STOCHASTIC THEORY OF ION MOVEMENT IN CHANNELS WITH SINGLE-ION OCCUPANCY

Application to Sodium Permeation of Gramicidin Channels

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ABSTRACT The electrodiffusion equations were solved for the one-ion channel both by the analytical method due to Levitt and also by Brownian dynamic simulations. For both types of calculations equilibration of ion distribution between the bath and the ends of the channel was assumed. Potential profiles were found that give good fits to published data on Na^+ permeation of gramicidin channels. The data were best fit by profiles that have no relative energy maximum at the mouth of the channel. This finding suggests that alignment of waters or channel charged groups inside the channel in response to an ion's approach may provide an energetically favorable situation for entry sufficient to overcome the energy required for removing bulk waters of hydration. An alternative possibility is that the barrier to ion entry is situated outside the region restricted to single-ion occupancy. Replacement of valine with more polar amino acids at the No. 1 location was found to correspond to a deepening of the potential minima near the channel mouths, an increase in height of the central barrier to ion translocation across the channel, and possibly a reduction in the mobility of the ion–water complex in the channel. The Levitt theory was extended to calculate passage times for ions to cross the channel and the blocking effects of ions that entered the channel but didn't cross. These quantities were also calculated by the Brownian dynamics method.

INTRODUCTION

Early methods for calculating fluxes through membranes used the formalisms of bulk electrodiffusion (Goldman, 1943) or the conductance approximation (Hodgkin and Huxley, 1952). When tracer studies revealed that ions in membranes did not move independently of each other (Hodgkin and Keynes, 1955), it became clear that for a detailed physical description of permeation, electrodiffusion formalisms that assumed the independence principle would not suffice. A natural and relatively simple way to incorporate ion–ion interactions into ion permeation calculations provided only a few ions can enter the channel at once is to use rate-theory representations of permeation. In these calculations, the ions are assumed to "hop" across the membrane in discrete jumps from potential minimum to potential minimum. Ion–ion interactions are introduced naturally as rules mitigating against the simultaneous occupancy of a site by more than one ion. This kind of theory is reviewed well by Hille (1984) and Cooper et al. (1985). The great advantage of this type of formalism is that the very difficult analysis associated with introducing ion–ion interactions into electrodiffusion theory is avoided. All calculations are reduced to linear algebra, in close analogy to the treatment of chemical reaction networks.

However, continuum diffusion theory is the more rigorously correct theory. This is clear from the original work of Einstein that showed the exact correspondence between microscopic random walk or thermal motion and macroscopic diffusion theory (Einstein, 1926). The addition of velocity-independent deterministic forces, such as gravity and electric fields, provides an essentially complete conceptual foundation for describing phenomena in which both thermal motion and deterministic forces play a role, such as molecular and ionic movement across membranes.

With the wide availability of high-speed digital computers, a relatively straightforward method of describing such phenomena in detail has become feasible. This is Brownian dynamics (Ermak and McCammon, 1978). In this technique, the thermal movement of particles is represented explicitly by the actual simulation of trajectories using strings of random numbers. The feasibility of this technique for calculating ion fluxes in membranes has been demonstrated (Cooper et al., 1985). The disadvantages of Brownian dynamics include the fact that it is relatively computationally intensive and that it is to some extent approximate. The approximations are of two sorts. One is that it may be difficult to run the Brownian dynamics program long enough on the computer to get a very large number of ions through the membrane. Thus, inaccuracies may be introduced by fluctuations of short duration, which would not have a significant effect on a measured average

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current in a channel. The second type of approximation in Brownian dynamics lies in the coarseness of the simulated movement. For most of the simulations presented in this paper, this coarseness is of the order of one-tenth of an Angstrom, i.e., that is how far an ion moves on the average in a single time step. While this movement is about a hundred times smaller than the "hops" typical of rate-theory barrier models, it is not a priori obvious that even this level of description is sufficiently fine to give only negligible errors in correlating current–voltage (I-V) curves to potential profiles in membrane channels. Accordingly, it is desirable to compare Brownian dynamics methods with exact analytic methods to the extent feasible, in order that particular Brownian dynamics programs and algorithms may be properly tested.

In this paper, we show the correspondence between the results of Brownian dynamics computations done in our lab and the analytical theory developed by Levitt (1986) for ion fluxes in a single-ion channel. We also extend the Levitt theory to calculate such quantities as mean passage times and blocking times for ions that occupy the channel but do not go all the way through the channel. For sample calculations we use particular potential profiles that correspond well to experimental data on Na^+ permeation of gramicidin. The correspondence between the Levitt theory and the Brownian dynamics is a manifestation of the correspondence between the diffusion and random walk first shown by Einstein (1926). The correspondence also gives us confidence that our methods for modeling entrance and exit of the ions at the channel mouth are a good representation of the equilibration condition at the mouth used by Levitt (1986).

METHODS

Existing Analytical Theory

We calculated fluxes according to the equations of Levitt (1986) for various potential profiles and mobilities within the membrane. In each case, we assumed equal permeant concentrations on both sides of the membrane. It should be stressed that the Levitt equations ignore inhomogeneities in either chemical or electrical potential in the bath outside the membrane. As such, they are an undoubted oversimplification of the true situation, but are worth studying as a first step towards a more complete understanding. They also provide an analytical check on the validity of stochastic dynamic methods away from equilibrium, which makes them a valuable tool for the development of stochastic dynamic methods for studying ion permeation.

In the calculations shown in Figs. 2, 3, and 6 the Levitt equations were evaluated by a "piece-wise analytic" method. In this method, the potential profile was represented by a lattice of points. In our case, we hypothesized a channel 26-Å long and defined the potential by a number at each angstrom. The potential profile was approximated everywhere by connecting the potential points with a series of straight lines. This approximation permitted us to find analytical expressions for the various integrals in the Levitt equations, since each integral could be made to reduce to a sum of terms of the form

$$\int_{x_1}^{x_2} e^{ax}\mathrm{d}x,$$

(a = constant), whose solution is well known. For other results shown, the numerical integration was by a "brute force" method, i.e., by dividing the channel into many small increments and linearly adding the contribution of each to the integral. We investigated potential profiles of the general form that there was a central barrier (presumed to be associated with "image forces") and a potential minimum near the mouth of the channel. Since the charge distributions in the neighborhood of an ion "relax" in the ion's vicinity in response to the ion's presence, this minimum is better thought of as a minimum in free energy rather than a minimum in electrostatic potential. Indeed, the entire potential profile in these calculations is a free energy curve (or "potential of mean force") along the ion's permeation pathway. In Eyring rate theory calculations of ion permeation, these potential minima are sometimes referred to as "binding sites." In the stochastic and continuum diffusion theories used in this paper, we do not picture ions as hopping over barriers from binding site to binding site, but rather as "crawling" across free energy surfaces. Thus, a potential minimum is only one of an infinite number of locations where an ion may be, albeit a relatively likely location.

Extensions of the Analytical Theory

In this section we extend the one-ion electrodiffusion theory by Levitt to calculate mean passage times and "blocking time," i.e., total time channel is occupied by ions that do not pass through the channel. All notation is written to be consistent with Levitt (1986).

Hypothesize a situation in which there are permeant ions on one side of the membrane only. In that situation, the mean passage time for ions to cross the channel would be the probability, or fraction of time, that the channel is occupied by a crossing ion divided by the flux, i.e.,

Mean passage time

$$= \frac{\text{Fraction of time occupied by crossing ion}}{\text{Flux (ions/s)}}. \quad (1)$$

The flux for the above situation is given by a trivial extension of Levitt (1986; Eq. 2A):

$$J = P_0 C e^{\psi_1}/H(1), \quad (2)$$

where J is the flux (ions/s), P_0 is the fraction of time the channel is empty, C is the bath concentration (ions/m^3), ψ_1 is the transmembrane potential in dimensionless units, and $H(1)$ is the resistance to flow across the channel, defined by

$$H(1) = \int_0^L e^{\psi(x)}\mathrm{d}x/(DA),$$

where D is the diffusion coefficient (M^2/s), A is the cross-sectional area of the channel (M^2), L is the length of the channel (m), and $\psi(x)$ is the dimensionless potential as a function of position within the channel.

The fraction of time the channel is occupied by crossing ions is just the channel cross-sectional area multiplied by the convolution integral over the channel length of the concentration in the channel and the probability that an ion at position x will leave the channel at the end opposite of which entered, i.e.,

Fraction of time occupied by crossing ion

$$= A \int_0^L \mathrm{d}x C(x) P(x), \quad (3)$$

where $C(x)$ is the concentration in the channel averaged over time as a function of position in the channel, and $P(x)$ is the probability that an ion at x will ultimately leave the channel at $x = L$ rather than $x = 0$, i.e., will pass through the channel.

From Levitt (1986), the expression for $C(x)$ is just:

$$C(x) = \{e^{-\psi(x)} P_0 C e^{\psi_1}[H(1) - H(x)]\}/H(1), \quad (4)$$

where $H(x)$ is just the resistance integral evaluated up to the position x in the channel rather than all the way across the membrane.

From Gardiner (1985), Eqs. 5.2.189 and 5.2.190,

$$P(x) = \int_0^x \mathrm{d}x e^{\psi(x)} \bigg/ \int_0^L \mathrm{d}x e^{\psi(x)} = H(x)/H(1). \tag{5}$$

Combining Eqs. 1–5 gives:

$$\text{Mean passage time}$$
$$= A \int_0^L \mathrm{d}x e^{-\psi(x}[H(1) - H(x)]H(x\,)/H(1). \tag{6}$$

Eq. 6 gives the mean passage time entirely in terms of notation introduced in Levitt (1986).

Another quantity of interest that can be calculated by extending the Levitt theory is the maximum possible net flux through the channel, which occurs when ions are present at a very high concentration on one side of the channel and are absent on the other side. Formally this is represented by dividing the flux in Eq. 2 by the fraction of time that the channel is occupied by ions, $(1 - P_0)$, i.e.,

$$J_{\max} = P_0 C e^{\psi_1}/[(1 - P_0)H(1)], \tag{7}$$

where $J_{\max}$ is the maximum possible net flux (ions/s) through the channel at a voltage ψ_1.

From the definition of P_0 it is clear that

$$1 - P_0 = A \int_0^L \mathrm{d}x C(x) \mathrm{d}x, \tag{8}$$

where $C(x)$ is as given in Eq. 4.

Substituting Eq. 4 in 8 and doing a little algebra,

$$P_0/(1 - P_0)$$
$$= H(1) \bigg/ \left\{ A C e^{\psi_1} \int_0^L \mathrm{d}x e^{-\psi(x)}[H(1) - H(x)] \right\}. \tag{9}$$

Combining Eqs. 9 and 7 yields

$$J_{\max} = \left\{ A \int_0^L \mathrm{d}x e^{-\psi(x)}[H(1) - H(x)] \right\}^{-1}. \tag{10}$$

Eq. 10 represents the maximum flux possible through a one-ion channel as a function of the energy profile within the channel.

Comparing Eqs. 10 and 6, we can calculate the relative time the channel is occupied by ions that go through the channel as opposed to the time that it is occupied by ions that ultimately leave the same end they go through. In particular, the fraction of time that the occupied channel contains ions that go through the channel is given by multiplying the RHSs of Eqs. 6 and 10:

$$\frac{\text{Time channel is occupied by ions going through}}{\text{Total time channel is occupied}}$$
$$= \text{Mean passage time} \cdot J_{\max}$$
$$= \frac{\int_0^L \mathrm{d}x e^{-\psi(x)}\,[H(1) - H(x)]H(x)}{H(1) \int_0^L \mathrm{d}x e^{-\psi(x)}\,[H(1) - H(x)]}. \tag{11}$$

$$\frac{\text{Time channel is occupied by ions that ``back out'' entry side}}{\text{Total time channel is occupied}}$$
$$= \frac{\int_0^L \mathrm{d}x e^{-\psi(x)}\,[H(1) - H(x)]^2}{H(1) \int_0^L \mathrm{d}x e^{-\psi(x)}\,[H(1) - H(x)]}. \tag{12}$$

It is of interest to extend Eqs. 11 and 12 to the situation in which there are bathing ions on both sides of the membrane. To see how to do this, let us define variables O_L, O_R, and E as the fraction of time the channel is occupied by ions entering from the left, occupied by ions entering from the right, and empty, respectively. Then we can write immediately:

$$O_L = (1 - P_{0,1})\,(1 - O_R) = (1 - P_{0,L})\,(E + O_L) \tag{13}$$

$$O_R = (1 - P_{0,R})\,(1 - O_L) = (1 - P_{0,R})\,(E + O_R) \tag{14}$$

$$E + O_R + O_L = 1, \tag{15}$$

where $P_{0,R}$ and $P_{0,L}$ are the fractions of time the channel would be empty if all the ions were on the right- and left-hand side of the channel, respectively. Solving Eqs. 13–15 for O_R and O_L and then dividing the expressions yields

$$O_L/O_R = [(1 - P_{0,L})P_{0,R}]/[(1 - P_{0,R})P_{0,L}]$$
$$= C_L K_{D,R}/(C_R K_{D,L}), \tag{16}$$

where C_L,C_R are the ionic concentrations on the left- and right-hand sides of the membrane, respectively. $K_{D,L}$ and $K_{D,R}$ are the dissociation constants that would be obtained if all ions were on the left- and right-hand sides of the membrane, respectively.

Eqs. 11, 12, and 16 can be used to calculate the relative amounts of time the channel is occupied by permeant and blocking ions from both sides of the membrane. Eq. 16 gives the relative amounts of time occupied by ions entering from the two sides, whereas Eqs. 11 and 12 give, for ions entering from one side, the relative amounts of time occupied by blocking and permeant ions.

Later in the paper, numerical results from Eqs. 6, 11, 12, and 16 will be presented. The evaluation of integrals in Eqs. 6, 11, and 12 is by "brute force," with the channel length divided into segments of 0.01 Å and the integrals evaluated by the linear method.

Stochastic Theory

We also calculated fluxes according to a Brownian dynamics method, as described in Cooper et al. (1985). In all of the computations reported in this paper, the assumption was made that only one ion could be in the channel at one time. This is thought to be a reasonable approximation for the case of sodium permeation of gramicidin channels since the conductance vs. concentration curves for NA$^+$ current obey a Michaelis–Menten relationship quite closely (Andersen, 1984) and the Ussing flux ratio exponent is found to be quite close to one (Finkelstein and Andersen, 1981). (Some have argued that a second Na$^+$ can enter the gramicidin channel [Hladky and Haydon, 1984], but if this occurs only occasionally, it will not significantly affect our analysis.)

To calculate the entry of ions into the ends of the channel, the "entrance tube," as described in Cooper et al. (1985), was used in a slightly modified form. The modifications relate to the requirement for detailed balance as the ions move across the interface between the bath and the channel interior. The formal mathematical statements for detailed balance are available in treatises on stochastic theory (i.e., Gardiner, 1985; Risken, 1984). Conceptually, detailed balance simply means that one can have no "Maxwell's demon" at the interface, sorting ions out and and ascribing different rules of motion for them according to the velocities with which they impinge on the interface. Although the principle is clear, the reasoning required to make a particular calculational method conform to that principle is sometimes subtle. We therefore adopted a "computer experimentalist's" approach to algorithms for ion entry, i.e., we tried various algorithms that might be reasonable and tested the results against relationships we know from analytical theory to be true. One criterion for validity of entrance algorithms is that they give zero net flux and the Boltzmann distribution of ions within the membrane whenever the two sides of the membrane are at thermodynamic equilibrium. For the one-ion approximation, the ratio of unidirectional fluxes away from equilibrium should be given by the Ussing relationship. The Levitt theory gives us an additional criterion for assessing the validity of our algorithms. Using it, for the special case of the single-ion channel, we can use the magnitudes of net fluxes and the ionic distributions away from equilibrium as criteria for the validity of possible entrance algorithms.

Of those we tried, the entrance algorithm that was found to give the best results ("best" defined as closest agreement to the Levitt analytical theory) was simply to put the following two modifications on the "entrance tube" as described in Cooper et al. (1985): (*a*) The mobility in the entrance tube is the same as the mobility in the channel. This condition is in lieu of constructing an explicit representation for the bath just outside the channel that includes the change in mobility as an ion moves from the bulk environment to the channel environment. (The full equations for Brownian dynamics in a viscous medium include a mobility gradient term [Ermak and McCammon, 1978, Eq. 15].) (*b*) The ion movement in the entrance tube is biased by one-half of the electric field that exists just inside the channel, i.e., the arithmetic mean between the field just outside the channel and just inside the channel.

It might seem at first to the reader that these conditions are contrary to intuitive sense. One's first instinct might be to make the mobility in the entrance tube equal to the mobility in the bath and the electric field for an ion trying to enter the electric field just outside the channel mouth. The misconception upon which this intuition is based is that the entrance tube is a representation of the bath just outside the channel. In fact, it is not; rather it is a computational device for presenting ions at the mouth of the channel at just such a frequency and with just such a depth of penetration as is appropriate for a bath that is homogeneous in concentration right up to the mouth of the channel. It is equivalent to the assumption in the Levitt (1986) theory that the ions are in equilibrium at the ends of the channel.

All computations presented were done on a Digital Equipment Corp. (Marlboro, MA) MicroVax II programmed in FORTRAN. Unless otherwise specified, the time-step used in all the Brownian dynamics calculations is 1 ps. The numerical integration method used is simple first-order, i.e., the electrical force on the particle during each time step is assumed to be that appropriate to the position at the beginning of the time step. The dimensions of the aqueous region inside the channel are assumed to be a diameter of 4 Å and a length of 26 Å.

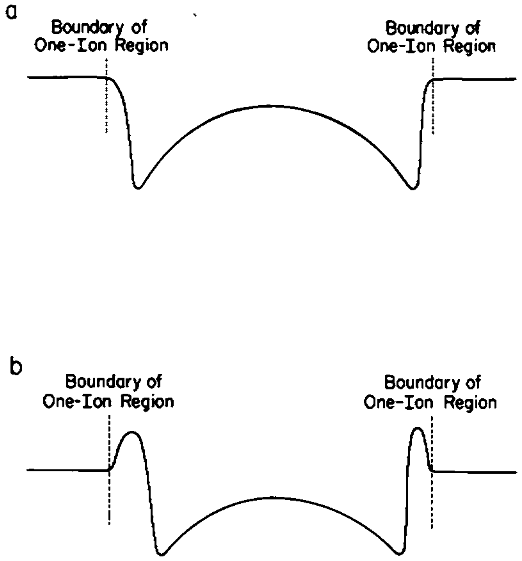

FIGURE 1 Postulated shapes of potential profile for ion translocation in channel due to interactions among ion, water, channel, and environment. Transmembrane potential is added to this potential to give total potential. *a* shows potential profile form assumed for computations presented in this paper. The potential drops at the surface because of changes in the hydration environment and specific interactions with the channel. The central barrier is due to "image forces" associated with moving the ion from the high-dielectric aqueous solution into a narrow channel embedded in a low dielectric lipid membrane. *b* shows the same profile with the addition of a potential barrier at the surface due to the energetic cost of stripping bulk water of hydration from the ion as it enters the channel. This type of profile was not found to give good fits to current–voltage data.

RESULTS

We decided to fit the theories to data presented by Barrett-Russell et al. (1986), which includes data for both normal gramicidin and also gramicidins with altered side chains. First, we considered a profile of the general form of Fig. 1 *a*.

We imagined, arbitrarily, that the potential minima were at a distance of 1 Å from the end of the tunnel. The central barrier was postulated to have the shape calculated by Jordan (1982, 1983; Professor Jordan kindly sent us numbers computed from his theory for a channel of "electrostatic diameter" of 5 Å). In units of kT at 300°K, those values at various distances from the end of the channel are: 1 Å, 2.875 kT; 2 Å, 4.046 kT; 3 Å, 5.154 kT; 4 Å, 6.161 kT; 5 Å, 7.016 kT; 6 Å, 7.739 kT; 7 Å, 8.374 kT; 8 Å, 8.847 kT; 9 Å, 9247 kT; 10 Å, 9.555 kT; 11 Å, 9.771 kT; 12 Å, 9.899 kT; 13 Å, 9.943 kT. We interpolate linearly between the above points. Since we have arbitrarily set the minimum of the potential well at 1 Å from the end of the channel, where the potential is determined as some fitting factor times the difference between the potential at a given position and the potential at 1 Å. It should be noted that this procedure completely ignores the part of the image barrier that extends past the mouth of the channel into the bath. The consequences of neglecting this will be discussed in the Discussion section. Given these constraints, the behavior of the channel is determined by just three numbers: (*a*) the depth of the potential wells, (*b*) the height of the central barrier, and (*c*) the mobility (i.e., diffusion coefficient) of the ion–water complex within the channel. A computer program was written to calculate the Levitt theory currents as a function of these three numbers. The currents predicted by each trial potential were compared with the Barrett-Russell et al. (1986) data. The criterion for best fit in each case was to minimize the sum of the squares of the fractional difference between the calculated conductances and the conductances reported by Barrett-Russell et al. (1986).

Fig. 2 *a* shows the variations in the "best-fit" potentials for data from Barrett-Russell et al. (1986) on conductance of gramicidin with more polar side chains substituted for valine at the number 1 location. Table I gives these variations in tabular form. In this case, the data were the low-voltage concentration vs. conductance values (Barrett-Russell et al., 1986; Table II) and the voltage dependence of the conductance (Barrett-Russell et al., 1986; Fig. 5). Fig. 2, *b* and *c* show the degree of goodness of fit for the data from Barrett-Russell et al. (1986) used to fit the potential function parameters.

The variations in Table I show a definite progression in the potential function parameters as the valine at the No. 1 location is fluorinated. The single fluorination appears to

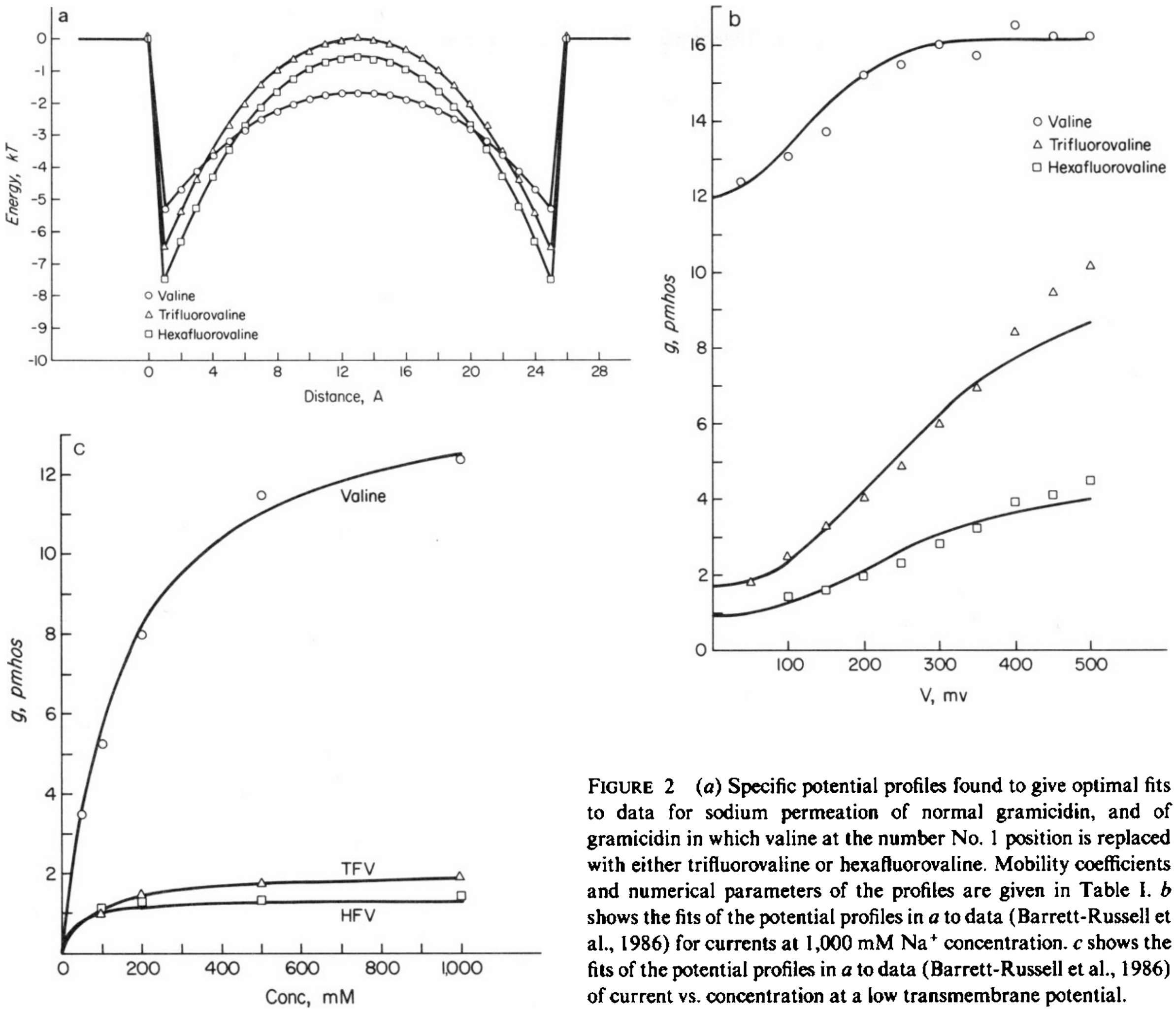

FIGURE 2 (*a*) Specific potential profiles found to give optimal fits to data for sodium permeation of normal gramicidin, and of gramicidin in which valine at the number No. 1 position is replaced with either trifluorovaline or hexafluorovaline. Mobility coefficients and numerical parameters of the profiles are given in Table I. *b* shows the fits of the potential profiles in *a* to data (Barrett-Russell et al., 1986) for currents at 1,000 mM Na^+ concentration. *c* shows the fits of the potential profiles in *a* to data (Barrett-Russell et al., 1986) of current vs. concentration at a low transmembrane potential.

have the effect of reducing the mobility, increasing the depth of the potential wells near the surface, and increasing the height of the central barrier. The second fluorination apparently increases these effects. The possible significance of these results will be discussed in the Discussion section.

We also explored the question of whether there should be a barrier to ion entry at the mouth of the channel. One line of physical reasoning for this is that there should be a hydration energy barrier associated with stripping waters of hydration from an ion so that it can enter the channel. To this end, we added one degree of freedom to the parameters defining the potential profile. We used as variable parameters the potential 1 and 2 Å into the channel (i.e., into the one-ion region) and, as before, the height of the central barrier. We expected that we might find a potential maximum at 1 Å (corresponding to a hydration barrier) and a potential minimum at 2 Å, corresponding to the sketch in Fig. 1 *b*. In fact, as we looked through the parameter space, we found neither relative error minima nor reasonably good fits to the data for any set of parameters for potentials of the form of Fig. 1 *b*. All relative minima of difference between data and computation either had a negative potential at the 1-Å position or a negative central barrier height (i.e., an energy well rather than barrier in the middle of the channel). Because of the firm physical basis for the existence of the central barrier (Parsegian, 1969; Levitt, 1978; Jordan, 1982), we consider such potential profiles to be implausible. We conclude that the data are not well fit by a potential of the form of Fig. 1 *b*. The significance of this negative result is considered in the Discussion section.

A comparison was made between the analytical results of the Levitt theory and Brownian dynamics simulations for the same potentials and mobilities.

For both the Brownian dynamics and also the analytical calculations, the results at each voltage can be character-

TABLE I

Amino acid at position No. 1	Diffusion coefficient	Well depth	Barrier height
	m^2/s	*kT*	*kT*
Valine	4.8×10^{-11}	5.3	3.6
Trifluorovaline	4.1×10^{-11}	6.5	6.5
Hexafluorovaline	3.0×10^{-11}	7.5	6.9

TABLE II
EFFECT OF DIFFUSION PARAMETERS ON CHANNEL CHARACTERISTICS

Parameter	Magnitude of G_{max}	Voltage dependence of G_{max}	Magnitude of K_D	Voltage dependence of K_D
Diffusion coefficient	Directly proportional	No effect	No effect	No effect
Well depth	Reduced by increasing well depth	Reduced by increasing well depth	Reduced by increasing well depth	Increased by increasing well depth
Barrier height	Reduced by increasing barrier height	Increased by increasing barrier height	Increased by increasing barrier height	Reduced by increasing barrier height

ized by the Michaelis–Menten parameters, i.e., a dissociation constant and a maximum conductance. Thus, we present the comparison between the K_d and G_{max} vs. V curves for analytical and Brownian dynamics calculations in Fig 3. Brownian dynamics runs were done at 1,000 mM concentration. The computer program keeps track of the fraction of time that the channel is occupied. Then the dissociation constant is given by

$$K_D = P_0C/(1 - P_0), \tag{17}$$

where C is the bath concentration in the simulation and P_0 is the fraction of time the channel is empty.

The maximum conductance is just given by

$$g_{max} = g/(1 - P_0). \tag{18}$$

The comparison of Brownian dynamics and Levitt theory results are given in Fig. 3 for the potential permeation parameters in Table I. Each point represents a 20-μs Brownian dynamics simulation. Our single-ion Brownian dynamics simulation runs ~4 μs/h of computer time on the MicroVax II, so each point represents 5 h of MicroVax II time. (By way of reference, 5 h on the MicroVax II is about the same amount of time on the VAX 780, ~100 h on an IBM PC-AT with high-speed math co-processor, ~24 min on the CYBER 175 mainframe at the University of Illinois, and <1 min on the CRAY XMP at the National Center for Supercomputing Applications on the University of Illinois campus.)

Fig. 3 *a* shows the K_d, or concentration at which the conductance is half-maximal, for the analytical theory and Brownian dynamics computations. Fig. 3 *b* shows the maximal conductance for the analytical theory and the Brownian dynamics computations. Fig. 3 *c* shows another test of the validity of the Brownian dynamics computations. For the one-ion assumption, it is necessary that the unidirectional fluxes obey the Ussing flux ratio (Hille, 1984). In Fig. 3 *c*, the points show the computed flux ratio as a function of voltage compared with the Ussing ratio. The numbers of ions were summed from computer runs at each of the three permeation parameters listed in Table I. All of the computations in Fig. 3 exhibit random fluctuations, since the simulated ion movements are driven by a random number generator.

In summary, Fig. 3 addresses the question of the accuracy of the Brownian dynamics computations by comparing them with analytical diffusion theory. Obviously the Brownian dynamics results are "noisy." Longer computer runs would be necessary to reduce the scatter in the Brownian dynamics results. It appears possible that the Brownian dynamics program systematically underestimates G_{max} at high voltage and K_D at low voltage. If so, this is presumably due to the relatively crude force-averaging we do over the trajectory of ions entering the channel. We do not think the errors are so large as to modify any of the conclusions we draw in this paper.

Fig. 4 shows the internal dynamics of the occupied channel, from the same Brownian dynamics computations as Fig. 3, and from the analytical theory (Eqs. 11, 12, and 16). In Fig. 4, *a–c* are shown the variance with voltage of the importance of different modes of ion movement, for each of the sets of permeation parameters of Table I. In particular, are plotted the fraction of time the channel is occupied by ions that enter from the high-voltage and leave the same side, by ions that enter the low-voltage side and leave from the low-voltage side, by ions that go through the channel from the high-voltage to the low-voltage side, and by ions that manage to buck the electric field and go through from the low-voltage to the high-voltage side. At low voltages, it is seen that most of the time the channel is occupied by ions that leave the same end they entered, and thus make no contribution to the current. At high voltages, the situation changes. Ions coming in from the high voltage side either leave very rapidly or else go on through the membrane and contribute to the current. In contrast, there remains, even at very high voltages, a significant blocking effect due to ions that enter from the low voltage side and remain in the channel for a significant period of time.

Fig. 4 *d* shows the mean passage time for ions to cross the channel. The symbols are from the Brownian dynamics computations and the solid lines are from the analytical theory (Eq. 6). The passage time varies with barrier height and mobility in ways one would expect, increasing as barrier height is increased and mobility declines. It declines significantly at high voltages. Note that the

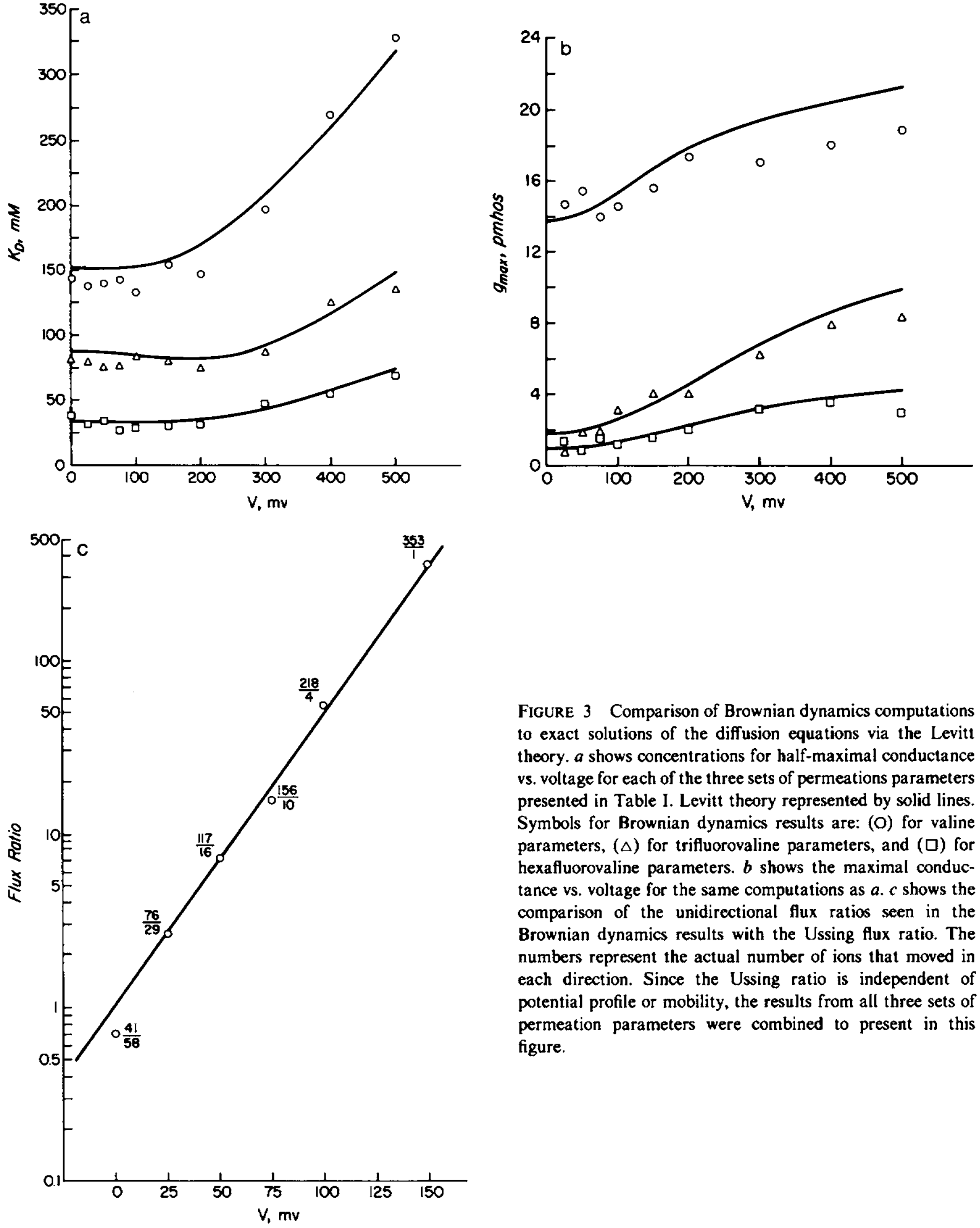

FIGURE 3 Comparison of Brownian dynamics computations to exact solutions of the diffusion equations via the Levitt theory. *a* shows concentrations for half-maximal conductance vs. voltage for each of the three sets of permeations parameters presented in Table I. Levitt theory represented by solid lines. Symbols for Brownian dynamics results are: (○) for valine parameters, (△) for trifluorovaline parameters, and (□) for hexafluorovaline parameters. *b* shows the maximal conductance vs. voltage for the same computations as *a*. *c* shows the comparison of the unidirectional flux ratios seen in the Brownian dynamics results with the Ussing flux ratio. The numbers represent the actual number of ions that moved in each direction. Since the Ussing ratio is independent of potential profile or mobility, the results from all three sets of permeation parameters were combined to present in this figure.

numbers above the symbols on Fig. 4 *d* represent the number of ions whose passage times were averaged for that symbol. When only one or a few ions constitute the sample, the mean passage times tend to have quite a large deviation from the analytical theory. There also appears to be a systematic difference between the analytical solution and the Brownian dynamics simulations, in that the BD passage times are somewhat too long. An interesting feature of the analytical mean passage times is that they are precisely symmetrical about zero voltage. In the situation for which we present the calculations, all of the asymmetry in going from high to low voltage lies in the probability of the passage event to occur; none of the asymmetry is in the duration of those passages that do occur.

Interestingly, one number we cannot calculate from these methods is how many ions enter the end of the

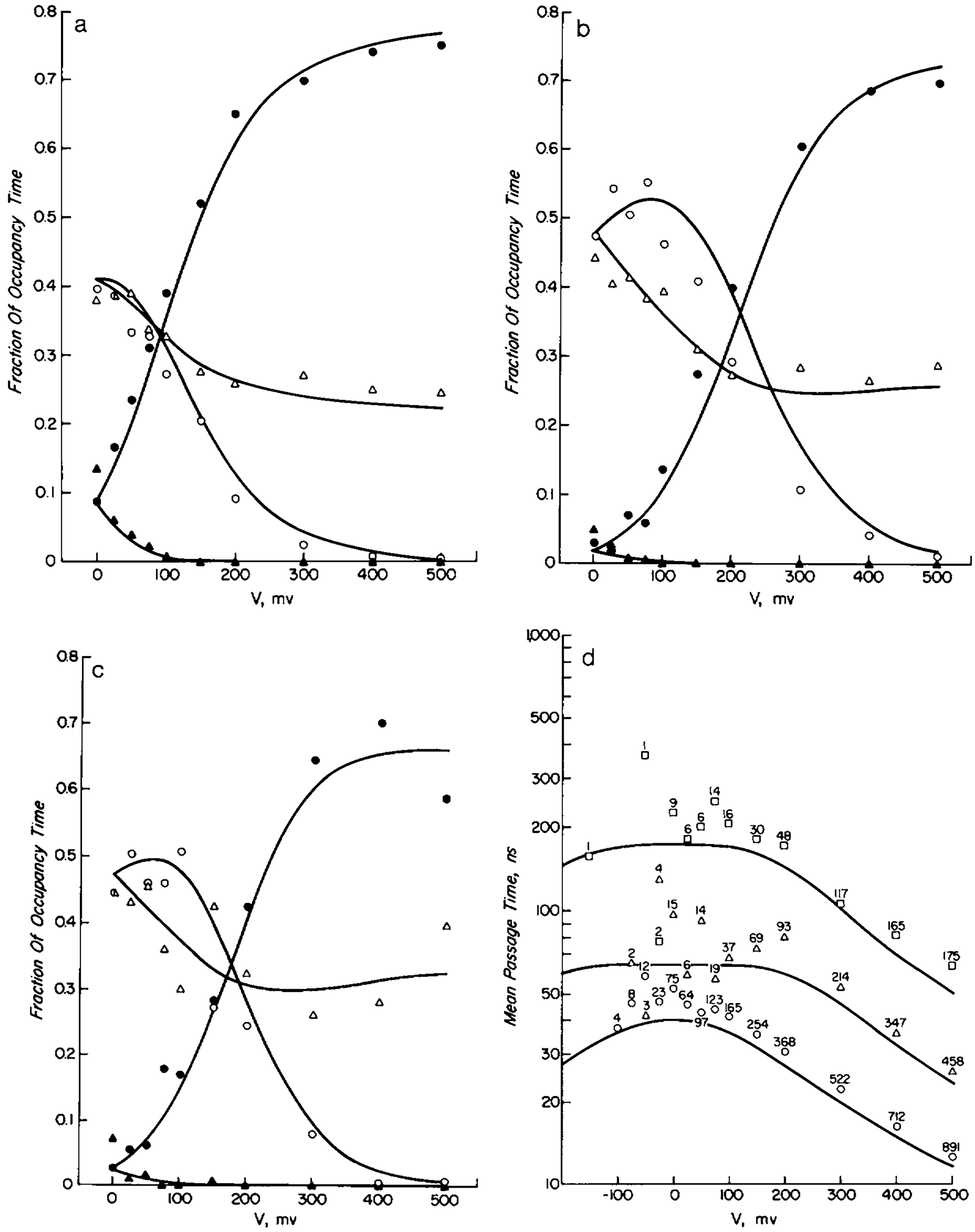

FIGURE 4 (*a–c*) The internal dynamics of an occupied channel, as seen by Brownian dynamics. Symbols represents fraction of time channel is occupied by ion that: (○) enters and leaves from high-voltage end; (△) enters and leaves from low-voltage end; (●) enters from high-voltage end and leaves from low-voltage end; (▲) enters from low-voltage end and leaves from high-voltage end. Solid lines are analytical solutions of Eqs. 11, 12, and 16. *a* shows results for valine parameters, *b* shows results for trifluorovaline parameters, and *c* shows results for hexafluorovaline parameters. *d* shows mean passage time for ions that cross the membrane vs. the difference between the entrance and exit voltages. Symbols are: (○) for valine parameters, (△) for trifluorovaline parameters, and (□) for hexafluorovaline parameters. Solid line is analytical solution for Eq. 6.

channel. To see this, let us derive an expression for how many ions per unit time penetrate at least a distance x, and designate this quantity $J(x)$. Then the rate at which ions impinge on the end of the channel would be given by $J(0)$.

To calculate $J(x)$, note that in Fig. 5,

$$J = J(x) - J_B(x), \tag{19}$$

where $J_B(x)$ is the rate at which ions ultimately return out the side of entry after reaching x. From the same reasoning as in Eq. 5

$$J/J_B(x) = H(x)/[H(1) - H(x)]. \tag{20}$$

Eliminating $J_B(x)$ between Eqs. 19 and 20 yields

$$J(x) = JH(1)/H(x). \tag{21}$$

As $x \rightarrow 0$, the RHS of Eq. 21 $\rightarrow \infty$. This result is related to the infinite speed paradox of Brownian motion, in which the apparent speed of a Brownian particle becomes infinite as the time and distance scale over which the particle's behavior is observed becomes infinitesimal. Of course the particle's speed doesn't really become infinite. What happens is that the Brownian motion description fails under these conditions, because the assumption underlying the description is that the motion is averaged over many collisions, so that the particle is everywhere assumed to be at a terminal velocity. This apparent paradox and its resolution is well explained in Einstein (1926). We have previously pointed out (Cooper et al., 1985) that the entry rate of ions predicted by the entrance tube in the BD channel computations increases without limit as the time step is decreased, as a manifestation of this apparent paradox.

Since the diffusion theory corresponds to the Brownian motion theory, it fails under the same circumstances. In the electrodiffusion theory, the assumption of being at terminal velocity at all times enters through the direct proportionality, in the Nernst–Planck equation, between flux and concentration and voltage gradients.

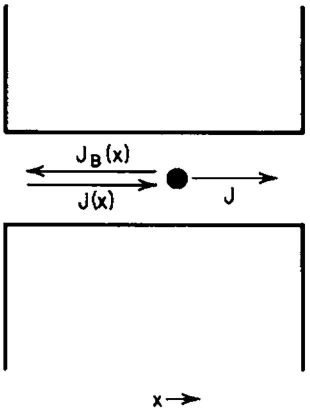

FIGURE 5 Schematic representation of flux of ions part way through the channel. Eqs. 19–21 in text show how many ions penetrate to what depth in the channel.

It must be stressed that these limitations in no way imply that the Brownian dynamics and electrodiffusion theories are wrong, but only that their domain of application does not extend into arbitrarily small time and distance scales.

The curves in Figs. 3 and 4 provide an explanation for the shapes of current–voltage curves. At high voltages, the passage time goes down and G_{max}, which increases at high voltage, is mainly limited by how rapidly ions can cross the membrane. However, the more rapid passage of ions crossing the membrane reduces the fraction of time the membrane is occupied, thus causing K_d also to rise with voltage. The increase in K_d and the increase in G_{max} tend to balance each other, the former reducing the conductance and the latter increasing the conductance. Which one dominates depends on the concentration. At high concentrations, the increase in G_{max} dominates and the I-V curve tends to be superlinear. At low concentrations, the K_d effect dominates and the I-V curve tends to be sublinear. This effect is demonstrated in Fig. 6, which is a family of I-V curves calculated by the Levitt theory from the valine parameters of Table I. These curves are very similar to those reported experimentally for sodium permeation of valine gramicidin by Andersen and Procopio (1980).

When seen experimentally, the sublinear low-concentration I-V curves such as in Fig. 6 have been attributed to limitations in access to the channel from the aqueous phase. That general interpretation can still be applied to our calculations with the proviso that, since we assume the access resistance to be negligible, the access limitation is the driving force (or chemical potential), i.e., the low concentration itself.

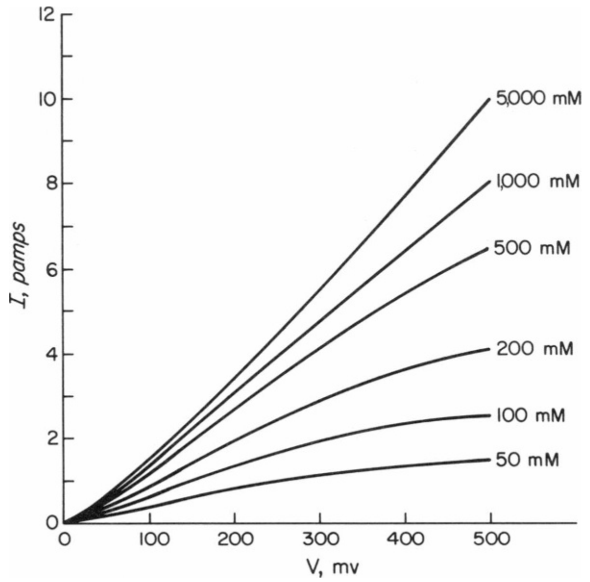

FIGURE 6 Family of current–voltage curves predicted from valine parameters of Table I, using Levitt theory. Note characteristic superlinearity at high concentrations and sublinearity at low concentrations. These curves are a fairly good fit to data presented by Andersen and Procopio (1980).

DISCUSSION

The close agreement between the Levitt theory results and the Brownian dynamics results suggests that both calculational methods are providing essentially correct descriptions of the single-ion channel.

An interesting result of our attempts to fit potential profiles to I-V curves is that we could find no evidence of a potential maximum near the channel mouth. This finding is at first puzzling, since it has commonly been thought there should be such a maximum due to the energy required to strip waters of hydration from the ion as it enters the channel. However, the maximum may be eliminated by the effects of water alignment in the channel as an ion approaches the mouth. The phenomenon of water alignment has been seen in the work of Mackay et al. (1984) and Etchebest and Pullman (1986) who have done explicit calculations of the energetics of ion entry and passage. Fig. 7 shows a schematic representation of how this alignment might facilitate ion entry. As the ion approaches the channel, the channel waters align by electrostatic forces in such a way as to attract the ion into the channel. This may compensate for the energy required to remove waters from the hydration shell sufficiently to eliminate a potential maximum on entry. It has also been postulated that polar groups in the channel structure near the mouth would bind strongly to cations (but not anions) as they enter the channel (Sung and Jordan, 1986), which would tend to eliminate a net barrier due to energy of dehydration. Another possibility is that there is a barrier just outside the channel, in the bulk solution. Exploring that possibility is outside the scope of the calculations presented in this paper, and will be dealt with in future work.

Professor Peter Wolynes (personal communication) has suggested to us that water alignment toward an ion already in the channel might also account for the relative difficulty of getting a second ion into the channel, since in this instance the waters are aligned in such a way as to repel the second ion's entry (Fig. 7 *b*).

It is important to assess the significance of the apparent effects of fluorinating the No. 1 valine. How sensitive is the analysis to the adjustable parameters? To answer this question we did the following sensitivity analysis: We fixed one of the three variable parameters and varied the other two to find the best fit to the data set under question, and also to find out how good that fit was. By varying the value of the "fixed" parameter and plotting the deviation between theory and experiment versus that value, we can see how sensitively that parameter is determined by the calculations we have done. The results of these calculations are shown in Fig. 8. The error function is the mean square of the fractional difference between the theoretical and experimental conductance.

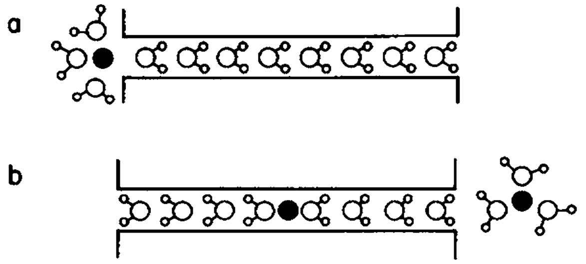

FIGURE 7 *a* is a schematic representation of mechanism whereby alignment of waters in channel may attract ion as it approaches channel, eliminating potential maximum associated with bulk waters of hydration. *b* is a schematic representation showing how alignment of waters towards ion already in channel reduces likelihood of second ion entry.

One result from Fig. 8 is that there appear to be clearly better fits to the data for valine gramicidin than for trifluorovaline (TFV) and hexafluorovaline (HFV). Whether this is systematic or because the HFV and TFV data are noisier (since the currents are smaller magnitude) we don't know. Looking at the particular figures, the results of Fig. 8 *a* on the diffusion coefficient cast some doubt on the extent to which fluorinating the valine does in fact reduce the diffusion coefficient. There seems little doubt that the diffusion coefficient for Na^+ in valine gramicidin, based on the data we are fitting, should be close to 5×10^{-11} m^2/s, but the error functions for TFV and HFV are shallow enough that it is not clear whether the diffusion coefficient for those cases is much changed from that for valine.

Dani and Levitt (1981) estimated the diffusion coefficient of Na^+ in gramicidin at 1.43×10^{-10} m^2/s, about three times our value. The reason for the discrepancy is that the Dani and Levitt value was calculated with an assumption of 34 pmho for the low voltage single-channel maximum conductance (Neher et al., 1978), whereas the data set from which we work (Barrett-Russell et al., 1986) indicates a value of only 13.8 pmho. The free energy profile (Fig. 2 *a*) determines the ratio of the single-channel conductance to the ion diffusion coefficient. In the Dani and Levitt study that ratio was 0.238 mho-s/m^2, whereas our potential profile gives a value of 0.288 mho-s/m^2, which is in reasonable agreement. This agreement means that the free energy profile in Fig. 2 *a* is consistent with the Dani and Levitt analysis, as well as with the Barrett-Russell et al. (1986) data. There remains, however, an unresolved discrepancy between the magnitudes of the single-channel conductances experimentally observed, which in turn leads to a difference in calculated values for the Na^+ diffusion coefficient.

For the barrier heights (Fig. 8 *b*) it is clear that the barrier height is increased by fluorinating the valine (although less clear that it is increased further by going from TFV to HFV). Fig. 8 *c* indicates a clear progression in depth of the well near the end of the channel as one goes from valine to TFV to HFV.

The careful reader may notice a couple of small differences between the positions of the minima in Fig. 8 and the best fit values of Table I. The values in Table I came from

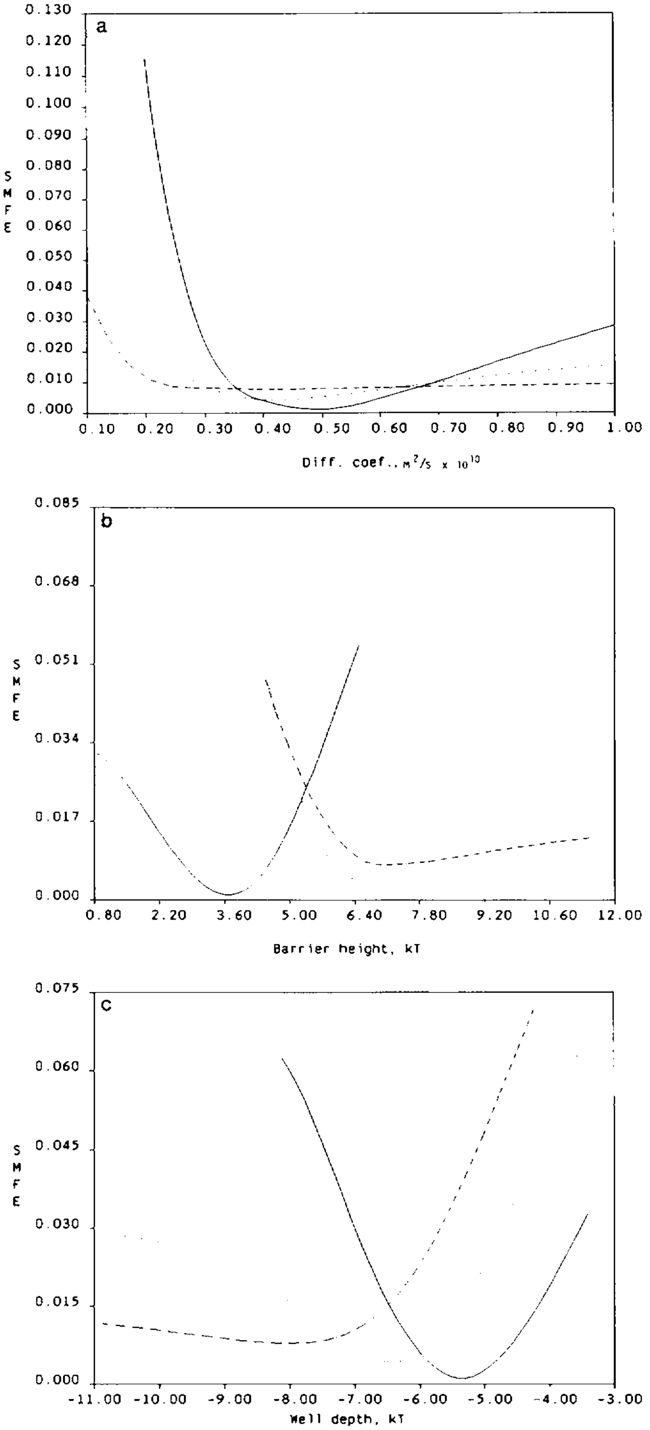

FIGURE 8 Calculations of sensitivity of curve-fitting to variables in permeation theory. Shows square of mean fractional error per data point of best fit vs. permeation variable. *a* is diffusion coefficient, *b* is height of central barrier, *c* is depth of potential well near channel mouth. ———, valine; ---, TFV; —·—·, HFV.

a program that used a straight-line interpolation between the values of potential at various positions, whereas the calculations of Fig. 8 used a cubic spline interpolation.

We can also represent in compact form qualitatively how various properties of the solutions depend on the numerical parameters put into the equations, based on our experience in the parameter fitting. These dependences are shown in Table II. We consider four basic quantities that define the channel: magnitude and voltage dependence of g_{max} and K_D. The diffusion coefficient affects only one of these; g_{max} is directly proportional to D. In contrast, increasing either well depth or the barrier height reduces g_{max}. For all of the other three quantities, increasing well depth and barrier height have opposing effects. Increasing the well depth reduces the extent to which g_{max} depends on voltage, reduces the magnitude of K_D, and increases the extent to which K_D depends on voltage. Increasing the barrier height increases the extent to which g_{max} depends on voltage, increases the magnitude of K_D, and reduces the extent to which K_D depends on voltage. Fitting the diffusion theory to the data is thus seen to be a balancing of these various dependencies.

Note that we have just three adjustable parameters and we are fitting four different experimentally determined quantities, so there are more experimental quantities to fit than there are adjustable parameters in the theory.

There are reasonable explanations for some of the changes in the permeation parameters associated with fluorinating the valine at the No. 1 location (as shown in Table I). The fluorinated valines are more polar than normal valine. The reduced mobility coefficients, if indeed they exist, might be reasonable as a consequence of the greater attraction between the polar amino acids and the polar channel waters. (It should be remembered that the entity that moves in the channel is not a bare ion but a chain of waters plus the ion [Mackay et al., 1984; Etchebest and Pullman, 1986].) Barrett-Russell et al. (1986) have suggested several possible explanations for how fluorinating the valine might increase the central barrier. While reasonable, all explanations should be regarded as tentative pending more extensive theory, such as theories including potentials extending into the bath, and molecular dynamics calculations that would actually calculate directly the potential associated with fluorinating the valine.

This paper explores what simple potential functions for sodium in gramicidin are consistent with data on ion permeation. Another way of theorizing about the potential function is to compute it from detailed molecular dynamics calculations. Our understanding of permeation might be judged complete to the extent that we can find a consistency between the results of molecular dynamics calculations and the results of computations of the sort presented in this paper. Several molecular dynamics studies including water–channel–ion interactions have been published that give explicit numbers for potentials inside the channel that govern Na^+ permeation of gramicidin (Lee and Jordan, 1984; Kim et al., 1985; Etchebest and Pullman, 1986). All of these studies concur that the energy of the system is reduced as the Na ion enters the channel, so that the Na ion tends to partition into the channel. This is in agreement with our findings. However, the magnitude of the energy reduction and the form of the intrachannel potential profile vary widely from study to study, as the studies make somewhat different assumptions about channel–ion–water interactions and the environment of the system. The results in closest agreement to what we find to be consistent with the current–voltage data are those of Lee and Jordan (1984). As calculational methods become more refined and complete, we can anticipate that a consensus will emerge on the correct potential functions for the permeation process. It should be noted that on the level of the molecular dynamics descriptions the distinction between the potential function and the diffusion coefficient, two distinct parameters in diffusion theory, is not so clear. The difference is one of degree rather than kind. What we call the local diffusion coefficient is determined by forces that vary very rapidly and over a short distance, giving rise to movement that on the Brownian time scale is rapidly fluctuating. What we call the potential is determined by forces that on the Brownian time scale are relatively constant in time, and whose effects may thus be described fairly well as a drift imposed on the rapid and apparently random fluctuations. In the calculations in this paper we have made the implicit assumption that there are no forces that fluctuate with an intermediate characteristic time, between picoseconds and microseconds. There is no a priori reason for assuming this on physical grounds; this assumption is simply implicit in both the Brownian dynamic and analytic realizations of diffusion theory presented in this paper, in which the diffusion coefficient and the potential are two distinct entities in this paper. Thus we can expect the type of theory presented in this paper, while more rigorous and physical than the previous Eyring rate theory, to still be an oversimplification realtive to the actual situation.

Further refinement will be desirable in the future in the types of calculations we present in this chapter. Refinement of the force-averaging algorithm for the trajectories involving ion entry should improve the accuracy of the Brownian dynamics calculations. It will be desirable to expand our methods to take account of potential gradients in the bath outside the channel. The existence of these gradients is inferred from both electrostatic (Jordan, 1982) and interfacial polarization (Andersen, 1983) effects, and will be dealt with in future work.

It will also be desirable to use the diffusion theory and molecular dynamics to explore coupling between ion and water movement. The presence or absence of an ion in the channel can have a number of different effects on water movement. Presumably the image force barrier to the ion, alluded to earlier in the paper, will retard the movement of the ion–water complex from one side of the channel to the other (Finkelstein and Andersen, 1981). In contrast, there should be some barrier to the movement of water itself across a narrow channel through a low-dielectric membrane, since the water is highly polar even though it carries no net charge. We know of no calculation of the magnitude of this effect. In one way the presence of an ion in the channel might facilitate water transport, and that is by making it easier for water to enter the channel. This effect has been seen clearly in molecular dynamics computations (Mackay et al., 1984).

As far as the effect of an ion in the channel on the rapidly varying forces that determine the diffusion coefficient of the ion–water complex, we really don't know from any physical principle how large or in which direction that effect should be. These effects are important and quite beyond the scope of the present paper.

The work should also be extended to the situation of multiple ion occupancy of the channel. It is clear that for single occupancy, the method of choice for theorizing about ion permeation is the analytical Levitt theory, since it is more precise and much less computer intensive than the Brownian dynamics. There also exists an analytical theory for the two-ion case (Levitt, D.G., personal communication) which might also be more efficient than Brownian dynamics for solving that situation. However, the computer time and memory necessary for the analytical theory are much stronger functions of the number of ions in the channel than are the computer requirements for the Brownian dynamics. Based on the present computer requirements for the two techniques, it appears that for more than two ions in the channel, the Brownian dynamics will be the method of choice.

Mr. Peter Gates wrote an early version of the Brownian dynamics program that was debugged and modified to do the computations presented herein. Drs. David Levitt and Roger Koeppe provided copies of papers before publication. Conversations with Drs. Olaf Anderson, Peter Jordan, David Levitt, and Peter Wolynes have been particularly useful.

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