrane (deLevie and Moreira, 1972; deLevie et al., 1972) and recently for diffusion at the mouth of a membrane channel (Peskoff and Bers, 1988). There are two major drawbacks to this approach. The first is that it's difficult, or at least tedious, to solve the Nernst-Planck equation and Poisson's equation simultaneously for the flux and the ionic concentration distributions. The second problem is that for systems of microscopic dimensions, which contain only one or a few ions at a time, this approach gives the wrong answer. The nature of the problem was suggested by Hladky (1984) and shown explicitly in the first Brownian dynamics computations of flux across membranes (Cooper et al., 1985), which failed to reproduce the deLevie et al. results for corresponding parameters and boundary conditions. The underlying reason for this discrepancy is the failure of such a small system to satisfy the assumption of ergodicity implicit in the continuum theory. Essentially this assumption implies that space-averaging and time-averaging for the ionic charge densities involved in the equations are equally valid. The assumption fails for a very small system because there is no mutual repulsion between a single ion at time t and itself at time $t + \Delta t$, but the continuum formalism "thinks" there is. Thus a comparison of the results of Cooper et al. and deLevie et al. reveal that the mutual repulsion of ions in a narrow multi-ion channel is greatly overestimated by the continuum formalism. Such an error, of unknown size, is undoubtedly also present in applying the full continuum theory to the space immediately outside the channel. Therefore we didn't feel that the mathematical complication of accounting for this effect in the context of continuum theory was worthwhile at the level of accuracy with which we are modeling the fluxes in this paper. In order to be confident of characterizing the access resistance to the channel more accurately than we have done in this paper, it would be necessary to map the potential outside the channel mouth using Poisson–Boltzmann theory (Jordan et al., 1988) and do a statistical analysis of Brownian dynamics trajectories leading to the mouth, as has been done for the diffusion-limited superoxide dismutase reaction (Sharp et al., 1987*a, b;* McCammon et al., 1986). Such an approach could also be used to test the accuracy of the Peskoff–Bers (1988) calculations.

Future work in understanding this system better will probably lie in more detailed physical calculations to deduce from physical theory on the atomic scale the system parameters that we have induced from fitting the data to the continuum theory. Then perhaps the array of calculational techniques and physical theory that are amassed in understanding the gramicidin channel may be brought to bear on understanding the functioning of more complicated channels of primary biological significance.

In the early stages of formulating the problem, conversations with Drs. Olaf Andersen, Kim Cooper, and David Levitt and Mr. Peter Gates were especially helpful. Dr. Andersen also made helpful suggestions at later stages of the work. In the electrostatics aspects of the problem, calculations by and conversations with Dr. Peter Jordan were very useful. During part of this work one of us (E. Jakobsson) enjoyed the hospitality of Dr. J. Andrew McCammon's lab for a sabbatical semester and received useful insights from Dr. McCammon on that part of the problem involving diffusion to the mouth of the channel. Recent conversations with Dr. Arthur Peskoff have been helpful to us.

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