The nature of ion and water barrier crossings in a simulated ion channel

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ABSTRACT Using a combination of techniques, including molecular dynamics, time-correlation analysis, stochastic dynamics, and fitting of continuum diffusion theory to electrophysiological data, a characterization is made of thermally driven sodium, water, and D_2O motion within the gramicidin A channel. Since the channel contents are constrained to move in a single-file fashion, the motion that corresponds to experimentally measurable rates of permeation of the membrane is the motion of the center of mass of the channel contents. We therefore emphasize channel contents center-of-mass motion in our analysis of molecular dynamics computations. The usual free energy calculation techniques would be of questionable validity when applied to such motion. As an alternative to those techniques, we postulate a periodic sinusoidal free energy profile (related to the periodic structure of the helical channel) and deduce the fluid dynamic diffusion coefficient and the height and spacing of the free energy barriers from the form of the mean-square-deviation function, using stochastic computations. The fluid dynamic friction in each case appears similar to that for aqueous solution. However, the diffusive motions are modulated by a spatially periodic free energy profile with a periodicity characteristic of an L-D pair of amino acids in the gramicidin helix, ~1.7 Å in the model we use. The barrier height depends on which substance is moving in the channel, but in each case is several times thermal energy. For barriers of this width and height, the motion is intermediate between the low-friction (transition-state) and high-friction (Brownian) limits. Thus, neither of these formalisms that have been used commonly to describe membrane permeation gives an accurate picture of the underlying physical process (although the Brownian description seems closer to correct). The non-Markovian Langevin equation must be solved to describe properly the statistics of the process. The "channel state of matter" characteristic of the channel contents appears to have some properties typical of the solid and some typical of the liquid state. The magnitude of the local friction and nature of the ion solvation are similar to the liquid state, but the periodicities of structure, free energy, and dynamics are somewhat solid-like. The alignment of water dipoles in the channel bears some resemblance to the orientational ordering of a nematic liquid crystal, but unlike a nematic liquid crystal, the waters have a degree of translational order as well. Thus, the "channel state" is not adequately described by analogy to either the solid or liquid states or to liquid crystals but must be dealt with as its own characteristic type of condensed matter.

INTRODUCTION

Historically, the process of permeation of ions in membrane channels has been described variously as electrodiffusion, as conduction in a fashion analogous to conduction in a wire, and as a "hopping" from site to site, much like an electron or a hole in a semiconductor. This literature is too voluminous to cite reasonably compactly but is reviewed in Hille (1992) and in some theoretical detail in Cooper et al. (1985). Such descriptions as those mentioned above have tended to be analogies rather than truly physical theories. By this we mean they describe permeation by saying what it would be like if it were like something else that we understand well, rather than starting with the physics of an ion channel and deducing from that physics the nature of the permeation process. Until the last few years, this was the best we could do because we did not have reliable detailed structural information on ion channels and thus did not have a foundation on which to construct applications to the membrane permeation problem of modern statistical mechanical theory and computational chemistry techniques. We also have been limited until recent years by relative lack of computational power. This situation has changed with the development of the gramicidin channel as a relatively simple (30 amino acids) model for developing a truly physical theory of permeation, combined with the rapid proliferation of powerful laboratory computers and increased access to powerful mainframes, including supercomputers. The first molecular dynamics simulation of gramicidin was published in 1984 (Mackay et al., 1984). The first few years of work were reviewed by Jordan (1987, 1988*a*). Since then, several groups have studied the dynamics of this system, using molecular dynamics (Jordan, 1988*b,* 1990; Chiu et al., 1989, 1991; Roux and Karplus, 1990, 1991*a, b*) and also diffusion theory and stochastic dynamics (Jakobsson and Chiu, 1988; Chiu and Jakobsson, 1989). These techniques have strengths and weaknesses that are complementary to each other. Molecular dynamics gives the most detailed physical picture of molecular events but is very computer intensive and can only be carried out for very short times (up to a few nanoseconds as a maximum). Stochastic dynamics is less computer intensive and can be carried out for longer times (up to microseconds) but requires one to assume the detailed physics of the system (e.g., the value of diffusion coefficients) rather than have the statistical physics emerge from the computations. Diffusion theory is less computer intensive even than stochastic dynamics but requires one to make assumptions about the statistics of ion–ion interactions in the channel, whereas those statistics arise from the stochastic dynamics. From the above discussion, one can see in outline how a total theory of ion permeation

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can be constructed from a combination of these techniques. Each technique can be used to calculate numbers to insert into the next most detailed description. For example, diffusion coefficients can be calculated from molecular dynamics and in turn can be used as input parameters for stochastic dynamics calculations to do simulations at long enough time scales to compare with experiments. The theme of this article is this "welding together" of computational techniques appropriate for different time scales and levels of physical detail to provide a more complete physical theory than has existed previously for permeation through a membrane channel.

THEORY AND METHODS

The diffusion theory and results cited in this article are given in Jakobsson and Chiu (1987) and Chiu and Jakobsson (1989). Molecular dynamics methods are given in Chiu et al. (1989, 1991*a*), both of which treat water in the gramicidin channel. The new molecular dynamics computations done for this article include D_2O in the gramicidin channel and the inclusion of sodium in the water-filled gramicidin channel. D_2O was constructed by giving the hydrogen atoms of the SPC water model an atomic weight of 2. The hydrogens in the gramicidin channel itself retained an atomic weight of 1, so the simulated experimental situation is one in which the experiment is done before the gramicidin has been deuterated by the D_2O. For the simulations including sodium, the standard GROMOS parameters for the sodium ion are used. In all molecular dynamics simulations presented in this article, a "soft" restraint (Chiu et al., 1991) with a time constant of 40 ps is used to maintain the right-handed β-helical structure of the gramicidin channel, with the reference structure described in Chiu et al. (1991*b*).

The stochastic dynamics computations presented in this article are similar to our previous Brownian dynamics computations (Jakobsson and Chiu, 1987, 1988) in that we treat the channel contents as a single entity characterized by a mobility, which is directly proportional to a diffusion coefficient. There is a major difference, however, in that one of the assumptions of our previous calculations is not satisfied in the present article. In Brownian motion (and hence our previous Brownian dynamics simulations), it is assumed that the sampling intervals are long compared with the time for velocities due to impulsive forces to die out on account of friction (Einstein, 1926). Another way of stating this is that the sampling time, or computed time step, is long compared with the velocity autocorrelation time. In our stochastic dynamics computations for this article, we found that we needed to use smaller time steps than could fill this condition, due to the narrowly spaced potential barriers associated with the structure of the gramicidin helix. It is therefore necessary to solve the Langevin equation. (For a clear discussion of the Langevin equation and time correlation analysis, see McQuarrie, 1976; Chandler, 1987.)

$$M(dv/dt) = -(k_BT/D)v - dU/dx + A(t), \qquad (1)$$

where M is the mass of the system whose motion is being analyzed, v is velocity, D is diffusion coefficient, U is free energy, and $A(t)$ is a time-varying fluctuating force of thermal origin.

This equation describes thermally induced motion more generally than does the Brownian description. It asymptotes to the Brownian description at long correlation times but is valid down to an arbitrarily fine time scale, as long as the motion is properly described by classical rather than quantum mechanics. The computer algorithmic realization of Eq. 1 that is used to drive the system, ΔX, in a time interval, Δt, is given by

$$\Delta v = \Delta t(-v(k_BT)/(MD) - (1/M)dU/dx) + GRN(2\Delta t/D)^{1/2}k_BT/M, \qquad (2)$$

$$\Delta x = v\Delta t, \qquad (3)$$

where GRN is a random number from a Gaussian distribution such that the mean value is zero and the mean squared value is one. The multiplying factor on GRN, essentially the magnitude of the fluctuating force, is given by the fluctuation-dissipation theorem. (See McQuarrie, 1976, ch. 20, or Chandler, 1987, ch. 8, for an introduction to the fluctuation-dissipation theorem. See Hängii et al., 1990, for a recent comprehensive review of the theory of thermally induced motion across energy barriers.) Trajectories computed according to the algorithms of Eqs. 2 and 3 are the basis of all the stochastic dynamic simulations presented in this article. The relationship between Eqs. 2 and 3 and molecular dynamics computations is that the diffusion coefficient D and the form of the free energy function U can be inferred from analysis of the molecular dynamics results, particularly from the mean-square-deviation (MSD) correlation function. By inspection, it is seen that D and U, together with the mass of the system, the temperature, and the Boltzmann constant, completely determine the behavior of the system. Thus, once D and U are determined from the short-time *MD* results, the long time behavior of the system can be calculated from Eqs. 2 and 3.

It should be pointed out that there are two kinds of barriers in the gramicidin channel that we do not deal with in this article. One is at the mouth of the channel associated with an ion's transition between the bulk hydration and the channel hydration environment. From fitting electrophysiology data to diffusion theory (Chiu and Jakobsson, 1989), from standard free energy molecular dynamics calculations (Roux and Karplus, 1990), and from other kinds of energetic calculations (Åqvist and Warshel, 1989), it is seen that this barrier consists of essentially partitioning ions into the channel; the free energy is lower inside the mouth than outside. The other kind of barrier that the results in this article do not deal with is the broad central barrier caused by image forces (Levitt, 1978; Jordan, 1982). Thus, the molecular dynamics (md) and stochastic dynamics (sd) results in this article relate entirely to the passage across the barriers in the interior of the channel that arise from the quasiperiodic structure of the helix. These are essentially the same as barriers seen in an infinitely long helix (Roux and Karplus, 1991*a*, *b*). It is the rate of crossing these barriers that determines the effective diffusion coefficient (Chiu and Jakobsson, 1989) for translocation across the channel.

RESULTS

Fig. 1 shows the MSD correlation function for several different molecular dynamics computed trajectories. All of these correlations were done on the z-coordinate motion or motion along the long axis of the gramicidin channel. The functions in Fig. 1 *A* were derived from a molecular dynamics run of a gramicidin channel with a sodium ion near the center and otherwise filled with water. It shows the MSD correlation function for the individual waters that remained in the channel for the duration of the simulation, for the sodium ion, and for the center of mass of the ion and chain of channel waters (6 waters, 3 on each side) taken as a unit. The reason the MSD for the individual waters is so much higher than for the sodium or the sodium-water chain is that the individual waters have substantial fluctuations about their individual locations and, much larger fluctuations than those shown by the sodium ion or by the center-of-mass of the entire chain. Fig. 1 *B* shows the corresponding curves for water, i.e., the MSD for individual water molecules from a molecular dynamics run with only water in the channel and for the center of mass of a chain of seven

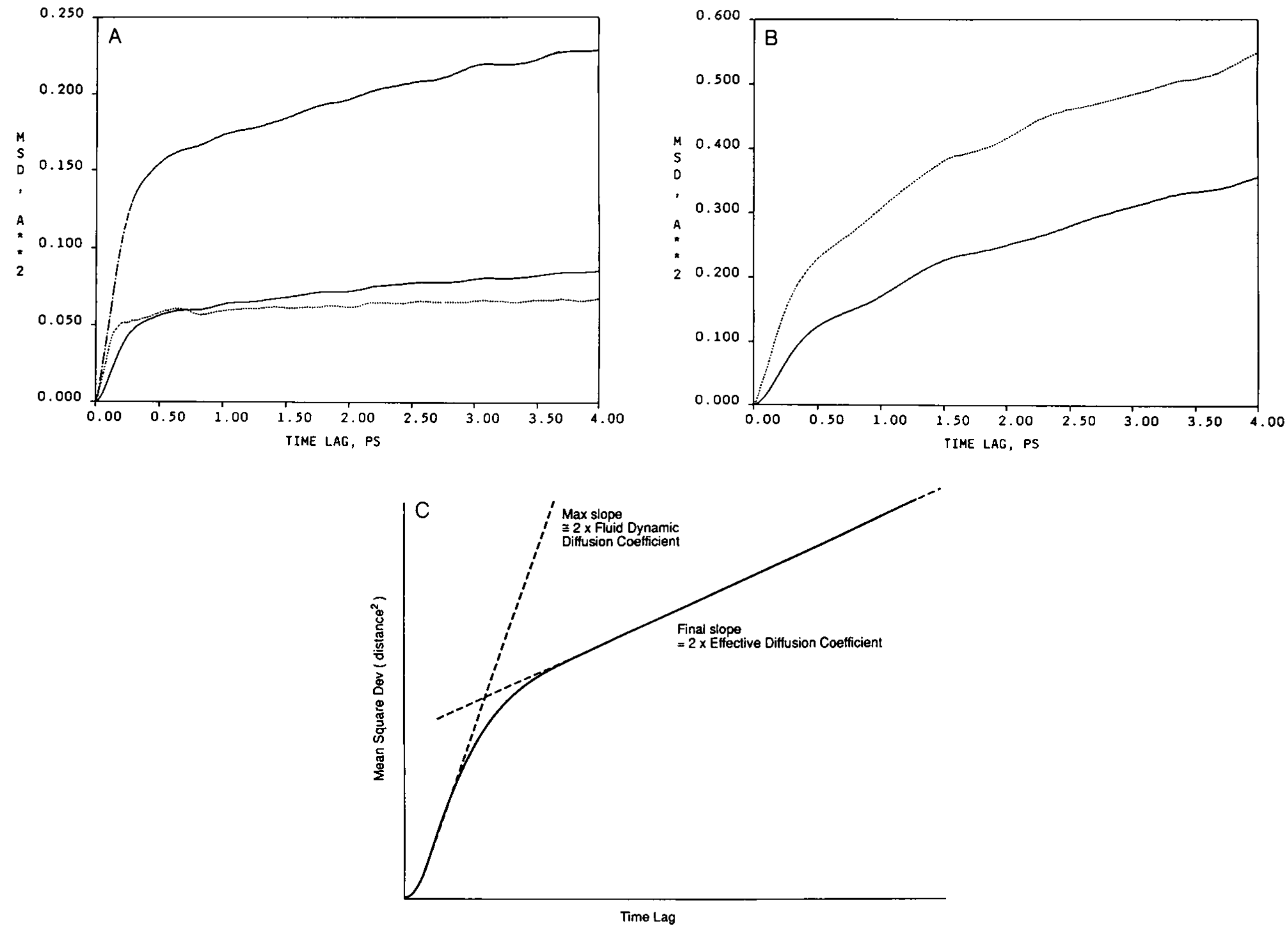

FIGURE 1 Mean-square-deviation (MSD) correlation function for molecular dynamics (MD) computed trajectories. (*A*) Simulation of a gramicidin channel containing one sodium ion in the center with the rest of the channel containing water molecules. The MSD function is shown for the sodium ion itself (*dotted line*), for the center of mass of the sodium ion plus six water molecules, three on each side (*solid line*), and for the individual water molecules (*dot-dash*). (*B*) MSD curves for only water in the channel. Shown is the curve for the individual water molecules (*dotted line*) and the center of mass of a chain of seven water molecules that remained in the channel for the duration of the simulation (*solid line*). (*C*) In schematic form, the significance of the curves of *A* and *B* as characteristic of thermally driven motion across a series of free energy barriers. The initial slope of the MSD curve indicates the fluid dynamic diffusion coefficient or local friction for fluctuations within individual potential wells. The final slope indicates the effective diffusion coefficient, which is a measure of how often the free energy barriers are crossed. The ratio of the two slopes is the "diffusion ratio" (Lifson and Jackson, 1962).

waters in the channel. The database for Fig. 1 *B* comprises the trajectories of seven water molecules that spend the entire simulation inside the channel. The MSD for the individual waters is the mean of the seven MSDs calculated for the individual waters. The MSD for the center-of-mass is calculated from the single trajectory derived from tracing the center-of-mass of the seven waters for the entire duration of the simulation. Fig. 1 *C* shows in schematic form the significance of the MSD functions in Fig. 1, *A* and *B*. They are characteristic of thermally driven motion across a series of free energy barriers. There is a sigmoidal shape at the origin of the MSD curve, which corresponds to the velocity autocorrelation time. Then the curve moves up in what would be a continued straight line if the motion were Brownian, with the diffusion constant equal to half the slope. In our case, the MSD curve bends over, suggesting that the motions are confined in local energy wells. The final slope is twice the effective diffusion coefficient, determined by the barrier crossing rate. The ratio of the final slope over the initial slope is called the "diffusion ratio," according to the usage of Lifson and Jackson (1962). In analogy to the Lifson and Jackson work on diffusion in a polyelectrolyte solution, the initial slope would be twice what they termed the "hydrodynamic diffusion coefficient." Since we are concerned with events in the channel rather than bulk aqueous solution, we will generalize the expression to "fluid dynamic diffusion coefficient."

One way to determine the free energy profile is to do a full free energy calculation (Roux and Karplus, 1991*a*). However, for the case of the center of mass of the channel contents as opposed to a single atom or molecule, it is not completely clear how to do such a calculation. We consider the motion of the center of mass of the channel contents because, in view of the obligatory single filing of the channel contents in the narrow gramicidin channel, it is the center-of-mass motion that most directly correlates to measurable transmembrane transport rates. (For the evidence for and discussion of single-filing in gramicidin, see Finkelstein, 1987, pp. 130–151.) As an alternative to the usual free energy calculations, we postulate a periodic sinusoidal free energy profile (related to the periodic structure of the helical channel) and deduce the fluid dynamic diffusion coefficient and the height and spacing of the free energy barriers from the form of the MSD function, using stochastic computations (Eq. 2). Since we do not include lipid in the molecular dynamics (MD) simulations, we do not include the image potential (Jordan, 1982) associated with the lipid-water system in the stochastic dynamics (SD) simulations. The algorithm is to insert the fluid dynamic diffusion coefficient from the MSD (Fig. 1 *C*) as *D* in Eq. 3 and do stochastic dynamics computations with various barrier heights and spacings to match the MSD curves from the molecular dynamics (Fig. 1, *B* and *C*). Because stochastic dynamics goes quite fast compared with molecular dynamics, it is feasible to explore many possible barrier parameters and arrive at good fits to the molecular dynamics MSD functions.

There is also a regular pattern to the form of the MSD curve as a function of the free energy barrier spacing and height that helps in the fitting. Simply put, for a given mass and fluid dynamic diffusion coefficient, the barrier height determines the effective diffusion coefficient (as measured by the final slope of the MSD). The effective diffusion coefficient is almost completely independent of the barrier spacing. (In the diffusive high-frequency limit, the effective diffusion coefficient is completely independent of the barrier spacing [Lifson and Jackson, 1962; Jakobsson and Chiu, 1988].) On the other hand, the barrier spacing determines the intercept of the final phase of the MSD with the vertical axis. The barrier height has very little effect on this intercept. These features are shown in schematic form in Fig. 2 *A*. Another helpful constraint is the channel structure. Since the barrier structure should be associated with regularities in the channel structure, there are just a few discrete values of barrier spacing that are sensible to test for fitting to the molecular dynamics data. Fig. 2 *B* shows that the appropriate barrier spacing for the sodium-water chain is the repeating unit of the gramicidin channel backbone—one L-D pair of amino acids. We found this to be true for all analyses of the center of mass of the channel contents: the sodium-water complex, the chain of water molecules only, and the chain of D_2O molecules only. Fig. 2 *C* shows the results of SD computations for the sodium-water chain with the appropriate barrier spacing and various barrier heights. It appears that the best fit is with a barrier height of 6.5 kT.

Fig. 3 shows the MSD correlations for the center-of-mass motions of pure water and pure D_2O. Fig. 3 *A* shows the curves for water generated by MD compared with the curves generated by SD with 15 barriers per channel and various barrier heights. It appears that the best fit of the SD curve to the MD curve is with a barrier height of 3.5–3.7 kT. Fig. 3 *B* shows that the apparent barrier height for D_2O is ~4.0 kT or a bit higher than that for water. Note that these barriers are many times higher than the free energy barriers for water motion calculated by Roux and Karplus (1991*a*, *b*); indeed, those authors judged that the water motion was essentially freely diffusive. We believe that this discrepancy is largely due to the fact that they considered individual water molecules, whereas we looked at the center-of-mass of the chain of waters. Thus, their analysis included many fluctuations of the individual water molecules relative to the chain of waters, whereas such fluctuations are eliminated from the center-of-mass analysis. We chose the center-of-mass analysis because of the obligatory single-filing of the waters. The early evidence for the single filing is presented in Finkelstein (1987; see especially pp. 130–151). Subsequently, every molecular dynamics study of gramicidin has verified single filing, up through Roux and Karplus (1991*a;* see especially Fig. 2) and the present study. This point will be discussed at greater length in the Discussion and Summary section of this article.

For reasons to be given below, the value of 6.5 kT given above for the sodium-water barrier height is actually very imprecise. We can say with confidence from the MSD analysis of the MD trajectories that the barrier heights for the sodium-water complex are substantially higher than for the water or D_2O chains, but our analysis will show that it is impossible to say accurately just from the MSD of the MD data how much higher the sodium-water barriers are.

The barrier spacing for the sodium ion itself is also one L-D pair, as is clear by the fact that the MSD for the sodium trajectories bends over at about the same position as does that for the center of mass for the sodium-water chain (Fig. 1 *A*). This is consistent also with the analysis of Roux and Karplus (1991*a*). However, the barrier spacing for individual water molecules in the sodium-water chain (Fig. 1 *A*) and in the all-water chain (Fig. 1 *B*) is substantially wider, as shown by the fact that the MSD function for individual water molecules rises much farther before it bends over than does the MSD function for the center of mass of the chain. Fig. 4 *A* shows the MSD from SD computations with various barrier spacings of individual water molecules in the sodium-water chain. Comparison of the SD curves with the curve derived from the MD computations suggests that the number of barriers for water motion is about nine barriers per channel. (Note that the curve for the MD is the same curve as for the single water molecules in Fig. 1 *A* and represents the mean MSD for all the waters in the channel, rather than any particular water molecule.) Nine barriers per channel correspond to a barrier spacing of ~26 Å/9 or 2.89 Å, which is about equal to

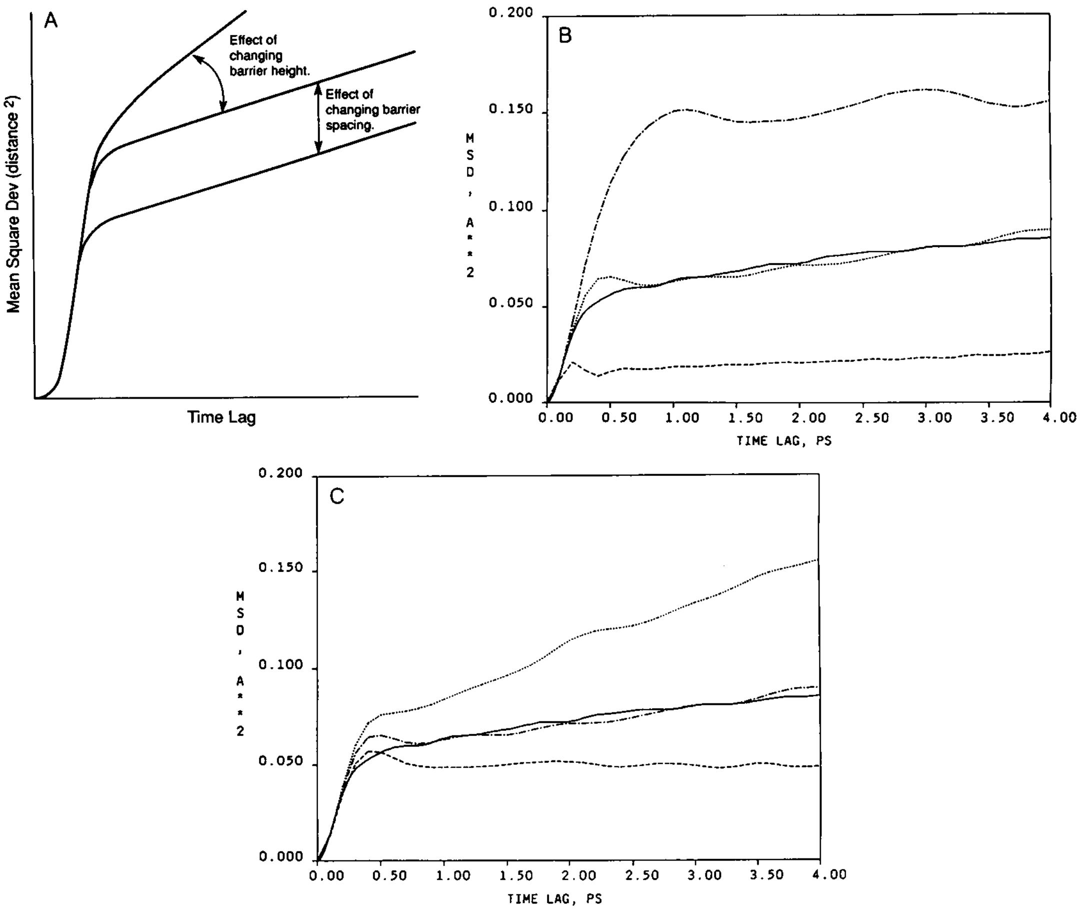

FIGURE 2 (A) Schematic representation for how features of MSD function relate to height and spacing of the periodic free energy barriers. Barrier height determines the final slope, which is proportional to the effective diffusion coefficient. Barrier spacing determines the vertical displacement or how far the MSD rises before it bends over. (B) Solid curve is MSD for center-of-mass of sodium-water chain for 640-ps MD run. Other curves are for SD runs of 640-ps duration of the center-of-mass of the sodium-water complex, each for the same assumed barrier height (6.5 kT) but different plausible barrier spacings: dot-dash is nine barriers (1 barrier for each distance increment between water molecules), dotted line is 15 barriers (every L-D pair), and dashed line is 30 barriers (every amino acid). From these types of calculation, it is seen that the final slope (effective diffusion coefficient) is almost independent of barrier spacing and that the L-D pair spacing is the best fit to the MD data. (C) Solid curve is MSD for center-of-mass of sodium-water chain for 640-ps MD run. Other curves are MSD for SD runs of 640-ps duration of the center-of-mass of the sodium-water complex, barrier spacing is every L-D pair, and barrier heights are 6 kT (*dotted line*), 6.5 kT (*dot-dash line*), and 7 kT (*dashed line*). Note that for same barrier spacing each curve would have about the same intercept on the vertical axis, but the slope is less the higher the barriers.

the spacing between adjacent waters. Thus, even though the overall motif for the translocation of material through the channel is single file, the periodicity of the potential for individual molecules can be different from the periodicity of the chain as a whole. This may be considered loosely analogous on the microscopic level to the macroscopic phenomenon of waves of different periodicity superimposed on each other on a water surface.

From Fig. 4 *B*, the best fit for the barrier height for individual water molecules in the sodium-water chain is a little over 6 kT. This is very similar to the barrier height of 6.5 kT that led to the best fit of the SD curves to the MD curve for the center-of-mass of the sodium-water chain (Fig. 2 *B*).

The molecular dynamics and stochastic dynamics runs above for the sodium-water system are 640 ps in duration. This is in fact too short to determine the relationship between the barrier height and the effective diffusion coefficient, because it is too short for the system to cross enough barriers to get a statistically valid relationship. The reasoning behind this statement is as follows. From fitting electrodiffusion theory to electrophysiological data, the effective diffusion coefficient for sodium-water translocation across the channel (D_{EFF}) is $\sim 0.94 \times 10^{-2}$ Å^2/ps. The barrier-to-barrier spacing (x_{B}) is ~ 1.73 Å for the sodium ion or sodium-water complex. The mean time to cross a barrier is then given by the following:

$$t = x_{\mathrm{B}}^2/(2D_{\mathrm{EFF}}) = 1.73^2/(2 \times 0.94 \times 10^{-2}) = 160 \text{ ps} \quad (4)$$

The fractional standard deviation for counting any number of steps N in a random walk is $1/\sqrt{N}$. The mean number of barrier crossings in a run of 640 ps is just 640/160 or four for the sodium ion or sodium-water

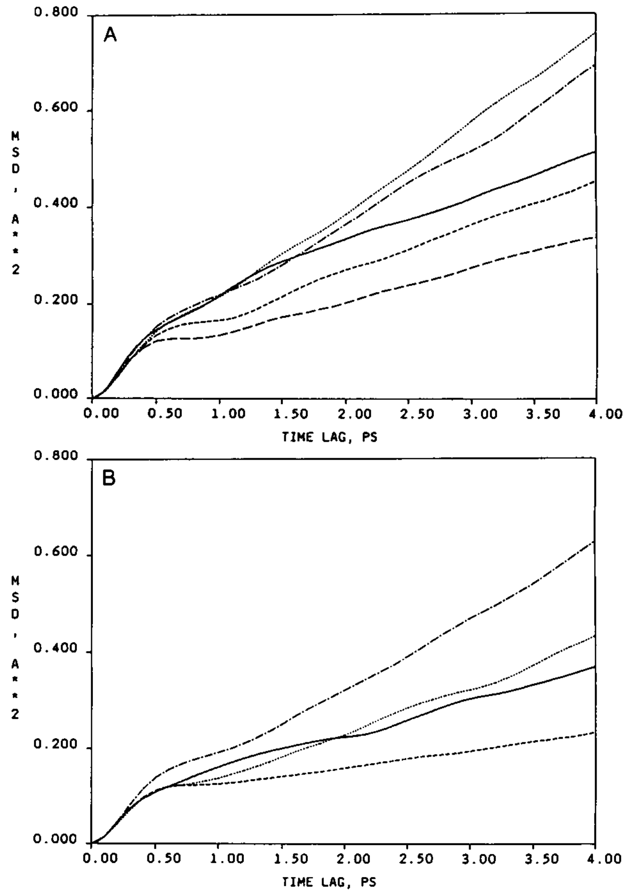

FIGURE 3 (*A*) MSD for center-of-mass of the chain of waters in the channel. Solid curve is from MD. Other curves are for 15 barriers per channel, and barrier heights of 4 kT (*long-dash line*), 3.7 kT (*short-dash line*), 3.5 kT (*dot-dash line*), and 3 kT (*dotted line*). (*B*) MSD for center-of-mass of a chain of D_2Os in the channel. Solid curve is from MD. Others are from SD with barrier heights of 4.2 kT (*dashed curve*), 4 kT (*dotted line*), and 3.6 kT (*dot-dash line*).

complex. This is clearly too few to gather enough data to compute the effective diffusion coefficient with reasonable accuracy. By corollary, the molecular dynamics runs are not long enough to determine the effective diffusion coefficient with reasonable accuracy. So we next turn to stochastic dynamics, for which much longer runs are feasible, since stochastic dynamics is much less computer intensive than molecular dynamics.

Fig. 5 shows the results of stochastic dynamics computations for the sodium-water complex. In these computations, we take from the MD calculations the parameters that we can reliably obtain from them, namely the fluid dynamic diffusion coefficient and the free energy barrier spacing. Then, we vary the barrier height and do a long enough stochastic dynamics run (10 ns) to determine the effective diffusion coefficient for each barrier height. The actual barrier height is then estimated as the barrier height that gives the same effective diffusion coefficient as does fitting the experimental data to electrodiffusion theory (Chiu and Jakobsson, 1989). We do not get an exact value for the barrier height, as there is some noise in even a long SD run. Our best estimate for the barrier height governing the motion of the sodium-water complex is 5.3 kT. This is a bit lower than the value of 6.5 kT derived from the full MSD function of the 640-ps MD run (Fig. 2 *B*). From inspection of Fig. 5, it is seen that a barrier height of 6.5 kT would give a D_{EFF} value of $\sim 3 \times 10^{-7}$ cm^2/s, which is about one-third the value we estimate from the electrophysiological data. It should be emphasized that the D_{EFF} value shown in Fig. 5 is derived from an extensive set of electrophysiological data, including both current-voltage and current-concentration relationships. Thus, although the present article is computational and theoretical, its conclusions derive as much from experimental as from theoretical considerations.

Superficially, the motion bears some resemblance to the "hopping" or Eyring transition state models that

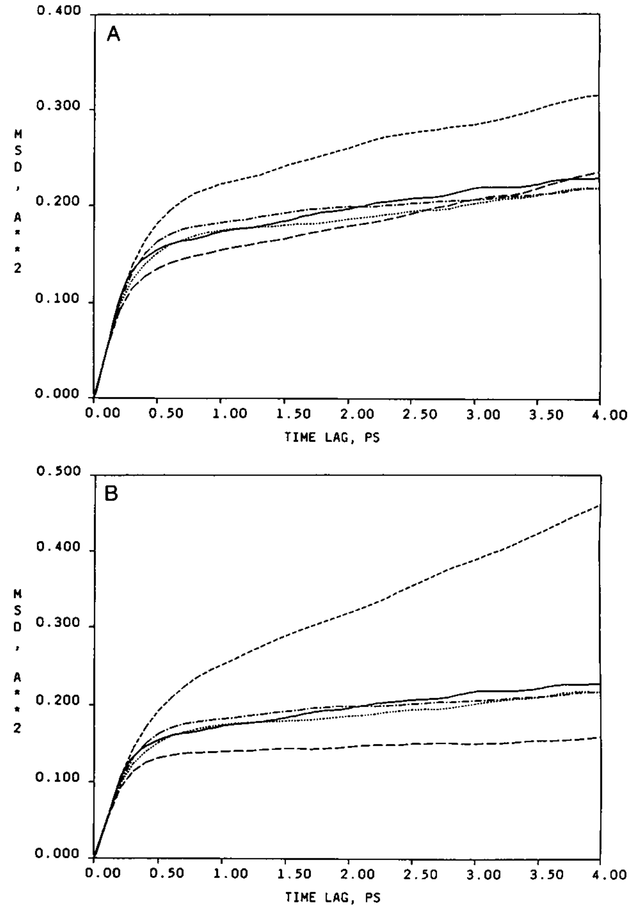

FIGURE 4 MSD for individual waters with a sodium ion in the channel. (*A*) Solid curve is from a 640-ps MD run with a sodium ion in the center of the channel. Other symbols are for stochastic dynamics runs with the following barrier structures: dotted line is 9 barriers per channel, 6.5 kT; dot-dash is 9 barriers per channel, 6.0 kT; short dash is 8 barriers per channel, 6.25 kT; and long dash is 10 barriers per channel, 6.25 kT. (*B*) Solid curve is from MD run with sodium ion in center of channel. Other curves are for SD runs with the following barrier structures: dotted line is nine barriers, 6.5 kT; dot-dash line is nine barriers, 6.0 kT; short dash is nine barriers, 5.0 kT; and long dash is nine barriers, 7.5 kT.

have been used widely to interpret data on ion permeation in channels. This resemblance is illustrated in Fig. 6, in which we plot the trajectory that provided the raw data for the 5.0 kT data point in Fig. 5. (The computations in Fig. 6 were done in a periodic potential much longer than a normal gramicidin channel so that the trajectory would remain in the periodic region for the full 8 ns of the simulation.) It is seen that the trajectory is a series of motions from one potential minimum to another, with the transitions being very abrupt relative to the time spent in the potential well. But the real question is this: Is the motion across the barriers a high-friction (Kramers) type of motion or a low-friction (Eyring) type of motion, or something in between?

One way to answer the question is to consider the "diffusion ratio" (D/D_{EFF}) defined by Lifson and Jackson (1962) as the ratio between the effective diffusion coefficient, D_{EFF} (the diffusion coefficient defined by the rate for the system to traverse the free energy barriers), and the fluid dynamic diffusion coefficient, D (the diffusion coefficient defined by the mobility of the system as it moves within a free energy well). In the Eyring limit, the ratio is given by

$$D/D_{\mathrm{EFF}} = e^{\phi}, \tag{5}$$

where ϕ is height of the barrier in kT.

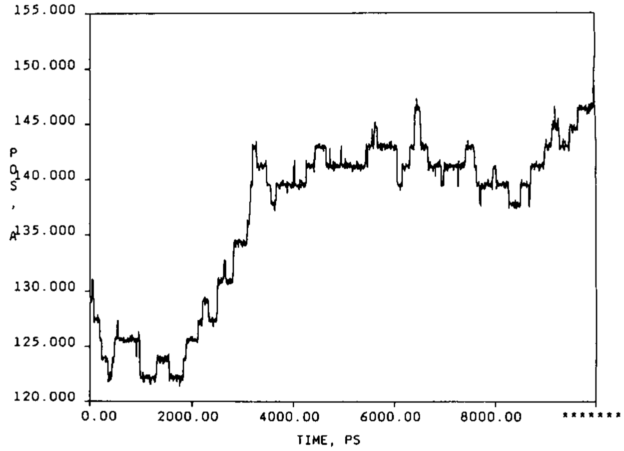

FIGURE 6 A barrier crossing trajectory computed by stochastic dynamics. The trajectory shown is for the sodium-water complex with a barrier height of 5.0 kT. This trajectory provided the data for one of the points in Fig. 5. Vertical axis is position, in ångströms. (The channel is made unrealistically long so that a long simulation can be run without the ion leaving the channel.) Barrier spacing is 1.73 Å. Trajectory resembles that for a "hopping" or Eyring model, but the resemblance is superficial, since the low friction assumption underlying the Eyring model is not valid.

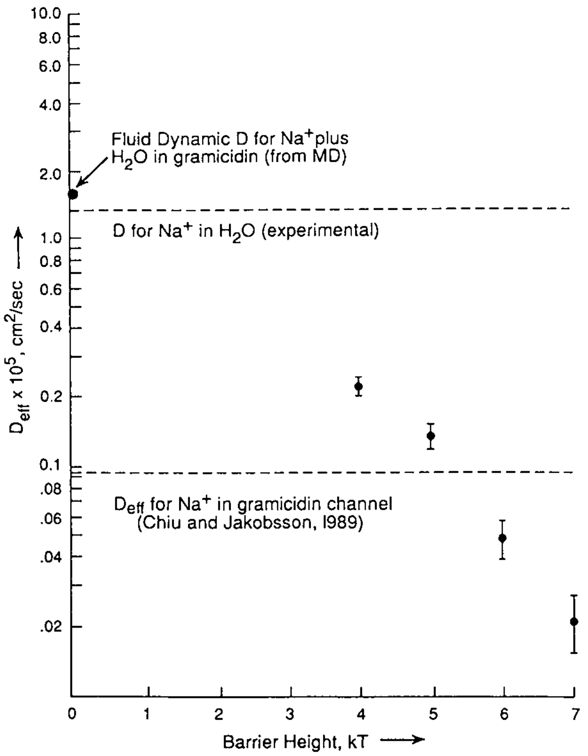

FIGURE 5 Effective diffusion coefficient versus barrier height for motions of the sodium-water chain. The fluid dynamic diffusion coefficient and barrier spacing are deduced from the molecular dynamics simulations and stochastics dynamics. The fluid dynamic diffusion coefficient is as shown. The barrier spacing is 15 barriers per 26-Å channel, or 1.73 Å. Then stochastic dynamics simulations of several nanoseconds duration each are done with various barrier heights. The effective diffusion coefficient is calculated form the final slope of the MSD correlation of the SD trajectory. The error bars are calculated from the "square-root-of N" rule; the fractional standard deviation is $1/\sqrt{N}$, where N is the number of barrier crossings in the stochastic dynamics simulation.

In the Kramers limit, the ratio is given by (Lifson and Jackson, 1962)

$$D/D_{\mathrm{EFF}} = (\tan \lambda/(2\lambda)(1 + \sin (2\lambda)/(2\lambda)), \tag{6}$$

where $\lambda = \cos^{-1} e^{-\phi/2}$.

For the 5.3-kT barrier height, the diffusion ratio is 200 for an Eyring process, 4.93 for a Kramers process, and 16.8 for the sodium-water system by our above analysis (Fig. 5). Thus, the sodium-water translocation process is neither purely transition state (Eyring) nor diffusive (Kramers) in character but rather characterized by intermediate friction, although it is much closer to the Kramers than the Eyring limit. The lesson of Fig. 6 is that a trajectory such as shown in that figure is not necessarily indicative of an Eyring type of process.

Fig. 7 *A* shows the normalized velocity autocorrelation function (NVACF) for the center-of-mass motion of the sodium-water system from MD simulations and from SD simulations with a 5.3-kT barrier height. We used the NVACF to analyze motions in gramicidin in an earlier paper (Chiu et al., 1991), and it is well established in the statistical mechanics of thermally driven processes (McQuarrie, 1976; Chandler, 1987). The two curves in Fig. 7 *A* are similar but not identical. A measure of their similarity is given by their spectral composition, as seen

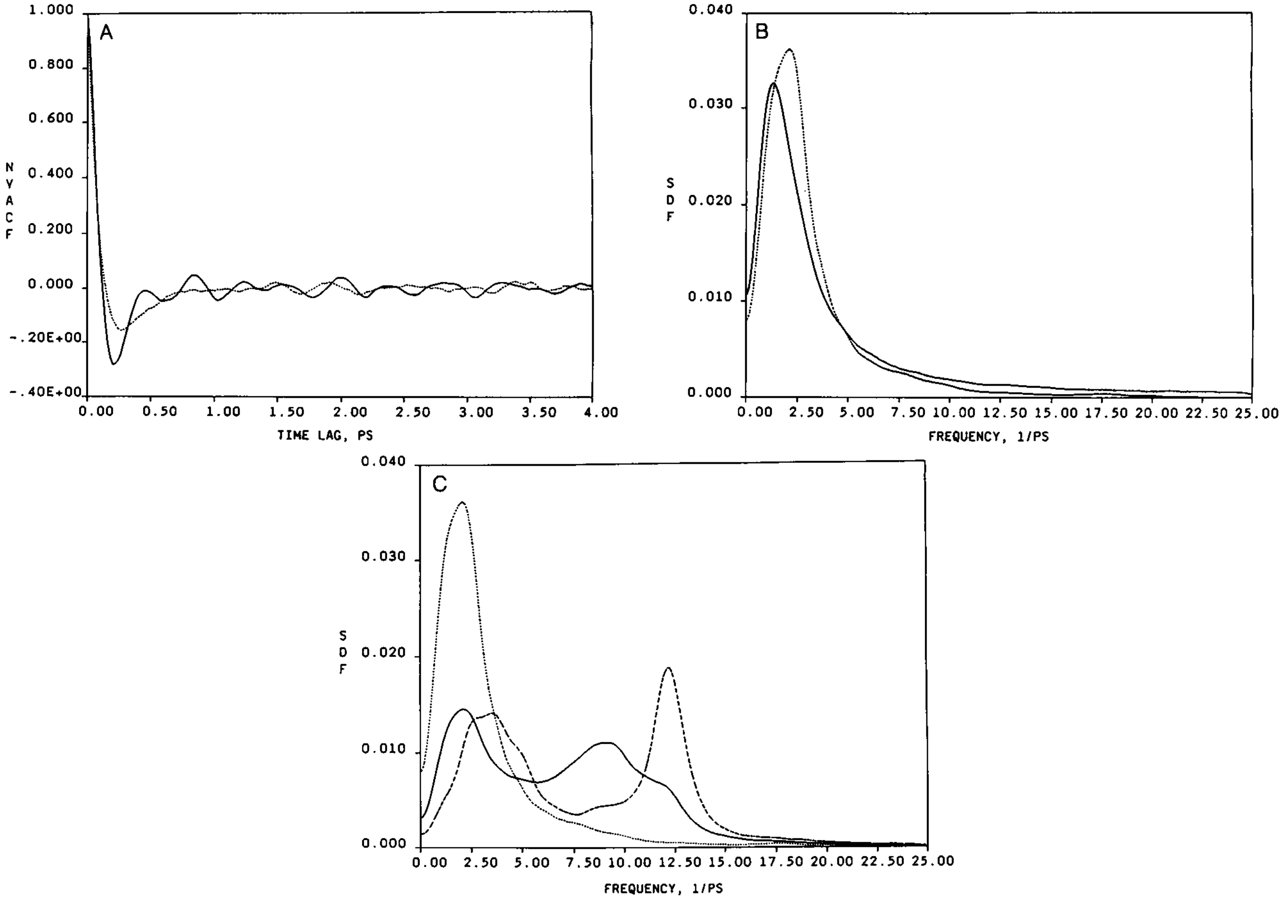

FIGURE 7 (*A*) Normalized velocity autocorrelation function (NVACF) from a 640-ps run for the center of mass of the sodium-water chain. Solid line is from MD simulation and dotted line from SD simulation with 5.3-kT sinusoidal barriers spaced at 1.73 Å intervals. (*B*) Spectral density functions for the curves in *A*. (*C*) Spectral density functions of the NVACF for different components of the channel contents from the MD run of *A*. Dotted line is the center of mass of the entire channel contents, solid line is the individual water molecules, and the dashed line is the sodium ion.

in Fig. 7 *B*. It is seen that both VACFs are strongly monotonic. The MD curve is slightly more monotonic, as evidenced by its slightly higher peak. The primary frequency of the MD motion is slightly higher than for the SD. These are small differences; the SD emulations clearly capture the essence of the barrier-crossing events described by the MD for the center of mass of the sodium-water system. On the other hand, the motions of the individual molecules in the sodium-water chain certainly have additional features. Fig. 7 *C* shows the spectral density function for the mean VACF of individual water molecules and the sodium ion in the sodium-water chain, as well as for the entire chain treated as a unit. The VACFs of individual water molecules in the channel differ only slightly from each other. It is seen that both the individual water and the sodium motions have characteristic peaks with higher frequency than that of the entire chain. The three dominant characteristic frequencies are 2.1 THz for the entire chain, 9.1 THz for the individual water molecules, and 12.2 THz for the sodium ion. For the individual waters, there is a bit of variation according to where in the channel the water is. The waters next to the sodium ion have about a 20% higher characteristic frequency than those next to the mouth. The curve for individual waters in Fig. 7 *C* is the arithmetic average of the curves for all individual waters in the channel. Except for the shoulder at the sodium frequency, it is very similar to the corresponding curve for a channel with only waters in it (Fig. 4b in Chiu et al., 1991*b*) and quite different from the corresponding curve for bulk or cap waters (Fig. 3b in Chiu et al., 1991*b*). Thus, the primary determinant of the individual water dynamic behavior is whether or not it is in the channel. Whether or not there is an ion in the channel, and the water's position relative to the ion, is a secondary determinant or modulator of the water's dynamic behavior.

Another way of exploring the nature of the barrier crossing process, using the results of Fig. 7 *A*, is to invoke the criterion of comparing the barrier width with the mean free path of the thermally driven motion (Frauenfelder and Wolynes, 1985; Fleming and Wolynes, 1990). The intuitive concept of "mean free path" is time between collisions. This intuitive concept is the operational one in considering a gas phase, but in the condensed matter situation that pertains in liquids or within ion channels, this intuitive concept is not meaningful because each particle is in almost continual contact with others. We replace the intuitive concept with the statistical mechanical concept that the mean free path is the distance traveled in the mean time for a particle's velocity to reverse direction. We can calculate the mean free path so defined by combining the MSD correlation with the VACF. We define the mean free path as the mean distance traveled (defined as the square root of the MSD function) in the time for the VACF to go to zero. In other words, this time is the persistence time of the velocity or the time for the velocity to become completely uncorrelated. The data plotted in Fig. 7 *A* give us a mean reversal time of 0.11 ps for the center of mass of the sodium-water chain, based on the MD computations. (The SD simulations, where the motion is approximated by the Langevin equation with static sinusoidal barriers, give a slightly longer reversal time. For the purpose of this discussion, the differences between the two are not significant.) Next, we look at the MSD function for the sodium-water chain (Fig. 1 *A*). The mean free path is just the square root of the MSD evaluated at the reversal time. This turns out to be 0.129 Å.

The other quantity we need to define to characterize the barrier-crossing motions is the barrier width. A reasonable rule of thumb is to look at how wide the barrier is 1 kT below the peak. For sinusoidal barriers of spacing x_B and height H kT, the width so defined is given by

$$W_B = (x_B/\pi)\cos^{-1}(1 - 2/H). \tag{7}$$

For barrier spacing of 1.73 Å and barrier height of 5.3 kT, the barrier width is 0.49 Å. Now that we have calculated the barrier width and the mean free path, we have the necessary criteria for characterizing the barrier-crossing motion as follows: if the mean free path is much longer than the barrier width, then the Eyring or transition state limit describes the motion. If the barrier width is much longer than the mean free path, then the Kramers or high friction limit describes the motion. If the barrier width and the mean free path are of comparable magnitude, then the motion is of intermediate friction, in which case the full Langevin equation is the simplest physically reasonable description of the barrier-crossing motion. For our values of 0.129 Å for the mean free path and 0.49 Å for the barrier width, we see that the intermediate friction situation pertains, just as we inferred from the calculation of diffusion ratios presented earlier in the paper. This result may be generalizable to ion and water motion in other ion channels, as indicated in the Discussion and Summary section of this article.

DISCUSSION AND SUMMARY

A comprehensive picture of motion in the gramicidin channel is built up by analyzing electrophysiological data using theory on three different levels of detail. In a previous article (Chiu and Jakobsson, 1989), electrophysiological data of Barrett-Russell et al. (1986) was combined with electrodiffusion theory to determine that the effective diffusion coefficient of the sodium ion in the gramicidin channel was about one-tenth that in bulk water. In this article, molecular dynamics computations show that the fluid dynamic diffusion coefficient for the channel contents is approximately that for aqueous solution. The MD further implies a periodic free energy profile for the center-of-mass motion of the channel contents, with barriers spaced each L-D pair along the membrane. Individual water molecules see barriers spaced farther apart, approximately at the water-water distance. From stochastic dynamics emulations of the molecular dynamics, the individual barriers are judged to be several times thermal energy in height. Of the three cases considered, the highest barriers to the center-of-mass motion are for sodium and water in the channel, the next highest are for D_2O, and the lowest are for ordinary water. Both by considerations of the diffusion ratio and also by comparing the mean free path with the barrier widths, it is seen that the barrier-crossing events are of intermediate friction. Thus, neither the low-friction (Eyring) nor high-friction (Kramers) limit provides an appropriate description of the motion through the channel. High friction, or diffusion, theory is useful (Cooper et al., 1988) but falls short of providing a fundamentally correct physical description of barrier-crossing in channels.

For sodium motions, the picture that we show is quite consistent with the molecular dynamics computations of Roux and Karplus (1991*a*, *b*), although our methods are somewhat different and some specifics of our molecular models are different. They used the CHARMM (Brooks et al., 1983) force fields (with some modifications for interactions between the sodium ion and the peptide, whereas we used the GROMOS (Hermans et al., 1984) force fields. They used periodic boundary conditions and a left-handed poly-alanine β-helix, whereas we used an explicit right-handed gramicidin channel without periodic boundary conditions. They evaluated the free energy profile by importance sampling, restraining the sodium ion with an artificial force. We evaluated the free energy profile by matching time-correlation functions from the MD, combined with effective diffusion coefficients inferred from electrophysiological data, with time-correlation functions from stochastic dynamics designed to emulate MD across periodic barriers. They considered the motion of the sodium ion alone, whereas we did our major analysis on the center-of-mass of the sodium-water chain. They characterized the nature of the barrier-crossing by activated-state dynamics. We characterized the nature of the barrier-crossing process by evaluating the diffusion ratios, mean free paths, and barrier widths for our best models for the process and comparing those with the Eyring and Kramers limits. For the instances in which we calculated the same quantities as Roux and Karplus, our results were remarkably similar. We both find the free-energy profile to have the periodicity of the L-D pairs in the backbone. Our barrier heights for sodium translocation are 5.3 kT, whereas theirs are 7.5 kT, or ~2 kT higher. The 2-kT difference, while having a substantial effect on transport rate, is within the realm of inaccuracy of classical molecular dynamics results. Roux and Karplus (1991*b*) calculated a transmission coefficient of 0.11 for barrier-crossing by sodium. The transmission coefficient is also given by the ratio of the diffusion ratios between the "correct" model and the Eyring model for barrier crossing. In our work then, the transmission coefficient would be (see Results) 16.8/200, or 0.084. Given the accuracy of molecular dynamics results, the difference between 0.11 and 0.084 is not significant. The conclusion from both numbers is that the barrier-crossing is not well described by Eyring theory.

In contrast to the case of sodium, for water there is a major difference between the views of the motion presented by us and by Roux and Karplus. They calculate barrier heights of a few tenths of a kilotesla, implying that the motion is essentially freely diffusive. We find the appropriate barrier height to be a few kT. We believe that the critical difference is that we are considering the motion of the center-of-mass of the water chain, whereas Roux and Karplus considered the motion of individual water molecules. Fig. 1 reflects the issue. Fig. 1 *A* shows that the MSD for sodium alone and for the sodium-water complex are very similar. An individual sodium ion does not execute large-scale excursions relative to sodium-water chain. Therefore, the Roux and Karplus calculations based on the sodium ion alone are fairly consistent with our calculations based on the sodium-water chain. Fig. 1 *B* shows that the MSD for the individual water molecules and the water chain are quite different. The individual water molecules do execute relatively large-scale motions relative to the water chain. Thus, the free energy calculation for the water individual molecules does not accurately reflect the nature of the translocation of the water chain. Because of the obligatory single-filing imposed by the channel dimensions (Finkelstein, 1987, pp. 130–151), it is the center-of-mass motion of the water chain that is directly correlated to water transport across the membrane.

From statistical mechanics, the correlation time and mean free path for thermally driven motion in fluids depend primarily on the mass of the particle and its fluid dynamic diffusion coefficient. Despite the long narrow geometry of the gramicidin channel, we find that these two numbers for a hydrated sodium ion in the channel are about the same as for such an ion in bulk solution. They therefore seem likely to be similar also for ions in a wider channel, which is the more usual situation in biological channels. It thus seems likely that for ion motion in other channels as for gramicidin, the mean free path will be a few tenths of an ångström. If local barriers are formed by polar or charged groups in the channel, the local barrier widths 1 kT from the top are likely to be of the same order as those in the gramicidin, i.e., ~1 Å. Therefore, it seems likely that barrier-crossing events in other channels as well as gramicidin will be found to be intermediate-friction processes. Note that this inference is completely independent of the long, thin geometry of the gramicidin channel, which is not likely to be characteristic of other channels. It depends only on roughly aqueous mobility in the channel and the characteristic dimensions of molecular groups such as amino acid side chains.

In addition to the localized barriers discussed in this article, the gramicidin channel contains a broad central barrier, the result of electrostatic image forces originally described by Levitt (1978) and in more detail by Jordan (1982). There are also several different kinds of data suggesting a drop in free energy at the mouth of the channel favoring the entry of sodium ions into the channel (Etchebest and Pullman, 1986; Chiu and Jakobsson, 1989; Roux and Karplus, 1990; Bobak et al., 1991). The present article does not deal explicitly with these larger features of the free energy profile. Chiu and Jakobsson (1989) assumed that the motion across these large features of the free energy profile was diffusive. By using the effective diffusive coefficient derived from the 1989 analysis, we have implicitly incorporated that assumption into our present analysis, specifically into the estimate of the local barrier heights as visualized in Fig. 5. Note that the "diffusive" process across the larger features of the free energy profile is a stepwise motion from one local potential well to the next in the fashion of Fig. 6. For this process, the effective mean free path will be the barrier-to-barrier spacing, or 1.73 Å. For the central barrier, we judged to give the best fit to the electrophysiological data, the barrier width 1 kT below the peak was 12.4 Å, or about seven times the mean free path. For the motion across the local barriers, we found the ratio of the barrier width to the mean free path to be 0.49 Å/0.129 Å, or 3.8. Thus, we expect the motion across the central barrier to be closer to the diffusive limit than is the motion across the local barriers. Since the diffusive limit provides the minimum possible diffusion ratio, any possible deviation from the diffusive limit for motion across the central barrier implies that our 5.3-kT estimate of the local barrier height is a bit too low. We think any systematic error due to this effect must be substantially <1 kT. The reasoning for this statement is as follows. In the vicinity of the "true" barrier height, the dependence of the diffusion ratio on the barrier height is slightly more than twofold per kT (Fig. 5). A barrier width/mean free path ratio of 3.8 gives a diffusion ratio about threefold greater than the diffusive limit (the results for the local barriers). A barrier width/mean free path ratio of seven (for the broad central barrier) should be much closer to the diffusion limit, well within a factor of 2. Thus, the diffusive assumption for the effective diffusion coefficient should be well within a factor of 2 of being valid, and thus should result in an error substantially <1 kT in the estimated local barrier height.

Fig. 7 shows that each thermally driven species, be it sodium ion, water molecule, or the entire sodium-water complex, has a characteristic frequency of motion within its local free energy well. This is somewhat analogous to the "attempt frequency" of transition-state theory. However, the physics of the situation differs from the transition-state case due to the higher friction.

The broadest methodological import of this article is in the "welding together" of transport theory across three levels of detail: molecular dynamics, stochastic dynamics, and continuum electrodiffusion theory. Results at one level of detail are used to parameterize the calculations at another level to produce a more comprehensive theory of transport than is available at any one level alone.

The most general conclusion of this article about transport in channels is that local barrier-crossing processes are likely to be intermediate friction rather than high-friction diffusive (Kramers) or low-friction hopping (Eyring). This intermediate friction motion reflects the fact that matter in the interior of the ion channel has both solid-like and liquid-like characteristics. Such mixed attributes are a general feature of liquids confined in narrow gaps (Granick, 1991). In the case of water in the gramicidin channel, the polarization of the water (Chiu et al., 1989; Chiu et al., 1991*b*) gives some of the organized character of a nematic liquid crystal (Chandrasekhar, 1977). The functional significance of this polarization is not obvious and is not explored in the present article. The solid-like attributes in the channel include relatively spatially fixed periodic energy barriers to motion, with motion proceeding by "jumps" across the barriers. The liquid-like attributes include a local solvation environment similar to aqueous solution, leading to a fluid dynamic diffusion coefficient similar to that in aqueous solution. Also, the periodic barriers are not absolutely fixed. The mobility of the peptide structures underlying the barriers is important to thermally driven motion through the channel (Chiu et al., 1991). Because of this mixture of solid-like and liquid-like attributes, the physics of the ion channel cannot be understood by analogy to anything else, solid or fluid. Rather, it must be understood on its own terms, by detailed statistical mechanical analysis of water and ion interaction with the most realistic possible models of channel-forming molecules.

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