A Molecular Theory for Nonohmicity of the Ion Leak across the Lipid-Bilayer Membrane

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ABSTRACT The current-voltage relationship of ion leak (i.e., ion transport involving neither special channels nor carriers) across the lipid-bilayer membrane has been observed to be log-linear above the ohmic regime. The coefficient of the linear term has been found to be universal for membranes and penetrants examined. This universality has been explained in terms of diffusion in an external field, where the ion position is described as a Markovian process. Such a diffusion picture can be questioned, however. It is also probable that a leaking ion gets over the potential barrier before experiencing sufficient random collision in the membrane, considering that each ion is surrounded with long lipid molecules aligned almost unidirectionally. As an alternative, we discuss this ion leak in terms of velocity distribution of the ions entering the membrane and density fluctuation of the lipids. We conclude that we can explain the universality without resorting to the diffusion picture.

INTRODUCTION

In the chemiosmotic theory (Mitchell, 1961; Williams, 1961) of oxidative phosphorylation at the inner membrane of mitochondria, one of the key assumptions is sufficient resistance of the membrane to ion flux. Mitchell and Moyle (1967) measured this large resistance, utilizing state 4 respiration, i.e., a state without ADP (see, e.g., Lehninger, 1975). It is assumed in this state that the current due to the leak (i.e., an ion transport process across the membrane involving neither special channels nor carriers) is balanced with the current driven by the respiratory chain, which produces the electrochemical potential gap (i.e., the sum of the chemical potential gap and the voltage gap multiplied by the charge) across the membrane. Subsequent experiments, sometimes using the K^+ ionophore valinomycin, have established the log-linear relationship between the ion flux and the voltage gap for the leak just above the voltage-gap regime showing linearity (Jackson, 1982; Pietrobon et al., 1983; O'Shea et al., 1984; Krishnamoorthy and Hinkle, 1984; Zoratti et al., 1986; Brown and Brand, 1986; Murphy et al., 1986; Falnes, 1987; Toninello et al., 1988; Garlid et al., 1988). Most researchers attribute the nonlinearity to nonohmicity of the membrane (see Pietrobon et al., 1983, and Zoratti et al., 1986, for an alternative discussion). We concentrate on this nonohmicity, limiting our discussion to a simple case where one kind of ion carries the current one-dimensionally across the flat membrane surrounded with a dilute electrolyte, as in previous publications. For convenience, we assume the charge of the ion (q) to be positive and set the z coordinate normal to the membrane so that the electric potential becomes lower as z becomes larger. The side with smaller z coordinates is referred to as the entry side and the other as the exit side (Fig. 1 *a*).

As discussed in detail later, the coefficient of the linear term in the log-linear relationship has been observed to be universal for membranes and penetrants examined. Nagle (1987) discussed the nonohmicity but did not mention the universality. Garlid et al. (1989) explained the nonohmicity and the universality by using the classical rate theory of Eyring (Eyring and Eyring, 1963). Its outline is as follows.

Let us begin by considering ion transport in the bulk of a dilute electrolyte in an external potential U. Water molecules collide with an ion so frequently and so randomly that the ion dynamics on a practical time scale can be reasonably expected to be a Markovian process with its position and velocity variables (see, e.g., van Kampen, 1981). On the assumption that the velocity is adiabatically eliminated (Gardiner, 1985) because U varies slowly enough with respect to space and because the friction coefficient of the ion (γ) is large enough because of frequent random collision, the ion density follows the Smoluchowski equation (see, e.g., Doi and Edwards, 1986):

$$\frac{\partial}{\partial t}c(z,t) = \frac{\partial}{\partial z}\left\{\frac{c(z,t)}{m\gamma}\frac{\partial U(z)}{\partial z}\right\} + \frac{kT}{m\gamma}\frac{\partial^2}{\partial z^2}c(z,t)$$

$$= \frac{\partial}{\partial z}\left\{Dc^{eq}(z)\frac{\partial}{\partial z}\frac{c(z,t)}{c^{eq}(z)}\right\} \quad (1)$$

where k is the Boltzmann constant, m is the ion mass, t is the time, T is the temperature, $D \equiv kT/(m\gamma)$ is the diffusion coefficient, and c^{eq} is the equilibrium ion density:

$$c^{eq}(z) \propto \exp\left[-\frac{U(z)}{kT}\right] \quad (2)$$

The leak across the membrane was regarded as such a diffusion process with a constant diffusion coefficient in an external field with a double-well potential. The potential felt by an ion would be higher in the middle of the membrane because of its hydrophobic environment. Let A, B, and C be

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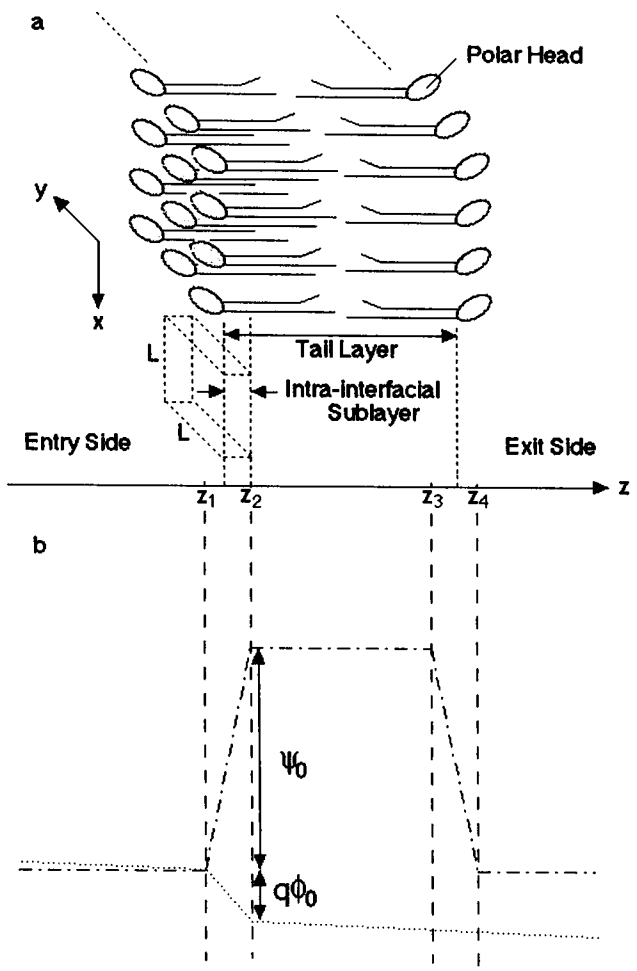

FIGURE 1 Scheme for our notation. (*a*) A piece of the lipid bilayer membrane (~5 nm in thickness) is shown. The z coordinate is normal to the membrane; the x and y coordinates are tangential to the membrane. The surface roughness of the membrane (see, e.g., Egberts et al. 1994) is omitted for simplicity. The electric potential is supposed to be higher on the entry side than on the exit side. The hydrophilic environment bulks on both the sides coincide with the ranges of $z < z_1$ and $z > z_4$, respectively. The hydrophobic environment bulk coincides with the range $z_2 < z < z_3$. The interfacial regions coincide with the ranges $z_1 < z < z_2$ and $z_3 < z < z_4$. In particular, the former region is simply referred to as the interfacial region. The plane $z = z_1$ is named the entry plane. The intrainterfacial sublayer is a sublayer of the tail layer that falls into the interfacial region (on the entry side). In the third section of this paper, a portion in this sublayer with the cross-sectional area of L^2, which is indicated by a box bordered with dashed lines, is supposed to have a segment mass relevant to the leak. (*b*) A simplified profile of the potentials ψ and $q\phi$ is shown (see the second section of the text). Actually, there should be variations in them other than those drawn here. Nevertheless, we expect that the potential wall at the interfacial region (on the entry side) has a height of $u_w \approx (\psi_0 - q\phi_0)$ and an approximately constant slope, where the voltage gap across the membrane approximately coincides with ϕ_0. A part of the interfacial region outside the tail layer coincides roughly with the region 2 defined by Marrink and Berendsen (1994), and $z = z_2$ coincides roughly with the middle of their region 3. Our term, "interfacial region," implies a transitional region from the hydrophilicity to the hydrophobicity, where ψ changes significantly; note the different usage of Marrink and Berendsen (1994, 1996).

the z coordinates of the bottom of the valley located on the entry side, the hilltop located within the membrane, and the bottom of the valley located on the exit side, respectively. If the potential has a sharp peak with the height (measured from the bottom at A) much larger than kT, Kramers' method (Kramers, 1940; Gardiner, 1985) tells that the total ion number ($N_C(t)$) in the electrolyte bulk on the exit side evolves as

$$\frac{\partial N_C}{\partial t} \approx \text{constant} \times \exp\left[-\frac{U(B)}{kT}\right] \cdot \left\{N_A \exp\left[\frac{U(A)}{kT}\right] - N_C \exp\left[\frac{U(C)}{kT}\right]\right\} \quad (3)$$

where the constant term is proportional to the diffusion coefficient in the membrane. The ion flux J is given by the left-hand side, and the voltage gap $\Delta\phi$ is given by $U(A) - U(C)$ divided by q. If the peak is located at the center of the membrane, $U(B) - U(A)$ can be assumed to be equal to a constant minus $(q\Delta\phi/2)$. Thus, neglecting the back-flux for the high-voltage gap, and using the electrochemical potential as U after Eyring and Eyring (1963), Garlid et al. (1989) derived a log-linear relationship, $\ln J = \ln J_0 + q\Delta\phi/(2kT)$, where J_0 is independent of $\Delta\phi$ and is proportional to N_A and the diffusion coefficient. Furthermore, they introduced a parameter β to generalize the above result:

$$\ln J = \ln J_0 + \beta\left(\frac{q\Delta\phi}{kT}\right) \quad (4)$$

where the β value deviates from 1/2 for a different shape of the potential.

Some sets of data for the proton leak across various membranes are replotted in Fig. 2, showing that the β value is around 1/2. Toninello et al. (1988) examined TEA^+(tetraethylammonium) and spermine. The data for TEA^+ agree with $\beta \approx 1/2$, which suggests that TEA^+ is transported by an ion leak process. However, spermine, usually tetravalent, was shown to give $\beta \approx 1/4$. Later, Toninello et al. (1992) presented evidence that spermine is transported by a specific uniporter, unlike TEA^+. This uniporter also carries spermidine and putrescine; the β value is not the universal value in each case. This strongly suggests that $\beta \approx 1/2$ is universal for the high-voltage gap-driven ion leak. Garlid et al. (1989) explained this by hypothesizing that the potential has a sharp peak at the center of the membrane near transmembrane proteins, where most of the ions pass, although its shape is trapezoidal elsewhere.

Apart from the friction force, the lipid molecules exert forces, like the van der Waals force, on an ion leaking across the membrane. Water molecules outside the membrane may also exert these forces on the ion. The length range of this attractive force is of molecular size. Thickness of the liquid-vapor interface, where local thermodynamic variables change significantly, is surely on this order (some Å) far from the critical point. The water-membrane interface

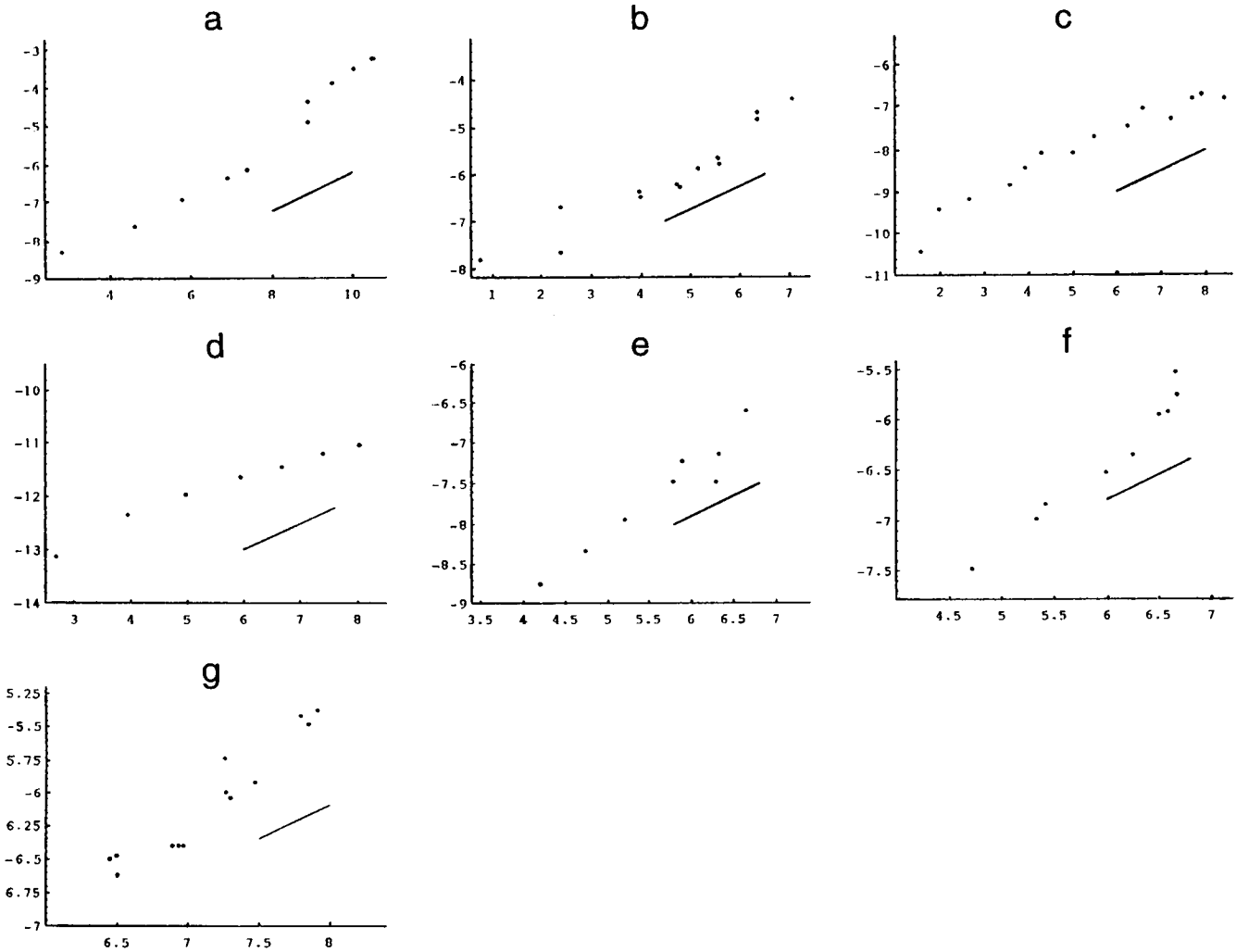

FIGURE 2 Experimental data. Some sets of experimental data for the proton leak from the literature are replotted. The Napierian logarithm of the flux (nmol/(s cm^2)) (the ordinate) is plotted against $q\Delta\phi/kT$ (the abscissa), where $\Delta\phi$ denotes the voltage gap and $q/kT = (1.6 \times 10^{-19}\ \text{C})/(4 \times 10^{-21}\ \text{J}) = 0.04(\text{mV})^{-1}$. The β value coincides with the slope in these replots; the solid lines are there to guide the eye and have a slope of exactly 1/2, corresponding with $\beta = 1/2$. (*a*) The flux-$\Delta\phi$ relationship across the cytoplasmic membrane of *Rhodopseudomonas capsulata* is measured at pH 7.0 by the use of carotenoid absorption (from the closed circles in figure 2 of Jackson, 1982). (*b*) Across the liposome membrane, around pH 7.0, obtained by the use of the K^+ ionophore (from the closed squares and circles in figure 2 and the closed circles in figure 4 of O'Shea et al., 1984). (*c*) Across the membrane of the rat liver mitochondria, at pH 7.3, obtained by the use of the K^+ ionophore (from the open and closed squares in figure 3 of Krishnamoorthy and Hinkle, 1984). (*d*) Across the liposome membrane, at pH 7.1, obtained by the use of the K^+ ionophore (from the open circles in figure 5 of Krishnamoorthy and Hinkle, 1984). (*e*) Across the membrane of the rat liver mitochondria, at pH 7.0, obtained by the use of the K^+ ionophore (from figure 2 of Brown and Brand, 1986). (*f*) Across the membrane of the rat liver mitochondria, at pH 7.4, obtained by the use of the respiration rate (from the squares and the triangles in figure 1b of Murphy et al., 1986). (*g*) Across the membrane of the rat liver mitochondria, at pH 7.2, obtained by the use of the K^+ ionophore (from table A 1.1 of Falnes, 1987).

is thicker; the z range where a lipid molecule is located is widely distributed in typical configurations of the system (Egberts et al., 1994). The potential felt by a cation at some place and caused by the interactions like the van der Waals force with water and lipid molecules depends on their temporal configuration. Roughly speaking, the potential in a typical configuration is identified with the local free energy on the condition that the cation is there. The molecular dynamics (MD) simulation by Marrink and Berendsen (1994) showed that the free energy changes significantly in an interval of $\sim$1 nm from the boundary of their region 1 and region 2 to the middle of their region 3 (see their Figures 6 and 7). We use the term "interface" or "interfacial" for this transitional region between hydrophilicity and hydrophobicity (Fig. 1), where the potential, or the free energy, changes significantly. Note the different usage of Marrink and Berendsen (1994, 1996).

As discussed by Danielli (1970) and Blank (1979), a leaking ion is not surrounded with many smaller particles but with larger lipid molecules, which are almost aligned in the z direction. Lieb and Stein (1969) suggested that a penetrant molecule within the membrane moves from one

transient pocket of free volume (or "hole") to another, like the movement within a soft polymer. Details are discussed in recent MD simulation studies. Bassolino-Klimas et al. (1995) observed that a benzene molecule makes a large jump (~0.8 nm) in the direction normal to the membrane within an elongated free volume. According to them, a jump appears to be a ballistic motion with an average velocity estimated from the Boltzmann distribution. The alignment was also pointed out (Xiang, 1993; Egberts et al., 1994); Marrink and Berendsen (1994, 1996) found that the tail alignment is predominantly in the z direction in their region 3, which is most resistant to permeation.

Thus, a leaking ion, driven by the high-voltage gap, may pass through the thin interface before experiencing sufficient random collision. Ion dynamics involving neither slowly varying potential nor frequent random collision may be relevant to the high-voltage gap-driven ion leak. Hence, even if the position dependence of the diffusion coefficient is taken into account, as suggested by Marrink and Berendsen (1994, 1996), it can be questioned whether the Smoluchowski equation is valid within the membrane, particularly when the high-voltage gap is imposed. We will discuss this point further in the last section.

As we shall see, we can derive the log-linear relationship with $\beta \approx 1/2$ for the high-voltage gap-driven ion leak by using a completely different model. We give an outline of our theory in the next section and discuss the actual application to the membrane in the third section.

GENERAL FRAMEWORK

Our starting point is to assume that ions cannot easily enter the tail layer, although they can easily leave it, because of steric hindrance due to the tails and the potential barrier. The electrolyte and the polar heads of the lipids form a hydrophilic environment, whereas the tail layer forms a hydrophobic environment. The main resistance to the current is thus assumed to be located at the interfacial region between the hydrophilic environment bulk on the entry side and the hydrophobic environment bulk, which is simply referred to hereafter as the "interfacial region" (Fig 1 *a*). Its thickness (l_{int}) is ~1 nm, as described in the Introduction, and it includes only a superficial part of the tail layer. Let us refer to this superficial part of the tail layer as the intrainterfacial sublayer (of the tails). For simplicity, we assume a surface separating the interfacial region from the hydrophilic environment bulk to be perpendicular to the z axis, and refer to this boundary surface as the entry plane.

Let us write ψ for the typical potential mentioned in the Introduction, which is typically felt by a cation at some place and caused by the interactions like the van der Waals force with water and lipid molecules. Neglecting the double-layer potential due to charges fixed at the polar heads (see e.g., Andelman, 1995), we regard the potential ψ as a constant in each of the hydrophilic and hydrophobic environment bulks. Let ϕ denote the electric potential due to the external field. Because the main resistance is assumed to be at the interfacial region, variation in ϕ is negligible elsewhere. This asymmetrical shape of ϕ with respect to the membrane center is not strange. Roughly speaking, in the present situation, an electric field is imposed on a system that is separated into two conducting subsystems by an insulator located at the interfacial region. In this picture, it is easy to see a constant electrical potential on each side of the interfacial region after charge redistribution.

Thus the sum of ψ and $q\phi$ (denoted by u) varies significantly only in the interfacial regions. We assume that, although the variation in u is negligible in the bulks as compared with in the interfacial regions, the variation in the hydrophobic environment bulk is large enough to bring ions to the exit side with negligible back-flux for the high-voltage gap. We suppose here that the lipid molecules can exert a large friction force on the leaking ion, not through frequent random collision. Note that we do not consider a case where a difference in the ion concentration drives the ion leak across the membrane (see Discussion).

For simplicity (Fig. 1 *b*), suppose

$$\psi(z) = \begin{cases} 0, & \text{for } z < z_1 \text{ and } z > z_4 \\ \{(z - z_1)/(z_2 - z_1)\}\psi_0, & \text{for } z_1 < z < z_2 \\ \psi_0(\text{: constant}), & \text{for } z_2 < z < z_3 \\ \{(z_4 - z)/(z_4 - z_3)\}\psi_0, & \text{for } z_3 < z < z_4 \end{cases} \tag{5}$$

where the hydrophilic environment bulk on the entry side, the interfacial region, the hydrophobic environment bulk, the interfacial region on the exit side, and the hydrophilic environment bulk on the exit side are bordered at $z = z_1$, z_2, z_3, and z_4, respectively, and

$$\phi(z) = \begin{cases} \sim 0, & \text{for } z < z_1 \\ -\{(z - z_1)/(z_2 - z_1)\}\phi_0, & \text{for } z_1 < z < z_2 \\ \sim -\phi_0(\text{: constant}), & \text{for } z_2 < z \end{cases} \tag{6}$$

Here we have $l_{\text{int}} \equiv z_2 - z_1 = z_4 - z_3$. We can regard $\phi_0(>0)$ as the voltage gap across the membrane $\Delta\phi$ mentioned in the Introduction.

For $z < z_3$, the sum of Eqs. 5 and 6 is

$$u(z) = \begin{cases} \sim 0, & \text{for } z < z_1 \\ (\psi_0 - q\phi_0)(z - z_1)/(z_2 - z_1), & \text{for } z_1 < z < z_2 \\ \sim \psi_0 - q\phi_0, & \text{for } z_2 < z < z_3 \end{cases} \tag{7}$$

Although the actual shapes of the potentials should be more complicated, we expect that the potential u has roughly the above shape. The potential wall has a height of

$$u_w \approx \psi_0 - q\phi_0 \tag{8}$$

which is assumed to be positive.

A leaking ion experiences not only the above potential force, but also the friction force due to the lipid molecules. We assume that the cation cannot enter the tail layer without density fluctuation of tail segments in the intrainterfacial

sublayer. It can enter there when the space between the tail segments becomes wide enough, i.e., the local density in the intrainterfacial sublayer (ρ) becomes small enough because of movement of the lipids. A wide space between tail segments soon becomes narrow; then the cation that has entered the tail layer is trapped because of the substantial friction. When the space becomes wide again, the freed cation will be brought back to the hydrophilic environment bulk on the entry side if it is trapped before reaching the top of the potential wall, and be brought to the exit side otherwise. We suppose here that the ion cannot be kicked in totally random directions by almost unidirectionally aligned tails. We introduce a threshold (ρ_h) for simplicity; the cation can freely pass in the sublayer (i.e., the "hole" is open) if $\rho < \rho_h$, whereas it loses the velocity completely (i.e., the "hole" is closed) otherwise.

Thus, in our simplified picture, the cation should have finished "climbing up" the potential wall while the "hole" is open, to leak across the membrane. Once the cation has passed through the interfacial region to enter into the hydrophobic environment bulk, it is assumed to be brought to the electrolyte bulk on the exit side. Assuming that the leaking ion never experiences sufficient random collisions in the membrane, we suppose that the slope of u in $z_2 < z < z_3$ is, although small, still large enough to make back-flux negligible. We further expect that it is possible to incorporate effectively the z-directional movement of the lipid molecules during the ion passage by adjusting the l_{int} value.

The distribution of ρ can be assumed to be Gaussian:

$$\Pr(\rho) = \frac{1}{\sigma\sqrt{2\pi}} \exp\left[-\frac{1}{2}\left(\frac{\rho - \bar{\rho}}{\sigma}\right)^2\right] \tag{9}$$

where $\bar{\rho}$ denotes the average of ρ, and σ is the standard deviation. Later we will relate ρ and σ to measurable quantities. Then the membrane compressibility is involved as discussed in Nagle and Scott (1978) and Blank (1979).

Let us next consider the velocity distribution of the cations reaching the entry plane per unit area per unit time from the hydrophilic environment bulk on the entry side. Averaged over the configurations, the number of such cations with the velocity from $\mathbf{v}$ to $\mathbf{v} + d\mathbf{v}$, where the z component v_z is positive, coincides with the cation number within a tilted-column-shaped region, which has a volume v_z and protrudes from a region with unit area on the entry plane into the hydrophilic environment bulk on the entry side. For simplicity, we neglect possible structural hindrance to ion entry due to the polar heads. Let c_b be the density of the cations in this bulk, and the above cation number is

$$f(\mathbf{v})d\mathbf{v} = c_b\left(\frac{m}{2\pi kT}\right)^{3/2} v_z \exp\left[-\frac{mv^2}{2kT}\right]d\mathbf{v} \tag{10}$$

Suppose that a cation reaches a place on the entry plane with a velocity $\mathbf{v}$ ($v_z > 0$) at $t = 0$ and that the density ρ is then smaller than ρ_h around this place. The time τ when the density subsequently increases to ρ_h for the first time is a stochastic variable. Its distribution is a function of the initial value of ρ; let it be denoted by $F(\tau; \rho(t = 0))$. The minimum of v_z required for the cation to reach the hydrophobic environment bulk before the density increases to ρ_h for the first time is a function of τ. Let this minimum be denoted by $v_z^{min}(\tau)$. As shown in the Appendix, we obtain

$$v_z^{min}(\tau) = \begin{cases} \tau u_w/(2ml_{int}) + l_{int}/\tau, & \text{for } \tau < \tau_B \equiv l_{int}\sqrt{2m/u_w} \\ \sqrt{2u_w/m}, & \text{for } \tau \geq \tau_B \end{cases} \tag{11}$$

In our picture, provided that a cation reaches the entry plane with $v_z \geq v_z^{min}(\tau)$, where the initial density $\rho(t = 0)$ is smaller than ρ_h, the cation leaks across the membrane. Here for simplicity we neglect the possible constraints on v_x and v_y required for the cation to leak. Hence the ion flux is, on average,

$$J = \int_0^{\rho_h} d\rho \Pr(\rho) \int_0^{\infty} d\tau F(\tau; \rho) \int_{v_z^{min}(\tau)}^{\infty} dv_z \int_{-\infty}^{\infty} dv_x \int_{-\infty}^{\infty} dv_y f(\mathbf{v})$$

$$= \frac{c_b}{2\pi\sigma}\sqrt{\frac{kT}{m}} \int_0^{\rho_h} d\rho \exp\left[-\frac{1}{2}\left(\frac{\rho - \bar{\rho}}{\sigma}\right)^2\right] \tag{12}$$

$$\times \left\{I_1 \exp\left[-\frac{u_w}{2kT}\right] + I_2 \exp\left[-\frac{u_w}{kT}\right]\right\}$$

where

$$I_1 \equiv \int_0^{\tau_B} d\tau F(\tau; \rho) \exp\left[-\lambda_1^2\tau^2 - \frac{\lambda_2^2}{\tau^2}\right] \tag{13}$$

and

$$I_2 \equiv \int_{\tau_B}^{\infty} d\tau F(\tau; \rho) \tag{14}$$

where

$$\lambda_1 \equiv \frac{u_w}{\sqrt{8mkTl_{int}}} \quad \text{and} \quad \lambda_2 = \sqrt{\frac{m}{2kT}}\, l_{int} \tag{15}$$

Let us stop here for a rather qualitative discussion before using some particular expressions for ρ and $F(\tau; \rho)$ in the next section. We have two factors determining the ion flux J; one is a "hole"-opening interval, and the other is steepness of the potential wall. The first factor is expressed by $F(\tau; \rho)$, the distribution of the time τ needed for the density to increase from ρ to the threshold ρ_h for the first time. The density ρ is rarely far from its average value $\bar{\rho}$ because of its Gaussian distribution $\Pr(\rho)$. Thus we have only to look at the density range just below the threshold value ρ_h in considering the ion flux J. For such an initial density ρ, the density should quickly reach the threshold ρ_h on average, which means that $F(\tau; \rho)$ should decrease rapidly as τ becomes larger. The second factor is expressed by λ_1 for the

following reason. Leaving aside a number coefficient, we can regard $\sqrt{kT/m}$ as the average of the initial velocity (see Eq. 10). Thus λ_1^{-1} roughly coincides with the time needed for its deceleration to zero due to the negative acceleration $-u_w/(ml_{int})$, and λ_2 roughly coincides with the time needed for an ion to pass through the interfacial region if it keeps the initial velocity without the deceleration. Thus the integrand of I_1 decreases because of both the terms $F(\tau; \rho)$ and $\exp[-\lambda_1^2\tau^2]$ as τ increases. When the voltage gap is large, the "hole"-opening interval is expected to be rate-limiting because of the reduced steepness. Then, as τ increases from zero to τ_B, the integrand of I_1 first increases because of the term $\exp[-\lambda_2^2/\tau^2]$, and next decreases rapidly, mainly because of the term $F(\tau; \rho)$. As a result, I_1 becomes almost independent of τ_B, λ_1, and therefore u_w. Furthermore, because of the rapid decrease in $F(\tau; \rho)$ with respect to τ, I_2 may be negligible compared with I_1. Using Eq. 8, we thus have

$$J \approx J_{const} \times \exp\left[\frac{q\phi_0}{2kT}\right] \approx J_{const} \times \exp\left[\frac{q\Delta\phi}{2kT}\right] \quad (16)$$

where

$$J_{const} \approx \frac{c_b}{2\pi\sigma}\sqrt{\frac{kT}{m}}\exp\left[-\frac{\psi_0}{2kT}\right] \times \int_0^{\rho_h} d\rho \exp\left[-\frac{1}{2}\left(\frac{\rho-\bar{\rho}}{\sigma}\right)^2\right]\int_0^{\infty} d\tau F(\tau;\rho)\exp\left[-\frac{\lambda_2^2}{\tau^2}\right] \quad (17)$$

Here, J_{const}, corresponding to J_0 in Eq. 4, is independent of the voltage gap. The above expression of J_{const} is different from that of J_0, which contains the diffusion coefficient, although both are proportional to the cation concentration in the electrolyte bulk on the entry side, which is expressed by c_b here. Thus we obtain the log-linear relationship with $\beta \approx 1/2$. Note that this 1/2 comes from the expression $mv^2/2$ for the kinetic energy of a cation in our formulation, although it is involved with the shape of the potential barrier in the theory of Garlid et al., (1989).

A deviation of β from 1/2 with $1/2 < \beta < 1$ can be derived from Eq. 12 when I_2 cannot be neglected, as discussed in the last section. Some sets of data (Fig. 2, *c* and *d*) show deviations with $\beta < 1/2$, however. Such deviations may be explained if we note that the voltage gap $\Delta\phi$ is slightly larger than ϕ_0 (see Eq. 6). For simplicity, still regarding ϕ_0 as the voltage gap $\Delta\phi$, we proceed to the following discussions.

ESTIMATION

In this section we show that the relationship between the flux and the voltage gap computed from Eq. 12 with some material constants now available can be consistent with experimental data for the proton leak. Assuming that a proton leaks as an oxyonium ion H_3O^+, we use $m = 19 \times (6 \times 10^{23})^{-1}$ g. We use $T = 300K$ (i.e., $kT = 4 \times 10^{-21}$ J) and $q = 1.6 \times 10^{-19}$ C.

Let us first estimate the ψ_0 value. Experiments revealed that the nonlinearity (i.e., the log-linear relationship) becomes explicit when the voltage gap becomes more than ~100 mV. This suggests that, for such a voltage gap value, $q\phi_0$ becomes comparable with ψ_0 (see Eq. 8). Thus we estimate $\psi_0 \approx 10^{-20}$ J. This is consistent with the free-energy difference estimated by Markin and Volkov (1989) and Marrik and Berendsen (1996). When $q\phi_0 > \psi_0$, the potential wall disappears according to Eq. 8. Actually, the membrane is experimentally shown to be perforated at some voltage gap value less than ~300 mV (El-Mashak and Tsong, 1985).

Let us next discuss the density fluctuation. A cation should pass the space surrounded with several (say three to six) tails; let us define L^2 to be the area of a section normal to the z axis where, on average, such a number of tails pierce the intrainterfacial sublayer. We may formulate opening and closing of "the hole" in terms of mass (m_L) of the tail segments in a portion of the sublayer with a cross-sectional area of L^2, which is indicated in Fig. 1 *a* by a box bordered with dashed lines. We stipulate that a cation freely passes through the sublayer when m_L is smaller than a threshold m_{Lh} (i.e., the "hole" opens). Otherwise, a cation just reaching the entry plane cannot enter the tail layer, and a cation within the tail layer loses the velocity completely (i.e., the "hole" closes).

Assuming that L is much larger than the correlation length, we regard the probability distribution of m_L as Gaussian:

$$P(m_L) = \frac{\alpha}{\bar{m}_L\sqrt{2\pi}}\exp\left[-\frac{\alpha^2}{2}\left(\frac{m_L-\bar{m}_L}{\bar{m}_L}\right)^2\right] \quad (18)$$

where $\bar{m}_L$ is the average of m_L and α is so defined that $\bar{m}_L/\alpha$ gives the standard deviation. This equation corresponds to Eq. 9.

For simplicity, we use here the single-chain model, in which each lipid molecule has one tail (mass: M_{tail}), after Ben-Shaul (1995). Assuming that each monolayer has the same total tail number N, we can relate its fluctuation to the isothermal compressibility of the lipid bilayer membrane:

$$\kappa_T \equiv -\frac{1}{A}\frac{\partial A}{\partial \Pi}\bigg|_{N,T} \quad (19)$$

where A is the area of the whole membrane Ω and Π is the two-dimensional pressure of the bilayer. Ben-Shaul (1995) claimed that the isothermal compressibility of the lipid bilayer membrane comes mainly from the free energy of the tail layer and that interaction between the two layers of the lipids is negligible for this free energy. Let $\delta\tilde{n}(\mathbf{x})$ denote the deviation at $\mathbf{x} = (x, y)$ of the number of polar heads per unit area in a monolayer from its average N/A. We then have

(see, e.g., Chapter 9 of Reichl, 1980)

$$\int_\Omega d\mathbf{x} \int_\Omega d\mathbf{x}' \langle \delta\tilde{n}(\mathbf{x})\delta\tilde{n}(\mathbf{x}')\rangle = 2\kappa_T kT \frac{N^2}{A} \tag{20}$$

where $\langle \cdots \rangle$ implies the ensemble average.

Defining $\delta\tilde{m}(\mathbf{x})$ as the deviation in mass of the tails per unit area in a monolayer from its average and $\delta m(\mathbf{x})$ as the deviation in mass of the tail segments per unit area in the intrainterfacial sublayer from its average, we introduce a parameter N_s as

$$\begin{aligned} N_s\langle \delta m(\mathbf{x})\delta m(\mathbf{x}')\rangle &= \langle \delta\tilde{m}(\mathbf{x})\delta\tilde{m}(\mathbf{x}')\rangle \\ &= M_{tail}{}^2\langle \delta\tilde{n}(\mathbf{x})\delta\tilde{n}(\mathbf{x}')\rangle \end{aligned} \tag{21}$$

Suppose that the monolayer part of the tail layer can be separated into sublayers, the most superficial of which coincides with the intrainterfacial sublayer, and that the density of the tail segments in each sublayer varies independently. Then the number of these sublayers coincides with N_s and $M_{tail} \times NL^2/(A\bar{m}_L)$ (= the average tail mass in the area of L^2 of the monolayer divided by the average segment mass in the same area of the intrainterfacial sublayer). Actually, the tail has ~20 C-C segments, each of which is ~1 Å long (see, e.g., Sackmann, 1995) and cannot move independently. Furthermore, the intrainterfacial sublayer would include some C-C segments. Thus N_s should be much less than ~20. Our hydrophilic environment bulk coincides roughly with the region 1 of Marrink and Berendsen (1994), and our hydrophobic environment bulk begins roughly at the middle of their region 3. Thus we may estimate

$$N_s \approx M_{tail} \frac{NL^2}{A} \frac{1}{\bar{m}_L} \approx 2 \tag{22}$$

Because L is assumed to be far smaller than the correlation length, Eqs. 20 and 21 produce

$$\langle (m_L - \bar{m}_L)^2\rangle = \frac{L^2 M_{tail}^2}{N_s} \times 2\kappa_T kT \frac{N^2}{A^2} \tag{23}$$

Ben-Shaul (1995) claims that the average area per tail is ~30 Å^2 and $\kappa_T \approx 2$ Å$^2/kT$. Experimentally, the average area per lipid (with two tails) and κ_T were shown to be 70 Å^2 (Mimms et al., 1981) and 3–12 Å$^2/kT$ (Kwok and Evans, 1981; Mueller and Chien, 1983; Smeulders et al., 1990), respectively. Adopting $L^2 \approx 400$Å^2 and $2\kappa_T kT \approx 5$ Å^2, we have from Eqs. 18 and 23

$$\alpha = \left(M_{tail} \frac{NL^2}{A} \frac{1}{\bar{m}_L} \right)^{-1} L \sqrt{\frac{N_s}{2\kappa_T kT}} \approx 6 \tag{24}$$

The stochastic variable m_L is Markovian because the lipids collide so randomly with each other. Thus $F(\tau; \rho)$ would satisfy a differential equation of the first order with respect to τ. Considering that our present aim is only an order estimation of J, we assume that the distribution of $m_L(t)$ under the initial condition that m_L ($t = 0$) is definite remains a sharp peak around its average, instead of solving the differential equation explicitly. We expect that this is permitted for the short-time behavior of $m_L(t)$, which is relevant to J because the contribution to J from the $m_L(t = 0)$ range far smaller than m_{Lh} would be negligible because of Eq. 18. The average should evolve as (see, e.g., Chapter 13 of Reichl, 1980)

$$\langle m_L(t)\rangle - \bar{m}_L = \{m_L(t = 0) - \bar{m}_L\}e^{-\Gamma t} \tag{25}$$

because L is assumed to be much larger than the correlation length. Thus the distribution of the time τ needed for the density to increase to m_{Lh} for the first time is given by

$$F(\tau; m_L(t = 0)) = \delta(\tau - \tau_s(m_L(t = 0))) \tag{26}$$

where δ means Dirac's delta function and

$$\tau_s(m_L(t = 0)) \equiv \frac{1}{\Gamma} \ln \frac{m_L(t = 0) - \bar{m}_L}{m_{Lh} - \bar{m}_L} \tag{27}$$

As an estimate of the damping coefficient Γ, we may take the time characteristic of chain conformational transition. Here we use $\Gamma \approx 10^{12}$ s^{-1} (Mayer et al., 1988). This estimate is consistent with the results of Bassolino-Klimas et al. (1993, 1995) and Marrink and Berendsen (1994).

Now we can rewrite Eq. 12 as

$$\begin{aligned} J &\approx \int_0^{m_{Lh}} dm_L P(m_L) \int_{v_z^{min}(\tau_s(m_L))}^{\infty} dv_z \int_{-\infty}^{\infty} dv_x \int_{-\infty}^{\infty} dv_y f(\mathbf{v}) \\ &= \frac{\tilde{\alpha} c_b}{2\pi} \sqrt{\frac{kT}{m}} \int_0^{\eta_u} d\eta \exp\left[-\frac{1}{2}\tilde{\alpha}^2(1 + \eta)^2 - G(\eta)^2 \right] \end{aligned} \tag{28}$$

where

$$\eta \equiv \frac{m_{Lh} - m_L}{\bar{m}_L - m_{Lh}}, \quad \eta_u \equiv \frac{m_{Lh}}{\bar{m}_L - m_{Lh}}, \quad \tilde{\alpha} \equiv \frac{\alpha}{1 + \eta_u} \tag{29}$$

and

$$G(\eta) \equiv \begin{cases} (\lambda_1/\Gamma)\ln(1 + \eta) + \lambda_2\Gamma/\ln(1 + \eta), \\ \qquad \text{for } \eta < \eta_B \equiv \exp[\Gamma\sqrt{\lambda_2/\lambda_1}] - 1 \\ \sqrt{u_w/kT}, \\ \qquad \text{for } \eta \geq \eta_B \end{cases} \tag{30}$$

We can choose the parameter values consistent with the above discussions so that the values of the right-hand side of Eq. 28 fit well with the data in Fig. 2, *a* and *g*, as shown in Fig. 3, *a* and *b*, respectively. Our aim here is not to predict the parameter values in each system, but to show that our result, Eq. 12, can be consistent with experimental data under some reasonable parameter values.

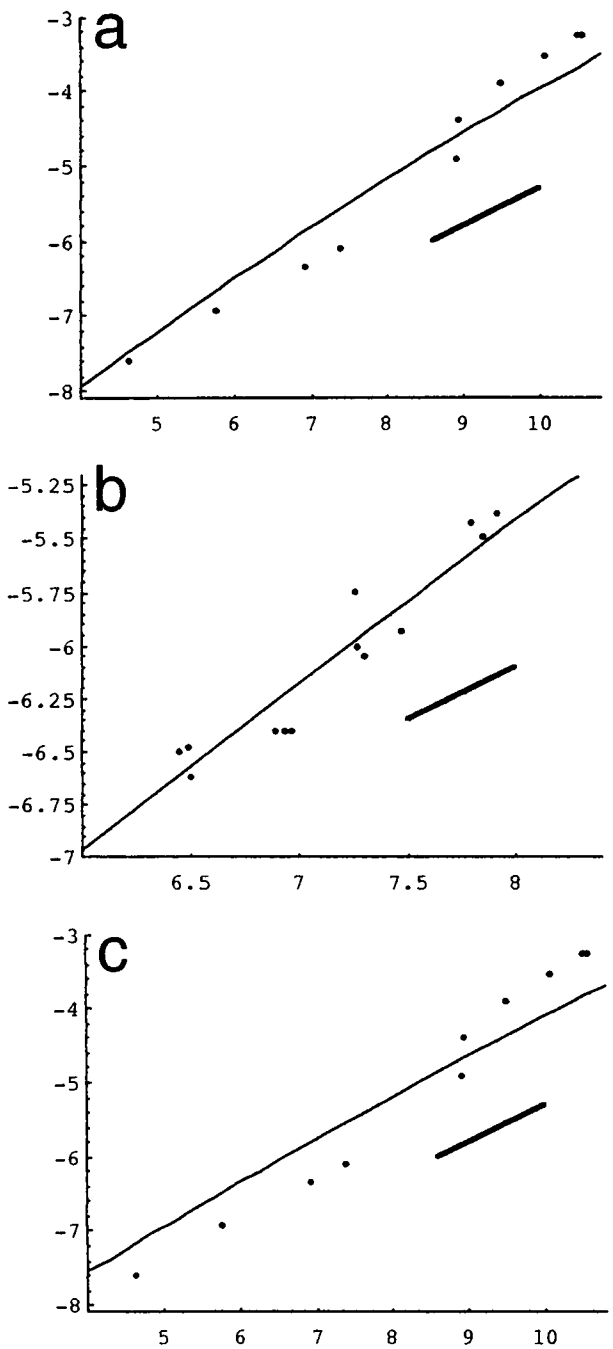

FIGURE 3 Plots of Eq. 28. The Napierian logarithm of J (nmol/(s cm^2)) is calculated by computer according to Eq. 28, and is plotted against $q\phi_0/kT$, as in Fig. 2 (*thin solid curves*). The thick solid lines are there to guide the eye and have a slope of exactly 1/2, corresponding to $\beta = 1/2$. We used $\alpha = 6$ and $\psi_0 = 4.8 \times 10^{-20}$ J; see below and the beginning of the third section of the text for the other parameter values used. (*a*) The points come from the data shown in Fig. 2 *a*. Because the nonlinearity is thought to become explicit above ~100 mV, only this range is shown here. $\Gamma l_{int} = 8 \times 10^2$ m/s, $c_b = 10^{-7}$ mol/liter, and $\eta_u = 3.09$ (i.e., $m_{Lh} = 0.76\ \bar{m}_L$). (*b*) The points are the same as those in Fig. 2 *g*. $\Gamma l_{int} = 3 \times 10^2$ m/s, $c_b = 10^{-7.2}$ mol/liter and $\eta_u = 1.51$ (i.e., $m_{Lh} = 0.60\ \bar{m}_L$). (*c*) The points come from the data shown in Fig. 2 *a*, as in Fig. 3 *a*. $\Gamma l_{int} = 8 \times 10^2$ m/s, $c_b = 1 \times 10^{-1}$ mol/liter and $\eta_u = 0.92$ (i.e., $m_{Lh} = 0.48\ \bar{m}_L$).

DISCUSSION

This paper focuses on the nonohmicity of the ion leak across the lipid-bilayer membrane. In the ohmic range, the current-voltage relationship for the ion leak has been studied primarily to gain insight into the conductance, which can be related to the permeability coefficient (see, e.g., Marrink et al., 1996a). Two anomalies of the proton leak are well known (Deamer, 1987; Deamer and Nichols, 1989): one is its permeability coefficient, which is much larger than expected from data for other monovalent cations, and the other is the relative independence of its conductance from the ambient pH value. The high-voltage-gap regime should also have both anomalies, but our result fails to explain them (note $J_{const} \propto c_b$ in Eq. 17). The current explanation for them is based on the transient hydrogen-bonded chain (tHBC) (Nagle, 1987) along the water pore across the membrane produced by thermal fluctuation (Nichols and Deamer, 1980). A similar explanation for anomalously high proton conductance through the gramicidin channel has been substantiated (see, e.g., Pomès and Roux, 1996). However, a recent MD simulation study (Marrink et al., 1996a) showed that this model, as it stands, fails to explain experimental data of the proton leak and suggested that the high proton concentration near the membrane due to the existence of buffers should be taken into account. Our model for the high-voltage gap-driven ion leak may also be improved by consideration of this buffer effect. Garlid et al. (1989) also argued that the effective concentration is not equal to the concentration in the bulk. This anomaly problem clearly needs further investigation. As in the preceding section, we can choose the parameter values to fit our result for the data, even if we set c_b much larger than expected from the pH value of the electrolyte bulk (Fig. 3 *c*). This is possible because the computed current value is sensitive to the density threshold (m_{Lh}).

In each of Fig. 3, *a–c*, the slope of the computed curve, indicating the β value, decreases slightly to approach 1/2 as the voltage gap increases. The reason is as follows. As the imposed voltage gap increases, the steepness of the potential wall becomes smaller and less effective in determining the ion flux; the "hole"-opening interval becomes more effective instead. Then, as discussed just below Eq. 15, the β value is reduced to approach 1/2. However, we cannot expect to detect such a slight change in the experimental data now available.

We can similarly explain why the overall slope of the computed curve of Fig. 3 *b* is the largest in Fig. 3. A smaller Γl_{int} value results in a steeper potential wall in Fig. 3 *b* than in any other graph of Fig. 3; the λ_1 value for the same voltage gap is larger in Fig. 3 *b*. This makes the "hole"-opening interval less effective in determining the ion flux. As a result, the slope of the computed curve in Fig. 3 *b* deviates the most from 1/2.

Garlid et al. (1989) attributed high permeability of biomembranes as compared with pure lipid bilayers to reduc-

tion and thinning of the energy barrier near a membrane protein. However, this high permeability may come from the modification in size, duration, and frequency of the elongated free volume near a membrane protein. Thus the sharp peak near a membrane protein is not the only explanation for the high permeability. Rather, we guess that the barrier shape remains trapezoidal, even near a transmembrane protein, because many hydrophobic amino acid residues of intramembrane portions of a transmembrane protein also repel water molecules.

O'Shea et al. (1984) and Krishnamoorthy and Hinkle (1984) reported that the relationship between the current (or ion flux) and the pH gap remains linear, even for a high pH gap regime, unlike the current-voltage relationship. Thus mechanisms are expected to be different between the case where the driving force is the electric field and the case where it is the concentration difference (or chemical potential difference). (See Verkman (1987) and Deamer and Nichols (1989) for different results.) Surely our present model holds only for the former case. The back-flux cannot be neglected in the latter case because the leaking ion should feel a flat potential in the hydrophobic environment bulk. (See the third paragraph of the second section of this paper.)

When we look at a time scale or a length scale on which the velocity correlation is negligible, we can use the diffusion picture where the position of a penetrant molecule is described as a Markovian process, even in the soft polymer (Müller-Plathe et al., 1992). Even if there is an external potential, as long as it does not change appreciably on this scale, we can still use the Smoluchowski equation in the diffusion picture. In the high-voltage gap-driven ion leak, however, it is probable that the diffusion picture breaks down all the more because the rate-limiting step occurs at the thin interfacial region.

In the absence of a voltage gap, MD simulations suggest that a transient pore across the membrane and a large jump of an ion are rare. This appears to favor the diffusion of penetrants among the tails (Marrink and Berendsen, 1994, 1996; Bassolino-Klimas et al., 1995). However, Chakrabarti and Deamer (1992) and Jansen and Blume (1995) could not use this picture in interpreting their experimental data. Paula et al. (1996) suggested that diffusion among tails does not work well for the ion leak when the membrane is thin.

Even if the large jump is also rare in the high-voltage gap regime, it is possible that each ion of a significant part of all the ions that have leaked across the membrane got over the potential wall by means of a large jump. If this is the case, change of the average position of the whole ions (or the ion flux) is decided by this rare large-jump process, and thus cannot be described by the diffusion picture.

Whether the diffusion picture is valid for the high-voltage gap-driven ion leak is not yet decided. Here we have shown that we can derive the log-linear relationship with the universality $\beta \approx 1/2$ for the high-voltage gap-driven ion leak without resorting to the diffusion picture.

APPENDIX

Here we assume that the hole is always open. As in the text, v denotes the velocity with which a cation reaches the entry plane. If we neglect possible constraints to v_x and v_y, the cation can enter the hydrophobic environment bulk only if $v_z > \sqrt{2u_w/m}$ because of the energy conservation law. Then the time required for the cation to enter this bulk is

$$t_{esc} = \frac{v_z - \sqrt{(v_z)^2 - 2(u_w/m)}}{u_w/(ml_{int})} \tag{A1}$$

which is the smaller one of the solutions of an integral of Newton's equation of motion:

$$v_z t_{esc} - \frac{u_w}{2ml_{int}} t_{esc}^2 = l_{int} \tag{A2}$$

Equation A1 yields

$$v_z = \frac{t_{esc}u_w}{2ml_{int}} + \frac{l_{int}}{t_{esc}}, \quad \text{for } t_{esc} < l_{int}\sqrt{\frac{2m}{u_w}} \tag{A3}$$

which leads to Eq. 11.

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