# ION MOVEMENT THROUGH GRAMICIDIN A CHANNELS

## Studies on the Diffusion-controlled Association Step

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ABSTRACT The permeability characteristics of gramicidin A channels are generally considered to reflect accurately the intrinsic properties of the channels themselves; i.e., the aqueous convergence regions are assumed to be negligible barriers for ion movement through the channels. The validity of this assumption has been examined by an analysis of gramicidin A single-channel current-voltage characteristics up to very high potentials (500 mV). At low permeant ion concentrations the currents approach a voltage-independent limiting value, whose magnitude is proportional to the permeant ion concentration. The magnitude of this current is decreased by experimental maneuvers that decrease the aqueous diffusion coefficient of the ions. It is concluded that the magnitude of this limiting current is determined by the diffusive ion movement through the aqueous convergence regions up to the channel entrance. It is further shown that the small-signal (ohmic) permeability properties also reflect the existence of the aqueous diffusion limitation. These results have considerable consequences for the construction of kinetic models for ion movement through gramicidin A channels. It is shown that the simple two-site-three-barrier model commonly used to interpret gramicidin A permeability data may lead to erroneous conclusions, as biionic potentials will be concentration dependent even when the channel is occupied by at most one ion. The aqueous diffusion limitation must be considered explicitly in the analysis of gramicidin A permeability characteristics. Some implications for understanding the properties of ion-conducting channels in biological membranes will be considered.

### INTRODUCTION

An important feature of the gramicidin A channel selectivity among alkali metal cations and $H^+$ is that it ranks similarly to the ion mobilities in aqueous solution: $P_{Li} < P_{Na} < P_K < P_{Rb} \approx P_{Cs} < P_H$ (Myers and Haydon, 1972). This does not imply that the gramicidin A channel is simply a water-filled hole in the membrane through which the ions permeate together with their hydration shells. The dimensions of the channel (the luminal diameter is ~4 Å [Urry, 1972; Finkelstein and Andersen, 1981]) and the single-file flux-coupling between ions and water (Levitt et al., 1978; Rosenberg and Finkelstein, 1978) are both incompatible with this possibility. An alternative interpretation of the selectivity data is obtained by noting that the relative permeabilities of ion conducting channels are largely determined by the ratios of the association rate constants (Bezanilla and Armstrong, 1972). The approximate correspondence between the permeability characteristics of the gramicidin A channel and the aqueous diffusion coefficients of the permeant ions could thus indicate that the magnitude of the overall association rate constant for ion entry into the channel is significantly determined by the diffusion-controlled rate constant for ion movement through the aqueous phase up to the channel entrance.[1] (Noyes, 1961; Amdur and Hammes, 1966; Schurr, 1970; and Eigen, 1974 should be consulted for discussions of diffusion limitations in chemical kinetics.)

Whether aqueous diffusion limitations in fact pose significant problems for ion movement through gramicidin A channels has been the subject of considerable debate. The arguments have mainly been theoretical, based upon comparisons of measured small-signal single-channel conductances and their theoretical upper limits (see Theory). It has generally been concluded that aqueous diffusion limitations play little role, if any, in determining the rate of ion movement through the channels (Hladky and Haydon, 1972; Läuger, 1976; Sandblom et al., 1977; Tredgold and Jones, 1979; Urban et al., 1980). The alternative view, that aqueous diffusion limitations may be an important determinant of the channel permeability characteristics, has

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[1]This is not a unique conclusion because the association rate constant could be radius dependent but not diffusion controlled, because, for example, the rate constant for ion entry into the channel is limited by the rate constant for exchange of $H_2O$ in the inner coordination shell of the ions (Eigen, 1963). The approximate correspondence between selectivity and aqueous diffusion coefficients would, in this case, show that both phenomena depend upon the ionic radii.

nevertheless had some support (Andersen, 1978; Levitt, 1978 *b*; Andersen and Procopio, 1980). The disagreement, and the meager experimental effort put into resolving this problem, could imply that the question is unimportant, but this is not the case. On the contrary, neglect of diffusion limitations that may exist will lead to quite erroneous conclusions about the molecular details of ion movement through the channel: the number of ions that can simultaneously occupy the channel may be overestimated, and estimates of dissociation constants for the first ion binding into the channel may be seriously in error. It is thus of considerable importance to establish to what extent aqueous diffusion limitations can affect the permeability characteristics of gramicidin A channels.

The purpose of the investigations reported in this series of articles was therefore to undertake an experimental investigation of the importance of aqueous diffusion limitations for ion movement through gramicidin A channels. The impetus for these studies was the observations that gramicidin A single-channel currents at low permeant ion concentrations and very high potentials appeared to reach a voltage-independent limiting value, and that the magnitude of this limiting current was consistent with diffusion-controlled ion movement through the aqueous phases up to the channel entrance (Andersen, 1978). This suggested that the question of aqueous diffusion limitations could be approached as follows: First, single-channel current-voltage characteristics should be measured at low permeant ion concentrations to ascertain whether a voltage-independent limiting current can, in fact, be observed at very high, but still attainable, potentials; second, the characteristics of these limiting currents should be studied to establish whether their magnitudes are reasonably consistent with a diffusion-controlled process and whether their magnitudes vary appropriately when the aqueous diffusion coefficient of the permeant ions is varied; third, the (diffusion-controlled) limiting currents should be used to calculate upper limits on the small-signal (ohmic), single-channel conductances, and these limiting conductances should be compared to the measured small-signal conductances to achieve a measure of how severe the aqueous diffusion limitations are. This approach, while indirect, has the advantage that it circumvents the problems associated with a direct calculation of the diffusion-controlled limit on the small-signal conductance (see the Discussion).

The first article (Andersen, 1983 *a*) showed that this approach is feasible: It is possible to obtain single-channel current measurements at very high potentials, where the currents at low permeant ion concentration become almost voltage independent. It was also shown that the magnitudes of the currents are qualitatively consistent with a diffusion-controlled process. The second article (Andersen, 1983 *b*) showed that the residual voltage dependence of the currents at this high potentials is an artifact, due to the interfacial polarization associated with applying the large potentials across a membrane-bound channel. The purpose of the experiments reported in this article is to study what physico-chemical factors may determine the magnitude of the voltage-independent limiting currents observed at high potentials. It will conclude that these currents indeed are diffusion controlled. Some of this material has been presented in preliminary form (Andersen and Procopio, 1978; 1980).

## THEORY

A measured single-channel conductance will always reflect the magnitude of the conductance of the aqueous convergence regions at both ends of the channel as well as the magnitude of the intrinsic conductance of the channel itself.[2] The small-signal (ohmic) conductance of the channel $[g(c', c'')]$ can be expressed as (Läuger, 1976)

$$1/g(c', c'') = 1/g'_a(c') + 1/g_i(c', c'') + 1/g''_a(c'') \qquad (1)$$

where $g'_a(c')$ and $g''_a(c'')$ are the small-signal conductances of the left and right aqueous convergence regions, respectively; $g_i(c', c'')$ is the intrinsic channel conductance; and $c'$ and $c''$ are the permeant ion concentrations in the left and right aqueous phases, respectively. There are two limiting conditions of Eq. 1 that are of special interest. When $g_i(c', c'')$ is much less than both $g'_a(c')$ and $g''_a(c'')$

$$g(c', c'') = g_i(c', c''). \qquad (2)$$

The measured channel conductance will in this case provide an accurate reflection of the interactions that occur between the channels and the permeating ions. But when $g_i(c', c'')$ is much larger than both $g'_a(c')$ and $g''_a(c'')$, the single-channel conductance will approach its maximal value for the given permeant ion concentrations, $g_{max}(c', c'')$:

$$g_{max}(c', c'') = g'_a(c') \cdot g''_a(c'')/ [g'_a(c') + g''_a(c'')] \qquad (3)$$

or, for a symmetrical channel and symmetrical aqueous phases ($c' = c'' = c$),

$$g_{max}(c) = g_a(c)/2. \qquad (4)$$

The measured single-channel conductance is in this case determined exclusively by the aqueous convergence regions. It provides therefore very different information about the channel than could be provided by $g_i(c)$. It is thus important to be able to estimate the magnitude of the convergence conductance to test whether or not $g(c)$ is close to $g_{max}(c)$.

The aqueous convergence conductance can be expressed as

$$g_a(c) = e \cdot F \cdot p_a \cdot c/kT \qquad (5)$$

where $e$ is elementary change, $F$ is Faraday's constant, $k$ is Boltzmann's constant, $T$ is the temperature in Kelvin and $p_a$ is the access permeability or convergence permeability of the aqueous phase (in $cm^3/s$). The magnitude of the convergence permeability is determined by the proper-

[2]The intrinsic conductance of the channel is defined as the conductance which would be measured in a *Gedanken* experiment where the aqueous diffusion coefficient of the permeant ion is made infinitely large, while all other properties of the aqueous solution and channel are kept constant. This definition is useful, but also somewhat artificial. The spatial extent of the aqueous convergence regions is so limited (<10 Å for the gramicidin A channel) that their transport characteristics will be unaffected by stirring the bulk aqueous phases. The convergence regions are therefore not conventional unstirred layers; on the contrary, the aqueous convergence regions are integral characteristics of a channel and the surrounding membrane.

ties of the channel itself and by the properties of the permeating ion (Läuger, 1976)

$$p_a = f \cdot r_o \cdot D \tag{6}$$

where $f$ is a factor whose magnitude depends upon the particular geometry assumed for the channel entrance (it will in the following be assumed that $f = 2 \cdot \pi$).[3] $r_o$ is the capture radius of the channel for the ion in question (see below), and $D$ is the aqueous diffusion coefficient of the ion.[4]

The capture radius is an operational parameter, which expresses how accessible the channel is to the ion in question. The magnitude of $r_o$ depends upon the spatial extent of the channel entrance, the attractive or repulsive forces which act between the channel and the ion and, inexorably, the actual geometry of the convergence region and the transport properties of the aqueous phase close to the channel-matters about which considerable ignorance exists. In the simplest case, where the channel is represented as a right-circular cylinder perpendicular to the plane of the membrane and the characteristics of the convergence regions are assumed to be similar to those at the bulk aqueous phase, $r_o$ can be expressed as (Ferry, 1936; Läuger, 1976)

$$r_o = r_c - r_i \tag{7}$$

where $r_c$ is the luminal radius of the channel while $r_i$ is the relevant radius of the permeant ion. If the ion has no lateral hydration as it moves through the channel this radius is the radius estimated from crystals of the ion with oxygen-containing ligands (see Hille, 1975 *a*). From Eqs. 4–7 one finally obtains a reasonable approximation to $g_{max}(c)$:

$$g_{max}(c) = e \cdot F \cdot \pi \cdot r_o \cdot D \cdot c/kT \tag{8}$$

$$= e \cdot F \cdot \pi \cdot (r_c - r_i) \cdot D \cdot c/kT. \tag{9}$$

The comparative coarseness of this estimate makes it necessary to have a more direct estimate of $g_{max}(c)$. Such an estimate is possible because the measured single-channel currents at very high potentials should ideally (in the absence of interfacial polarization) approach a voltage-independent limiting current, $i_{lim}$. If there are no voltage-independent transitions for ion movement intrinsic to the channel the magnitude of this current will be determined by ion movement through the aqueous convergence regions. $i_{lim}$ should in this situation equal the purely diffusion-controlled current, $i_D$, when there are no electrical potential gradients in the aqueous convergence regions (Läuger, 1976):

$$i_{lim} = i_D = F \cdot p_a \cdot c \tag{10}$$

$$= F \cdot 2 \cdot \pi \cdot r_o \cdot D \cdot c \tag{11}$$

or

$$g_{max}(c) = e \cdot i_{lim}/(2 \cdot kT). \tag{12}$$

[3]Explicit expressions for $f$ can be obtained for certain simple geometries (see Appendix A). While all these expressions are of questionable validity at the molecular level, the description of ion movement through the convergence regions becomes particularly simple if the channel entrance is assumed to be a hemispherical surface protruding from a plane membrane solution-interface (Hille, 1970; Läuger, 1976). In this case $f = 2 \cdot \pi$.

[4]The gramidicin A channels move by lateral diffusion in the plane of the membrane. $D$ must be therefore be considered an effective diffusion coefficient. The magnitude of $D$ will be a function of the aqueous diffusion coefficient of the ion, $D_{aq}$, and of the lateral diffusion coefficient of the channel, $D_l$. This latter parameter is on the order of $10^{-7}$ cm$^2$/s (Tank et al., 1981). The correction for lateral diffusion should therefore be minimal in the present case, as $D_{aq} \simeq 100 \times D_l$, and $D$ should be indistinguishable from $D_{aq}$.

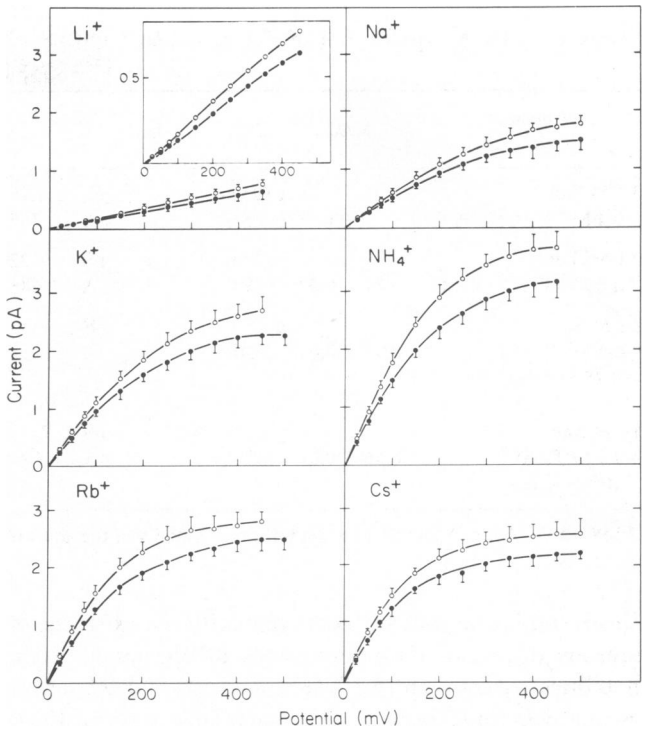


FIGURE 1 Gramicidin A single-channel current-voltage characteristics in $H_2O$ and in $D_2O$. Each point indicates the mean value of the current, plus respectively minus the standard deviation. ○, experiments with 0.1 M salt (as chloride) + 0.4 M TEACl in $H_2O$. ●, similar experiments but using $D_2O$ as the solvent. To facilitate the visual comparison of different records, they are all drawn to the same scale (except for the $Li^+$ *inset*). The solid curves have no theoretical significance. DPhPC, 25°C.

## MATERIALS AND METHODS

The experiments were done as single-channel current measurements on gramicidin A channels in either diphytanoylphosphatidylcholine/*n*-decane (DPhPC) or glycerolmonooleate/*n*-decane (GMO) membranes using an isolated bilayer patch clamp technique. The procedures and materials were those described in Andersen (1983 *a*).

## RESULTS

### Effects of Changing the Aqueous Diffusion Coefficients of the Permeant Ions

*Experiments with Diphytanoylphosphatidylcholine Membranes.* Fig. 1 illustrates gramicidin A single-channel current-voltage characteristics obtained with 0.1 M XCl (where $X^+$ represents the permeant ion) + 0.4 M TEACl and using either $H_2O$ and $D_2O$ as solvent. The currents measured in the $D_2O$ solutions were at all potentials less than the currents measured in $H_2O$ solutions.[5]

[5]A few experiments were done with $Li^+$ in GMO membranes to confirm that the substitution of $D_2O$ for $H_2O$ has qualitatively the same effect as in DPPC membranes [$i$(100) decreased from 0.192 ± 0.022 pA (mean ± SD) to 0.167 ± 0.024 pA]. The possible discrepancy between this result and the result of Tredgold and Jones (1979) will be taken up in the Discussion.

TABLE I

GRAMICIDIN A SINGLE CHANNEL CONDUCTANCES AND CURRENTS AS FUNCTIONS OF THE CONDUCTIVITY OF THE AQUEOUS PHASES

| Aqueous phase | $\lambda_s/\lambda_0$ | SD | Units | $Li^+$ | $Na^+$ | $K^+$ | $Rb^+$ | $Cs^+$ | $NH_4^+$ |
|---|---|---|---|---|---|---|---|---|---|
| 0.1 M Salt | | $g$ (25) | pS | 1.46 ± 0.28 | 6.26 ± 1.21 | 11.8 ± 1.8 | 18.7 ± 2.2 | 17.4 ± 1.8 | 19.1 ± 3.2 |
| +0.4 M TEACl in $H_2O$ | 1.00 | $i$ (450) | pA | 0.765 ± 0.095 | 1.74 ± 0.13 | 2.68 ± 0.31 | 2.80 ± 0.16 | 2.58 ± 0.20 | 3.78 ± 0.29 |
| 0.1 M Salt | | $g$ (25) | pS | 1.22 ± 0.19 | 5.07 ± 0.97 | 10.3 ± 1.5 | 14.5 ± 1.9 | 14.9 ± 1.8 | 16.4 ± 1.6 |
| +0.4 M TEACl in $D_2O$ | 0.81 ± 0.03 | $i$ (450) | pA | 0.645 ± 0.123 | 1.48 ± 0.15 | 2.26 ± 0.13 | 2.48 ± 0.19 | 2.22 ± 0.12 | 3.23 ± 0.32 |
| 0.1 M Salt | | $g$ (25) | pS | 1.33 ± 0.22 | 5.73 ± 0.99 | 11.4 ± 1.4 | 16.2 ± 1.8 | 15.3 ± 1.8 | 18.3 ± 1.9 |
| +0.4 M TEACl | 0.99 ± 0.03 | $i$ (450) | pA | | 1.66 ± 0.16 | 2.51 ± 0.22 | 2.68 ± 0.25 | 2.39 ± 0.17 | 3.54 ± 0.26 |
| +0.75 M Urea | | | | | | | | | |
| 0.1 M Salt | | $g$ (25) | pS | 1.36 ± 0.36 | 5.52 ± 0.93 | 10.0 ± 1.2 | 14.3 ± 1.7 | 14.5 ± 2.0 | 16.9 ± 1.6 |
| +0.4 M TEACl | 0.66 ± 0.01 | $i$ (450) | pA | 0.698 ± 0.056 | 1.58 ± 0.21 | 2.29 ± 0.27 | 2.33 ± 0.24 | 2.06 ± 0.19 | 3.29 ± 0.32 |
| +20% Sucrose | | | | | | | | | |

DPhPC, 25°C. Mean ± SD. $\lambda_s/\lambda_0$ denotes the conductivity of the aqueous phases relative to 0.1 M XCl + 0.4 M TEACl where $X^+$ is the permeant ion.

This result is qualitatively consistent with the existence of aqueous diffusion limitations, since alkali metal cation mobilities are between 16.5% ($K^+$ and $Cs^+$) and 21% ($Li^+$) less in $D_2O$ than in $H_2O$ (Chittum and LaMer, 1937; Longsworth and MacInnes, 1937; Swain and Evans, 1966). The average decrease in $i$(450) was 14 ± 2% (mean ± SD) for $K^+$, $Rb^+$, $Cs^+$, and $NH_4^+$, in reasonable agreement with the changes in diffusion coefficients.

Similar results were found when single-channel current-voltage characteristics measured in 0.1 M XCl + 0.4 M TEACl were compared with the characteristics measured in 0.1 M XCl + 0.4 M TEACl + 20% (0.58 M) sucrose. Table I summarizes the results from these experiments, as well as from experiments done with 0.1 M XCl + 0.4 M TEACl + 0.4 M TEACl + 0.75 M urea. (These last experiments were control experiments, to see what extent any current changes could be simply a result of the changes in bulk aqueous water activity produced by the sucrose addition; 0.75 M urea is isomotic with 0.58 M sucrose [Weast, 1972]. Urea was chosen as the reference solute because it is not permeable through the gramicidin A channel [Finkelstein, 1974; Andersen and Procopio, 1980], and does not affect the aqueous conductivity at low [urea] concentrations.) Table II compares the changes in $i$(450) and $g$(25) to the changes in aqueous diffusion coefficients of the permeant ions. A decrease in diffusion coefficient is, indeed, associated with a decrease in $i$(450) and $g$(25). But the effect of sucrose cannot be attributed solely to its effects on the aqueous viscosity (or the diffusion coefficient of the permeant ions). The addition of 0.75 M urea produces appreciable changes in both $i$(450) and $g$(25) without significant effects on the aqueous conductivity. The effect of sucrose is therefore probably best quantitated relative to the urea data. Table II lists both ways of expressing the data.

The aqueous diffusion coefficient of the permeant ion can also be altered by changes in temperature. Fig. 2 shows data obtained with 0.1 M CsCl + 0.4 M TEACl. $i$(450) varies between 1.57 ± 0.18 pA (mean ± SD) at 6.5°C and 3.74 ± 0.28 pA at 40.5°C. The Arrhenius activation energy for $i$(450), $E_a$(450), is calculated as (Amdur and Hammes, 1966):

$$d \ln [i(450)]/d\,(1/T) = -E_a(450)/R \qquad (13)$$

where $R$ is the gas constant. The value of $E_a$(450) is estimated to be 19.3 ± 0.9 kJ/mol (mean ± SD), which compares well with the activation energy for the aqueous diffusion coefficient for $Cs^+$, 16.6 kJ/mol.[6] The activation energy for $g$(25) is 23.9 ± 1.5 kJ/mol.

The current changes are graded, in the sense that they are more or less proportional to the changes in aqueous diffusion coefficients. This is illustrated in Fig. 3 which depicts the current changes seen when 10% (0.29 M) and 20% sucrose were added to 0.1 M CsCl. The current changes induced by the changes in diffusion coefficient changes are most pronounced for the most permeant ions: Very small current changes were seen when 20% sucrose was added to 0.1 M NaCl (Fig. 3). Sucrose cannot exert its effect simply by plugging up the channel entrance.

The data in Fig. 3 are affected by interfacial polarization effects because the experiments were done without support electrolyte (Andersen, 1983 *b*). The current-voltage characteristics cannot, therefore, reach a true voltage-independent limiting current. The single-channel currents at very high potentials will, instead, be linear functions of the applied potential (Andersen, 1983 *b*):

$$i(V) = F \cdot p_a \cdot c \cdot [1 + e \cdot C_m^* \cdot V/(kT \cdot \epsilon_o \cdot \epsilon_r^{H_2O}/L_D)] \qquad (14)$$

where $i(V)$ is the single-channel current as a function of $V$, the potential applied across the membrane, $C_m^*$ is the specific geometric capacitance of the membrane and chan-

[6]Calculated from the temperature variations of the limiting equivalent conductivity (Robinson and Stokes, 1965, Appendix, Table 6.2).

TABLE II

RELATIVE VARIATIONS IN GRAMICIDIN A SINGLE-CHANNEL CONDUCTANCES AND CURRENTS AS FUNCTIONS OF CHANGES IN THE DIFFUSION COEFFICIENT OF THE PERMEANT IONS

| | | | $Li^+$ | $Na^+$ | $K^+$ | $Rb^+$ | $Cs^+$ | $NH_4^+$ |
|---|---|---|---|---|---|---|---|---|
| 0.1 M Salt + 0.4 M TEACl in $D_2O$ | $D/D_0$ | | 0.79* | 0.829* | 0.835‡ | 0.84‖ | 0.83‡ | 0.80‖ |
| | $g/g_0$ | (25 mV) | 0.836 | 0.810 | 0.873 | 0.775 | 0.856 | 0.859 |
| vs. | | | | | | | | |
| 0.1 M Salt + 0.4 M TEACl in $H_2O$ | $i/i_0$ | (450 mV) | 0.843 | 0.851 | 0.843 | 0.886 | 0.860 | 0.854 |
| 0.1 M Salt +0.4 M TEACl +20% Sucrose | $D/D_0$ | | 0.61§ | 0.62§ | 0.63§ | 0.64‖ | 0.61‖ | 0.63‖ |
| | $g/g_0$ | (25 mV) | 0.932 | 0.882 | 0.847 | 0.765 | 0.833 | 0.885 |
| vs. | | | | | | | | |
| 0.1 M Salt +0.4 M TEACl | $i/i_0$ | (450 mV) | 0.912 | 0.908 | 0.854 | 0.832 | 0.798 | 0.870 |
| 0.1 M Salt +0.4 M TEACl +20% Sucrose | $D/D_0$ | | 0.66‖ | 0.65‖ | 0.66‖ | 0.64‖ | 0.67‖ | 0.66‖ |
| | $g/g_0$ | (25 mV) | 1.023 | 0.963 | 0.877 | 0.883 | 0.948 | 0.923 |
| vs. | | | | | | | | |
| 0.1 M Salt +0.4 M TEACl +0.75 M Urea | $i/i_0$ | (450 mV) | 1.01¶ | 0.952 | 0.912 | 0.869 | 0.862 | 0.929 |

DPhPC, 25°C. $D/D_0$ denotes the relative changes in diffusion coefficient.

*From Chittum and LaMer (1937).

‡From Swain and Evans (1966). It is assumed that the relative changes are unaffected by the presence of TEACl.

§From Robinson and Stokes (1965, Table 11.5). It is again assumed that the relative changes are unaffected by the presence of TEACl.

‖From conductivity measurements on the aqueous solutions. It is assumed that the transference numbers of the ions are unaffected by the solvent exchange or solute addition.

$g/g_0$ (25 mV) denotes the relative changes in $g$ (25)

$i/i_0$ (450 mV) denotes the relative changes in $i$ (450)

¶estimate based on measurements at 300 mV.

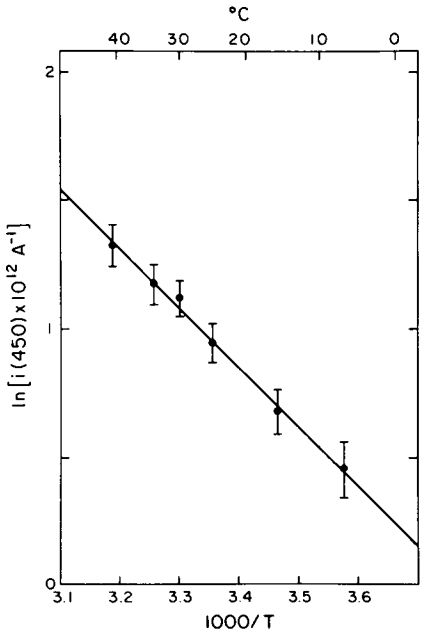


FIGURE 2 Temperature dependence of the gramicidin A single-channel currents at 450 mV applied potential (Arrhenius plot). Each point indicates mean (± SD) of the current. The solid line is determined by linear regression of the data to Eq. 13. ln $[i(450)] = 8.74 - 2.32 \times 10^3/T$, ($r = -0.996$). 0.1 M CsCl + 0.4 M TEACl, DPhPC.

nel, $\epsilon_0$ is the specific capacitance of free space, $\epsilon_r^{H_2O}$ is the dielectric constant of the aqueous phase, and $L_D$ is the Debye length of the aqueous phase. $i_{lim}$ can therefore only be estimated by subjecting the data in Fig. 2 to a linear regression analysis of the currents measured at high potentials ($V \geq 300$ mV).[7] As summarized in Table III, $i(500)$ and $i_{lim}$ are decreased by the addition of sucrose to the aqueous phases. The magnitude of the decreases is in fair agreement with the decrease in aqueous ion mobility. The small-signal conductance, $g(25)$, is likewise decreased by the addition of sucrose to the aqueous phases. This result is, of course, expected if $g$ is in the same order of magnitude as $g_a$.

[7]I am not quite sure whether the linear current increases seen at potentials above 300 mV in the presence of sucrose exclusively reflect simple interfacial polarization (where $p_a$ and $C_m^*$ are voltage independent). It is possible that the increased slopes of the asymptotic, linear, current-voltage characteristics reflect the existence of some additional voltage-dependent process. If this is the case the linear regression procedure will underestimate $i_{lim}$, and therefore overestimate the seriousness of the diffusion limitations. It is noteworthy, however, that the specific capacitance of unmodified DPPC membranes increases slightly, from 0.45 ± 0.02 $\mu$F/cm$^2$ (mean ± SD) to 0.49 ± 0.02 $\mu$F/cm$^2$ (Green and Andersen, unpublished results). The increased slopes are thus in qualitative agreement with the capacitance changes.

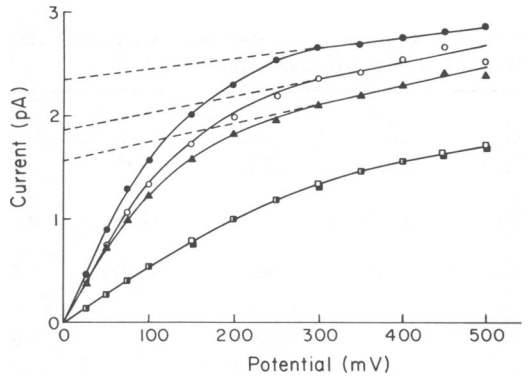


FIGURE 3 The effect of sucrose on gramicidin A single-channel current-voltage characteristics. Each point indicates the mean value of the current. ●, 0.1 M CsCl; ○, denotes 0.1 M CsCl + 10% (0.292 M) sucrose; ▲, 0.1 M CsCl + 20% sucrose. For each of the three experiments the straight lines are determined from a linear regression of the data obtained between 300 and 500 mV. The intercept at $V = 0$ provides an estimate of $i_{lim}$, see Eq. 14 and Table III. The squares denote results for $Na^+$: ■, 0.1 M NaCl, □, 0.1 M NaCl + 20% sucrose. The solid curves have no theoretical significance. DPhPC, 25°C.

Interpretation of the data in Table III is not quite straightforward, however, because addition of sucrose to the aqueous phases will decrease the water activity as well as increase the viscosity. This is important, because the single-channel conductance of the gramicidin A channel appears to be a function of the bulk phase water activity (see Table I). The effect of sucrose may thus in part be due to the change in water activity. To control for the decrease in water activity, other experiments were done where urea was added to the aqueous solutions instead of sucrose (Table III). The results of these experiments show that ~50% of the effect of sucrose may be related to the change in water activity. The mechanism by which a decrease in water activity affects $g(c)$ is not understood, but may, in part, reflect obligatory ion-water coupling in the single-filing gramicidin A channel.

The importance of aqueous diffusion can now be evaluted by combining Eqs. 1, 2, and 12 to obtain an expression for $g_i(c)$:

$$g_i(c) = e \cdot i_{lim} \cdot g(c)/(e \cdot i_{lim}(c) - kT \cdot g(c) \cdot 2). \quad (15)$$

Given the results for $g(25)$ and $i_{lim}$, $g_i$ (0.1) can now be estimated. The result is that $g_i$ (0.1) is considerably larger than $g(25)$, and that its magnitude is essentially constant throughout this series of experiments. Thus, $g_i$ (0.1) varies between 29.6 pS in 0.1 M CsCl and 27.8 pS in 0.1 M CsCl + 20% sucrose, and is 28.8 pS in 0.1 M CsCl + 0.7 M urea.

It should be noted, however, that the CsCl molality increased from 0.1 to 0.114 mol/Kg $H_2O$ in these experiments.

Similar experiments were done with glycerol (Table IV). Qualitatively, the effect is the same as with sucrose. But the quantitative interpretation is more problematical than for sucrose because the concentration of glycerol needed to produce a given change in viscosity is much higher than for sucrose. It is, therefore, not clear that appropriate controls exists for this experiment, as urea at these high concentrations itself changes the viscosity of the aqueous phases. That this osmotic effect may be a serious problem is most clearly seen by noting that $g_i$ (0.1) decreases throughout this series of experiments (from 29.6 pS in 0.1 M CsCl to 23.2 pS in 0.1 M CsCl plus 30% (3.25 M) glycerol while the CsCl molality increases to 0.130 mol/Kg $H_2O$). It should for comparison be noted that $g(25)$ is 12.9 ± 1.8 pS in 0.1 M CsCl + 3.0 M urea, where the CsCl molality is 0.115 mol/Kg $H_2O$. The results nevertheless provide substantial support for the notion that ion entry into the gramicidin A channel is significantly affected by diffusion limitations.

*Experiments with Glycerolmonooleate Membranes.* Fig. 4 illustrates gramicidin A single-channel current-voltage characteristics obtained with 0.1 M XCl +

TABLE III
EFFECT OF SUCROSE UPON $Cs^+$ CURRENTS IN GRAMICIDIN A CHANNELS

| Aqueous phase | $\eta_0/\eta_s$ | $\lambda_s/\lambda_0$ | $g$ (25) pS | $i$ (450–500) pA | $i_{lim}$ pA | $\frac{i_{lim}(S)}{i_{lim}(0)}$ |
|---|---|---|---|---|---|---|
| 0.1 M CsCl | 1.00 | 1.00 | 18.0 ± 0.5 ($n$ = 5) | 2.81 ± 0.11 ($n$ = 14) | 2.36 ± 0.04 | 1.0 |
| 0.1 M CsCl +10% sucrose | 0.76 | 0.83 | 16.1 ± 0.4* ($n$ = 5) | 2.60 ± 0.15* ($n$ = 8) | 1.9 + 0.2 | 0.81 |
| 0.1 M CsCl +20% sucrose | 0.55 | 0.67 | 14.7 ± 0.5‡ ($n$ = 5) | 2.40 ± 0.13‡ ($n$ = 11) | 1.6 ± 0.1 | 0.68 |
| 0.1 M CsCl +0.7 M urea | 0.98 | 1.01 | 16.3 ± 0.6 ($n$ = 6) | 2.73 ± 0.007 ($n$ = 6) | 1.92 ± 0.04 | |

DPhPC, 25°C. Mean ± SD (number of measurements).
$\eta_0/\eta_s$ denotes the change in viscosity (relative to 0.1 M CsCl), estimated from data for sucrose or urea in $H_2O$ (Weast, 1972). $\lambda_s/\lambda_0$ denotes the measured change in aqueous conductivity. $i_{lim}$ (0) and $i_{lim}$ (S) denote $i_{lim}$ in the absence and presence of sucrose. The percentages are (wt/vol)
*($p < 0.001$, compared to CsCl)
‡($p < 0.01$, compared to CsCl + 10% sucrose)

TABLE IV
EFFECT OF GLYCEROL UPON $Cs^+$ CURRENTS IN GRAMICIDIN A CHANNELS

| Aqueous phase | $\eta_0/\eta_G$ | $\lambda_G/\lambda_0$ | $g$ (25) | $i$ (450–500) | $i_{lim}$ | $\frac{i_{lim}(G)}{i_{lim}(0)}$ |
|---|---|---|---|---|---|---|
| | | | *pS* | *pA* | *pA* | |
| 0.1 M CsCl | 1.00 | 1.00 | 18.0 ± 0.5 (n = 5) | 2.81 ± 0.11 (n = 14) | 2.36 ± 0.04 | 1.0 |
| 0.1 M CsCl +10% glycerol | 0.78 | 0.85 | 16.7 ± 0.5* (n = 6) | 2.58 ± 0.16* (n = 9) | 1.9 ± 0.2 | 0.80 |
| 0.1 M CsCl +20% glycerol | 0.57 | 0.71 | 14.0 ± 0.6*‡ (n = 7) | 2.43 ± 0.09*‡ (n = 11) | 1.7 ± 0.1 | 0.72 |
| 0.1 M CsCl +30% glycerol | 0.42 | 0.54 | 12.1 ± 0.7*‡§ (n = 5) | 2.04 ± 0.6*‡§ (n = 10) | 1.3 ± 0.2 | 0.58 |

DPhPC, 25°C. Mean ± SD (Number of measurements).
$\eta_0/\eta_G$ denotes the change in viscosity (relative to 0.1 M CsCl) estimated from data for glycerol or urea in $H_2O$ (Weast, 1972). $\lambda_G/\lambda_0$ denotes the measured change in aqueous conductivity.
$i_{lim}(0)$ and $i_{lim}(G)$ denote $i_{lim}$ in the absence or presence of glycerol.
The percentages are (wt/vol).
*($p < 0.01$, relative to 0.1 M CsCl)
‡($p < 0.02$, relative to 0.1 M CsCl + 10% Glycerol)
§($p < 0.001$, relative to 0.1 M CsCl + 20% Glycerol)

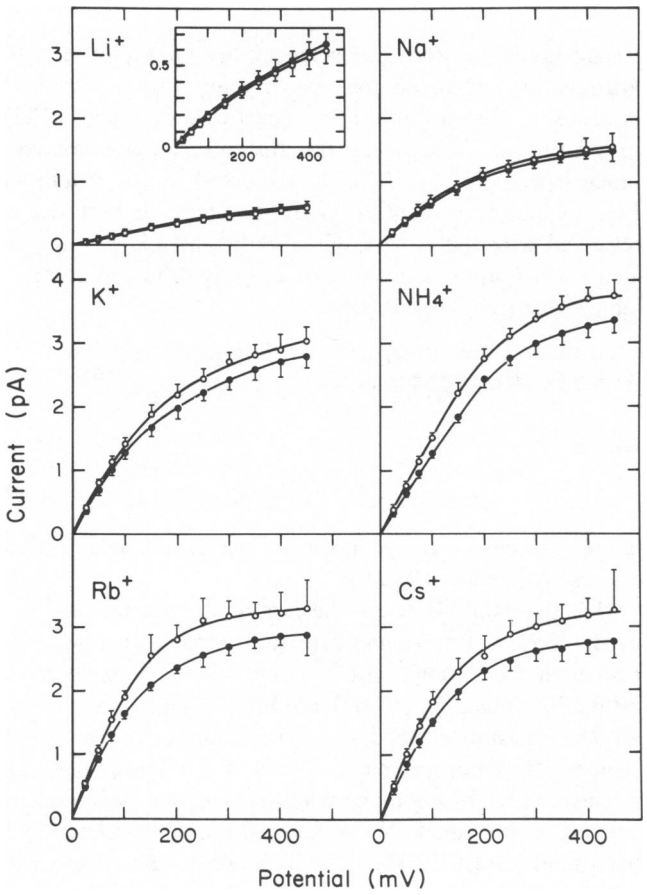


FIGURE 4 Effect of 20% sucrose on gramicidin A single-channel current-voltage characteristics. Each point indicates the mean value plus respectively minus the SD. ○, 0.1 M salt (as chloride) + 0.4 M TEACl. ●, 0.1 M salt + 0.4 M TEACl + sucrose. To facilitate visual comparison of the different records, they are all drawn to the same scale (except for the $Li^+$ *inset*). The solid curves have no theoretical significance. GMO, 25°C.

0.4 M TEACl and with 0.1 M XCl + 0.4 M TEACl + 20% sucrose in the aqueous phases. The currents are much more saturating than in the absence of TEACl (cf. Fig. 8 of Andersen, 1983 *a*) and their magnitudes are depressed by the addition of sucrose. Table V summarizes the values for $g(25)$ and $i(450)$. It was not technically possible to do the urea control experiments in this case. The changes in $i(450)$ may therefore overestimate the dependence of $g_{max}(c)$ on the aqueous diffusion coefficient of the permeant ion. The qualitative conclusion, that a decrease in the aqueous diffusion coefficient of the permeant ion produces a decrease in the measured single-channel conductance should, nevertheless, still hold.

## The Concentration-Dependence of the Single-Channel Currents at High Potentials

If the single-channel currents at high potentials represent the bimolecular association between the ions and the gramicidin A channel, with a minimal contamination by subsequent first-order reactions steps, these currents should vary linearly with the permeant ion concentration, see Eq. 10. This is indeed the case at low permeant ion concentrations. Fig. 5 illustrates data for $i(400)$ obtained with $Cs^+$ at a constant ionic strength of 0.5 M (maintained with TEACl). $i(400)$ is indeed a reasonably linear function of $c$ between 0.005 and 0.1 M, but deviates at higher $Cs^+$ concentrations. At 0.5 M CsCl, $i(400)$ is only about two-thirds of the value predicted from extrapolation of the data obtained at lower concentrations. In contrast to the behavior observed with $i(400)$, $g(25)$ deviates from linearity, even at the lowest $Cs^+$ concentrations where measurements were done. From the magnitudes of $i(400)$ at $c \leq 0.1$ M one can estimate $g_{max}(c)$, the single-channel conductance seen when only the access resistance is limiting, see Eq. 12. This estimate is illustrated by the stippled line in

TABLE V

GRAMICIDIN A SINGLE-CHANNEL CONDUCTANCES AND CURRENTS IN GLYCEROLMONOOLEATE MEMBRANES

| Aqueous phase | | Units | $Li^+$ | $Na^+$ | $K^+$ | $Rb^+$ | $Cs^+$ | $NH_4^+$ |
|---|---|---|---|---|---|---|---|---|
| 0.1 M salt | $g(25)$ | pS | 2.10 ± 0.44 | 8.36 ± 1.28 | 16.5 ± 1.5 | 22.2 ± 2.2 | 20.9 ± 2.8 | 15.1 ± 2.1 |
| +0.4 M TEACl | $i$ (450) | pA | 0.591 ± 0.080 | 1.55 ± 0.20 | 3.05 ± 0.21 | 3.30 ± 0.43 | 3.29 ± 0.62 | 3.76 ± 0.2 |
| 0.1 M salt | $g$ (25) | pS | 2.71 ± 0.36 | 7.79 ± 0.82 | 14.7 ± 1.8 | 19.6 ± 2.2 | 17.4 ± 1.6 | 12.9 ± 1.0 |
| +0.4 M TEACl | | | | | | | | |
| +20% sucrose | $i$ (450) | pA | 0.628 ± 0.059 | 1.49 ± 0.17 | 2.80 ± 0.19 | 2.87 ± 0.32 | 2.79 ± 0.26 | 3.37 ± 0.20 |
| 0.1 M salt | $D/D_0$ | | 0.61* | 0.62* | 0.63* | 0.64‡ | 0.61‡ | 0.63‡ |
| ±0.4 M TEACl | $g/g_0$ (25 mV) | | 1.033 | 0.932 | 0.891 | 0.883 | 0.833 | 0.854 |
| +20% sucrose | | | | | | | | |
| vs. | | | | | | | | |
| 0.1 M salt | $i/i_0$ (450 mV) | | 1.063 | 0.961 | 0.918 | 0.870 | 0.848 | 0.896 |
| +0.4 M TEACl | | | | | | | | |

GMO, 25° C. Mean ± SD. $D/D_0$ denotes the relative changes in diffusion coefficient.

*from Robinson and Stokes (1965, Table 11.5). It is assumed that the *relative changes* are unaffected by the presence of TEACl.

‡From conductivity measurements on the aqueous solutions. It is assumed that the transference numbers of the ions are unaffected by the solute addition. $g/g_0$ (25 mV) denotes the relative changes in $g$ (25). $i/i_0$ (450 mV) denotes the relative changes in $i$ (450).

Fig. 5. This estimate of $g_{max}(c)$ is still, however, affected by interfacial polarization effects and will be ~10% too high (estimated from the data in Andersen, 1983). The fairly close agreement between the present estimate for $g_{max}(c)$ and $g(25)$ at the lowest CsCl concentrations is, therefore, a strong indication that the permeability characteristics of gramicidin A channels may be very seriously affected by aqueous diffusion limitations (particularly since $g(25)$ may be up to 7% less than the ideal small-signal conductance [see Eq. B11]). This is reinforced by the results in Fig. 6 which show how the current-voltage characteristics observed with 0.002 M CsCl + 0.498 M TEACl have a shape which approaches that of a purely diffusion-controlled process (see Appendix B):

$$i(V) = F \cdot p_a \cdot c \cdot \frac{\sinh [e \cdot V/(2 \cdot kT)]}{\cosh [(1-2\alpha) \cdot e \cdot V/(2kT)]} \quad (16)$$

where

$$\alpha = C_m^*/(\epsilon_0 \cdot \epsilon_r^{H_2O}/L_D + 2 \cdot C_m^*) \quad (17)$$

is a parameter that accounts for interfacial polarization effects (Andersen, 1983 *b*).

The deviations between the experimental points and the predictions of Eq. 16 indicate that some resistance still resides in the channel. $g(25)$ is only ~85% of its maximal attainable value, $g_{max}(0.002)$, see Eq. 4, which occurs when all the resistance resides in the aqueous convergence regions. It is not yet clear if this is a reflection of the intrinsic barrier heights or whether it is due to ion occupany in the channel. No serious attempts have therefore been made to fit theoretical expression to the data. (The data in Fig. 6 represents more or less the best resolution of single-channel currents that is possible with the present system [where the average channel lifetime only is a few hundred ms]. Further progress in characterizing the limiting shape of the current-voltage characteristics at very low permeant ion concentrations will probably depend upon many-channel measurements, cf. Eisenman et al., 1980.)

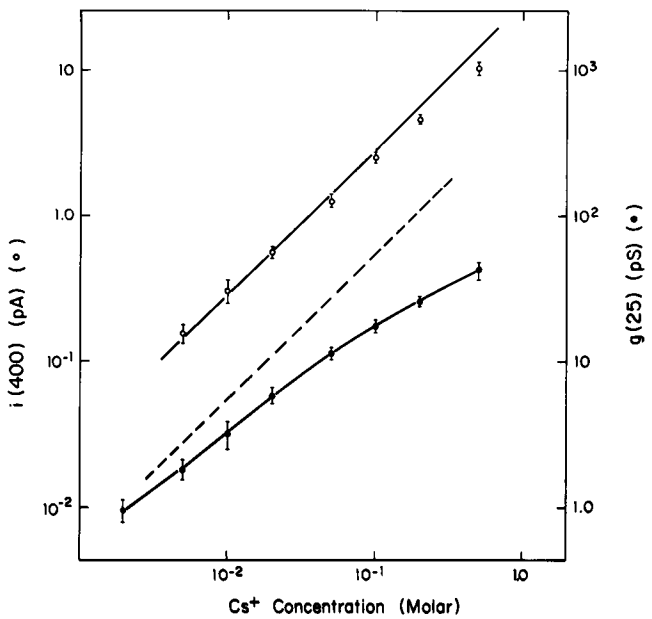


FIGURE 5 Concentration dependence of $i(400)$ and $g(25)$. Each point indicates the mean (± SD) of the current respectively conductance. ○ denotes current, and refers to the scale to the left; the straight line associated with these points has a slope of 1.0. The position of the line is determined by $i_{lim} = 27.9 \cdot c(i_{lim}$ in pA, $c$ in M). ● denotes conductance and refers to the scale to the right; the solid curves through the points have no theoretical significance, the stippled line indicates $g_{max}(c)$ as calculated from Eq. 12. Ionic strength is maintained constant at 0.5 M with TEACl. DPhPC, 25°C.

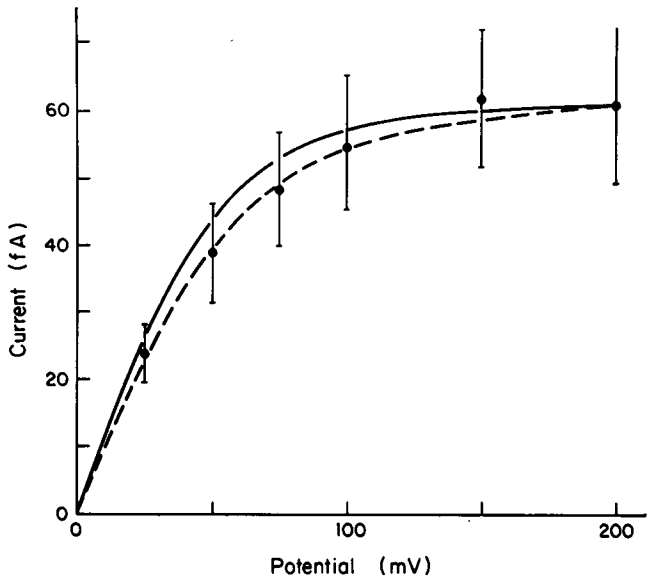


FIGURE 6 Gramicidin A single-channel current-voltage characteristics at 0.002 M CsCl + 0.498 M TEACl. The points indicate mean (± SD) of the currents. The solid line is drawn according to Eq. 16, with $\alpha = 0.0065$ ($C_m^* = 1.05$ $\mu$F/cm$^2$, Andersen, 1983 *b*). The interrupted line is drawn according to Eq. B11 assuming that $k_1 >> p_a = 1.7 \times 10^8$ l/(mol · s) and $k_1/p_a = 3 \cdot k_{-1}/l$ and correcting for interfacial polarization). It should be emphasized that this line only is drawn for illustrative purposes and that many alternative fits are possible although sufficient information to choose between them is not available.

## DISCUSSION

Section 1 contains a discussion of the main result of this investigation: that the magnitude of the currents at high potentials are determined by diffusion of the permeant ion through the aqueous phases up to the channel entrance. Section 2 is a discussion of the implications these results have for the development of kinetic models for ion translocation through gramicidin A channels, and generally for ion movement through membrane-bound channels. Unless specifically stated otherwise the discussion will focus upon the behavior observed with the most permeant ions ($K^+$, $Rb^+$, $Cs^+$, and $NH_4^+$). Section 3 contains a discussion of possible implications for understanding the characteristics of channels in biological membranes.

### The Limiting Currents are Diffusion-Controlled

It will in the following be assumed that the single-channel currents at high potentials are intrinsically voltage independent (Andersen, 1983 *b* should be consulted for a discussion of this point).

The distinction between a diffusion-limited ion entry and a bone fide voltage-independent association step can be made by analyzing the dependence of $i_{lim}$ (in practice $i(400)$ to $i(500)$, sometimes $i_{lim}$ estimated from linear regressions of the asymptotic currents) on the aqueous diffusion coefficient of the permeant ion. The major result of these studies is therefore that the single-channel currents at high potentials are decreased when the aqueous diffusion coefficient of the permeant ions is decreased (Figs. 1–3, Tables II, III, IV, and V). The magnitudes of the decreases depend upon the decrease in the diffusion coefficient (Tables III, IV). The four different experimental procedures: substitution of $D_2O$ for $H_2O$, changes in temperature, and addition of sucrose or glycerol to the aqueous solutions, provide results which are in general agreement with one another. These arguments suggest very strongly that the magnitudes of the limiting currents primarily are determined by diffusion of the permeant ions through the aqueous phase up to the channel entrance. It cannot be excluded, however, that the experimental maneuvers used here could affect the magnitude of the limiting current by mechanisms that do not depend on their effects on the diffusion coefficients of the ions.

Specific effects of sucrose (such as a physical plugging of the channel) are unlikely, because the dramatic effects of sucrose observed with the most permeable ions (Figs. 1, 3, and 4) are essentially nonexistent with the less permeable ions: $Na^+$ (Fig. 3) or $Li^+$ (Fig. 4). But a quantitative interpretation of the sucrose data is nevertheless difficult. The data in the top part of Table III suggest, for example, that the effect of sucrose on the permeant ion mobility is transmitted in toto to the convergence permeability. A more critical analysis of the sucrose effect (based upon the lower segment in Table II and a comparison with the urea data in Table III) indicates, on the other hand, that less than half of the mobility decrease is reflected in the convergence permeability. This latter result is probably the more believable. It is not in disagreement with the notion that the limiting currents are diffusion-limited, quite the contrary; it is to be expected. The Stokes' radius of sucrose is ~4.7 Å (Robinson and Stokes, 1965). The decrease in the diffusion coefficient within 10 Å or so of the channel entrance need not, therefore, be identical to the decrease in the bulk phase diffusion coefficient. There is, additionally, substantial evidence that sucrose is poorly solvated by the water close to the membrane-solution interfaces (Katz and Diamond, 1974; LeNeveu et al., 1977). The effective sucrose-excluding volume corresponds to ~13 $H_2O$/phosphatidylcholine, which is equivalent to a 5–6 Å thick layer of water covering the polar groups. The exclusion of sucrose from this layer is not absolute, however, because sucrose can partition, albeit poorly, into the water layer close to the polar headgroups (Katz and Diamond, 1974; LeNeveu et al., 1977).[8] The concentration of sucrose will, therefore, begin to deviate from the bulk solution value further from the bilayer interface than expected from the nominal volume of nonsolvent water. The quantitative

[8]The chemical potential of the water closest to the phospholipid head groups is less than the chemical potential of bulk water (LeNeveu et al., 1977). The sucrose exclusion can thus be attributed to a decrease in water activity brought about by the presence of the choline head groups.

effect of sucrose upon the limiting current should thus be considerably less than the effect on the bulk aqueous phase diffusion coefficients of the permeant ions. The sucrose results are consequently consistent with the notion that the limiting currents are diffusion controlled, although it cannot be excluded that sucrose has additional effects on the channel or membrane (the results of LeNeveu et al. suggest that the bilayer thickness increases slightly upon sucrose addition). It should, in particular, be noted that the decreases in $g(25)$ are too large in comparison with the decreases in $i(450)$ (Table II), whether the data are compared with the urea controls or not. The basis for this effect is not understood, although it may indicate that ion movement through the channel is associated with small fluctuations in channel structure, and that the rate or amplitude of these fluctuations somehow depends on the aqueous viscosity (see Gavish and Werber, 1979; and Beece et al., 1980 for a discussion of the effects of solution viscosity on polypeptide dynamics).

The data for glycerol (Table IV) are also qualitatively consistent with diffusion control of the limiting currents. These data suffer from essentially the same problems as the sucrose data: they are confounded by poor partitioning of glycerol into the water layers closest to the membrane (Katz and Diamond, 1974), and the small-signal conductances are disproportionally depressed in comparison with the decreases in the limiting currents. The latter problem is, in this case, partly due to a decrease in the bulk phase water activity.

The physical properties of $D_2O$ molecules are quite similar to those of $H_2O$ molecules (Arnett and McKelvey, 1969; Eisenberg and Kauzmann, 1969). The properties of $D_2O$ solutions are likewise similar to those of $H_2O$ solutions apart from a higher viscosity, melting point, and heat capacity, differences that may be related to a greater strength of deuterium bonds compared with hydrogen bonds (Nemethy and Scheraga, 1964). Substitution of $D_2O$ for $H_2O$ should thus be comparatively innocuous, at least for the analysis of the limiting currents. (It should, in particular, be noted that the dimensions of $NH_4^+$ and $ND_4^+$ are very similar [Taylor and Sabine, 1972]). The decreases in $i(450)$ are in acceptable agreement with the decrease in the permeant ion diffusion coefficients (Table II). Because ions and water move through the channel by a single-file mechanism (Levitt et al., 1978; Rosenberg and Finkelstein, 1978), one can exclude that the decrease in $i(450)$ results from a mechanism related to the ion movement through the channel interior (Andersen, 1983 *a*).[9] The decrease in the currents at high potentials should therefore be due to the decrease in the permeant ion diffusion coefficients in the aqueous phases. At low potentials, on the other hand, $D_2O$ may not be a perfect $H_2O$ substitute since $D_2O$ binds stronger than $H_2O$ to amide carbonyl oxygens (Kobayashi and Kobayashi, 1980). This can affect the small-signal single-channel conductance in several ways because both water and ions in the channel are solvated by carbonyl oxygens. Entry of an ion, $X^+$, into the channel will, for example, tend to be associated with the expulsion of a water molecule (see also Levitt, 1978 *b*):

$$C{=}O \cdots HOH + X^+ \rightleftarrows C{=}O \cdots X^+ + H_2O. \quad (18)$$

The dissociation constant, $K$, for ion binding into the gramicidin A channel will therefore depend upon the energetics of not only the ion-water and ion-carbonyl interactions but also of the water-carbonyl interactions. The stronger binding of $D_2O$ than $H_2O$ to the carbonyl groups lining the channel wall will shift the equilibrium in Eq. 18 towards the left in $D_2O$ solutions, and thus decrease the affinity of the channel for the ion. The stronger binding of $D_2O$ to the carbonyl groups will presumably also decrease the water permeability of the channel. This may act to decrease the channel conductances because the rate constant for ion translocation through the channel at low potentials is determined by the diffusional water permeability of the channel (Andersen and Procopio, 1980; Finkelstein and Andersen, 1981). A decrease in affinity (increase in $K$) and a decrease in translocation rate constant will tend to decrease the single-channel conductances at low salt concentrations ($c < K$). The magnitude of the decreases will depend upon the type and concentration of the permeant ion. This may account for the apparent discord between the present results with $Li^+$ (0.1 M) in $H_2O$ and $D_2O$, where substantial conductance changes are observed (Table I and II, and the lack of effect observed at higher $Li^+$ concentrations [0.5 M] by Tredgold and Jones, 1979). The results of Tredgold and Jones are, in fact, in excellent agreement with the notion that aqueous diffusion limitations are important.

The effects of temperature are likewise consistent with the notion that the magnitude of $i_{lim}$ is determined by the diffusion-controlled ion entry into the channel. The Arrhenius activation energy for $i(450)$ for $Cs^+$ (19.3 kJ/mol) is larger than for the diffusion in bulk solution (16.6 kJ/mol). The difference is small, however, and reflects most likely that ion movement through the water layers closest to the membrane-solution interface is less facile than through bulk water. This could be because ions are well solvated in this region, for example, because of electrostatic repulsion due to image potentials set up at the membrane solution interface (Neumcke and Läuger, 1969; Kauzmann, quoted in Perutz, 1978), or it could be because the effective diffusion coefficient of the ion is decreased in this region.

Two different mechanisms will contribute towards a

[9]Concentration-dependent changes in the shapes of single-channel current-voltage characteristics, similar to those seen by comparing Figs. 5 and 7 of Andersen (1983 *a*), are also seen in $D_2O$ solutions (data not shown). It is thus possible to use the arguments presented in relation to Fig. 13 of Andersen (1983 *a*) to show that the limiting currents seen in $D_2O$ must reflect ion entry into channel.

decreased diffusion coefficient of ions close to the channel entrance. The first is the hydrodynamic drag interaction between an ion and the membrane plus channel, the second is a bona fide decreased fluidity of the $H_2O$ close to the polar head groups. The former problem has been analyzed by Wolynes and Deutch (1976) for the diffusion-controlled association of uniformly reactive spheres. It was found that the diffusion-controlled association rate constants will be decreased by ~30%, from the values calculated for the absence of hydrodynamic interactions. A similar conclusion was reached by Emeis and Fehder (1970) based on a molecular dynamics simulation of a two-dimensional fluid. The latter problem was studied by Rossky and Karplus (1980) in their molecular dynamics simultations of the alanine dipeptide in water: It was found that the translational and rotational correlation times were prolonged for $H_2O$ near the methyl groups, such that the effective diffusion coefficient of these $H_2O$ molecules was fivefold less than the bulk phase diffusion coefficient.

The magnitude of a diffusion-controlled ion flux up to the channel will be affected by these complications, but I cannot evaluate exactly how serious the problems will be in the present case. It is nevertheless clear that there will not be a perfect correlation between changes in bulk diffusion coefficients and changes in the diffusion-controlled ion movement up to the channel entrance, and that the changes in the small-signal conductances may be different than predicted from the changes in the voltage-independent currents using a simple, essential, macroscopic theory of diffusion-controlled currents. It is, therefore, important that all four maneuvers used to change the aqueous diffusion coefficient produce reasonably sized changes in the currents at high potentials. The quantitative agreement between changes in the bulk phase diffusion coefficient and the current changes is not perfect for a diffusion-controlled system. But the agreement is qualitatively acceptable, considering that the magnitude of diffusion-controlled currents will be determined by the characteristics of the aqueous region within 5–10 Å from the channel entrance.

The conclusion that diffusion limitations are important determinants of the permeability characteristics of gramicidin A channels is critically dependent upon the current measurements at very high potentials. These measurements show that there exists an effectively voltage-independent step in the association reaction (Andersen, 1983 *a*, *b*), and the results in this article show that the rate constant associated with this voltage-independent step varies in a manner consistent with it being a diffusion-controlled step. The interpretation of each of the experiments is not unique, but the common factor in these experiments, vis-a-vis the finding of voltage-independent currents at high potentials, is the changes in the aqueous diffusion coefficients. It can thus be concluded, albeit circumstantially, that the magnitude of $i_{lim}$ primarily is determined by the magnitude of the aqueous diffusion coefficient of the permeant ion and that $i_{lim}$ is diffusion-controlled. (Electrostatic calculations of Levitt [1978 *a*] show that an appreciable fraction [a few percent] of the applied potential falls across an aqueous region stretching a few angstroms out from the channel entrance. This tends to exclude radius-dependent mechanisms that are not diffusion dependent, see footnote 1, because the voltage dependence of $i_{lim}$ implies that the reaction steps that determine the magnitude of $i_{lim}$ cannot involve a translational movement of the permeant ion across a region where any appreciable potential difference [associated with the applied potential] exists. That is, one can exclude reactions subsequent to the formation of an outer-sphere complex between ion and channel.)

Additional support for this conclusion is obtained by calculating the magnitude of the voltage-independent rate constant for association between ion and channel, $k_f^*$:

$$k_f^* = i_{lim}/(e \cdot c). \quad (19)$$

If the currents are diffusion limited $k_f^*$ will be equal to the aqueous access permeability, $p_a$,[10] and one should be able to calculate the capture radii for the ions using Eq. 6. (It must in this case be assumed that the electromigrative component to $i_{lim}$ [Läuger, 1976] is negligibly small. This assumption will be satisfied when the capture radius of the channel is less than the Debye length of the aqueous phases [Andersen, manuscript in preparation].) Table VI lists estimates for $k_f^*$ and for $r_o$ and $g(25)/g_{max}$ as calculated from $i(450)$ in 0.1 M XCl + 0.4 M TEACl. A comparison of the estimates for $k_f^*$ with diffusion-controlled rate constants for enzymatic reactions [$8 \times 10^7$ to $5 \times 10^8$ 1/mol · s) from Table II of Eigen (1974)] shows that the present rate constants are in general agreement with those found for diffusion-controlled movement up to small reactive sites on large surfaces. The magnitude of $p_a$ must therefore reflect the particular restrictions that face an ion as it attempts to make contact with the channel entrance.

The capture radius is remarkably similar for those alkali metal cations where an estimate is possible ($Na^+$ through $Cs^+$), and $r_o$ is somewhat larger for $NH_4^+$ (even larger values are found for $Ag^+$ and $Tl^+$ [Andersen manuscript in preparation]). It must be emphasized that no mechanistic significance should be attached to the magnitude of $r_o$, as it is, at this time only, a fudge factor that conceals the detailed sequence of events involved in ion entry into a gramicidin A channel. The variation of $r_o$ among groups of ions with different chemical characteristics is, therefore, not an argument against the importance of aqueous diffusion limitations, because the noble gas-like

[10] $k_f^*$ and $p_a$ reflect the same parameter but are evaluated differently: $k_f^*$ is a normal second-order reaction-rate constant [units; liter/(mol · s)] whereas $p_a$ is the convergence permeability for a single channel (units, $cm^3/s$), $k_f^*$ and $p_a$ are thus related by $k_f^* = 1{,}000 \cdot N \cdot p_a$. I will not adhere strictly to the formal units for these quantities but use whichever is most convenient.

TABLE VI
VOLTAGE-INDEPENDENT ASSOCIATION RATE CONSTANTS AND CAPTURE RADII OF THE GRAMICIDIN A CHANNEL

| | | $Li^+$ | $Na^+$ | $K^+$ | $Rb^+$ | $Cs^+$ | $NH_4^+$ |
|---|---|---|---|---|---|---|---|
| Lipid | | | | | | | |
| | Ion radius* (A) | 0.53 | 0.95 | 1.32 | 1.45 | 1.63 | 1.49 |
| | D‡ ($\times 10^5$) ($cm^2/s$) | 1.03 | 1.33 | 1.95 | 2.07 | 2.05 | 1.96 |
| DPhPC | $k_f^*$ ($\times 10^{-8}$ liter/(mol × s) | >0.5 | ≳1.1 | 1.7 | 1.7 | 1.6 | 2.4 |
| | $r_0$(Å) | >0.12 | ≳0.22 | 0.23 | 0.22 | 0.21 | 0.32 |
| | $g(25)/g_{max}$ | <0.098 | ≲0.18 | 0.23 (0.43) | 0.34 (0.54) | 0.35 (0.54) | 0.26 (0.51) |
| GMO | $k_f^*$ ($\times 10^{-8}$) liter/(mol × s) | >0.4 | ≳1.0 | 1.9 | 2.1 | 2.1 | 2.3 |
| | $r_0$ (Å) | >0.1 | ≳0.19 | 0.26 | 0.26 | 0.26 | 0.32 |
| | $g(25)/g_{max}$ | <0.18 | ≳0.28 | 0.28 | 0.35 | 0.33 | 0.21 |

0.1 M salt (as chloride) +0.4 M TEACl, 25°C.
The numbers in parenthesis denote values for 0.01 M salt + 0.49 M TEACl, 25°C.
*From Hille (1975*a*), except for $NH_4^+$ where the radius is from Khan and Baur (1972).
The coordination number for $NH_4^+$ is assumed to be four.
‡From Robinson and Stokes (1965, Appendix Table 6.2).

ions ($Li^+$ through $Cs^+$) probably will behave differently than ions such as $NH_4^+$, $Ag^+$, and $Tl^+$, which have different charge distribution and greater deformability, e.g., Krasne and Eisenman (1973).

## Implications for the Analysis of Ion Permeability Characteristics of the Gramicidin A Channel

The data in Table VI show that $g(25)$ at low salt concentrations becomes comparable to its maximal possible value, $g_{max}$ ($c$). But the possibility that aqueous diffusion-limitations may be a major, if not the dominant, rate limiting step in ion entry into the gramicidin A channel has generally been disregarded in analyses of its permeability characteristics. This neglect may have serious consequences for the choices of kinetic model used in the analysis of experimental data and for the significance of the values of rate constants estimated by fitting the models to the data. A complete discussion of these problems will go beyond the available space. I wish, however, to address two points: first, that neither current-voltage nor conductance-activity characteristics will, in general, reflect accurately the intrinsic permeability characteristics of the channel (this is discussed in Appendix B); second, that biionic potentials measured under strict biionic conditions (only one permeant ion in each aqueous phase with equal concentrations of the permeant ions) may vary as a function of their concentration, even when at most one ion can occupy the channel.

This latter result is very important, because a concentration-dependent permeability ratio, as evaluated from such biionic potential measurements, is generally considered to be a hallmark of multiple ion occupancy in single-filing channels.[11] But this is not necessarily the case for channels where $g_i \simeq g_a$.

Let X and Y denote two different permeant ion species, each present in only one aqueous phase, and let $P^y/P^x$ denote their permeability ratio which in general will be a function of potential. As derived in Appendix C for a singly-occupied channel, one finds that

$$P^y/P^x \approx \frac{p_y(y) \cdot c_y}{p_x(u) \cdot c_x} \cdot \frac{1 + (c_x/K_x) \cdot f_x(u) + (c_y/K_y) \cdot f_y(u) + 2 \cdot [p_x(u)/p_a^x] \cdot \cosh(u/2)}{1 + (c_x/K_x) \cdot f_x(u) + (c_y/K_y) \cdot f_y(u) + 2 \cdot [p_y(u)/p_a^y] \cdot \cosh(u/2)} \quad (20)$$

where $u = e \cdot V/kT$, $K_x$ and $K_y$ are the single-site dissociation constants for X and Y, respectively, and $f_x(u)$ and $f_y(u)$ are functions of the rate constants associated with the movement of X respectively Y through the channel (see Eqs. C13 and C14). In the limit where $c_x = c_y$ approach zero,

$$P^y/P^x = \frac{p_y(u)}{p_x(u)} \cdot \frac{1 + 2 \cdot p_x(u) \cdot \cosh(u/2)/p_a^x}{1 + 2 \cdot p_y(u) \cdot \cosh(u/2)/p_a^y} \quad (21)$$

[11] "Concentration-dependent" permeability ratios, as calculated from zero-current potentials measured at arbitrary ionic conditions, will generally be observed in singly occupied channels. This situation arises because the permeability ratio will be voltage dependent unless the "constant offset energy barrier" condition is satisfied (Hille, 1975 *a*). If the concentration of only one of the permeant ions is varied, one will thus find that the permeability ratio is concentration dependent, but this is a trivial consequence of the concentration-dependence of the zero-current potential. It is for diagnostic purposes important to measure the biionic potential *senso strictu*, or else to work with a constant activity ratio of the permeant ions. The permeability ratios should in these situations ideally be concentration independent in a singly occupied channel, unless aqueous diffusion limitations are important.

or, when $p_x >> p_a^x$ and, $p_y >> p_a^y$

$$P^y/P^x = p_a^y(u)/p_a^x(u). \quad (22).$$

The permeability ratio is in this situation determined exclusively by the aqueous convergence regions. But in the limit where $c_x = c_y$ approach infinity one finds that

$$P^y/P^x = p_y(u)/p_x(u) \quad (23)$$

and the permeability ratio is now determined exclusively by the intrinsic channel properties. Only in the rather unlikely event that $p_x/p_a^x = p_y/p_a^y$ will the permeability ratio be concentration independent. The physical reason underlying the concentration dependence of the permeability ratio is that the permeant ions at the channel entrance at low concentrations will be out of equilibrium with the bulk aqueous phases. The flux of X for example, will depend on the concentration of X at the two channel entrances, but these concentrations are themselves functions of the flux of X(and Y). At high permeant ion concentrations, when $g_i$ approaches its limiting value and $g/g_a \rightarrow 0$, then the permeant ion concentrations at the channel entrance will be in equilibrium with the bulk solution. $P^y/P^x$ will in this case only depend properties of the channel.

The prediction of concentration-dependent permeability ratios in singly-occupied channels has important implications for kinetic analyses of ion movement through gramicidin A channels, because Urban et al. (1980) found that permeability ratios (relative to $Na^+$) measured in GMO membranes indeed vary weakly with concentrations at low concentrations ($c < 0.1$ M). Since biionic potential measurements were the most precise (and sometimes the only) measurements at these low ion concentrations, it was concluded that the best fit of a two-site-two-ion model to the data demanded that the dissociation constants for the first ion be in the $10^{-3}$ M range. This analysis of the data (the so-called fit II, in Urban et al., 1978) had an important prediction: that the permeability ratios and the conductance ratios should begin to diverge at salt concentrations above $10^{-4}$ M (see Fig. 2 of Urban, 1978). This prediction is, however, contradicted by the experimental finding (Andersen, 1983 *a*) that the ratio of the single-channel currents measured in GMO membranes at 25 mV with 0.01 M KCl or 0.01 M NaCl is in essential agreement with the permeability ratio found with 0.01 M NaCl vs. 0.01 M KCl by Urban et al. (1980).

The most likely basis for this discrepancy is that the concentration dependence of the permeability ratios seen at low permeant ion concentrations is in part a reflection of aqueous diffusion limitations. This was not incorporated into the model used by Urban et al. The least-squares curve-fit procedure would, therefore, produce a set of rate constants that gave too-low dissociation constants for the first ion in the channel. This problem became particularly serious for $Na^+$ because it was the reference ion for the biionic potential measurements. It is thus likely that the dissociation constant for $Na^+$ is much higher than estimated by Urban et al. It is indeed possible that there is no double occupany of gramicidin A channels with $Na^+$ in GMO membranes, in agreement with the findings in DPPC membranes (Procopio and Andersen, 1979).[12] The question of $Na^+$ occupancy in GMO membranes is still open, however.

## Possible Implications for Channels in Biological Membranes

Voltage-gated ion-permeable channels in the membranes of excitable cells seem to be generally capable of selecting among the alkali metal cations (and other small monovalent cations) as well as maintaining a large a turnover rate of ions through the channel (a high single-channel conductance), see Hille (1975 *a*) for a review. It has long been recognized that these two characteristics are in conflict with each other: The ability to select among different ions depends on interactions between the permeant ions and some critical groups in the channel wall, while the large turnover numbers imply that the critical interactions must be of fairly small energy since the selectivity depends upon the relative heights of the peak energy barriers for the different ions (Hille, 1975 *a*, *b*).

These difficulties led to the notion that the selectivity of ion conducting channels is governed by a principle of selective exclusion (Bezanilla and Armstrong, 1972; Hille, 1975 *a*; Mullins, 1975). According to this scheme, there exists a limited range of ion sizes and geometries that allow ions to enter some critical section of the channel, the "selectivity filter," and interact with the polar groups lining the channel wall in this region. It is thus immaterial whether or not some excluded ions could, in principle, interact favorably with these groups. Only ions that can enter this selectivity filter and have favorable interactions with the groups lining the walls (presumably by a combination of the energetic arguments proposed by Eisenman, 1962, and Krasne and Eisenman, 1973, and the "intimate fit" scheme reviewed by Mullins, 1975, will be permeable. Considerable support for this picture was provided by Hille for both $Na^+$ channels (Hille, 1971, 1972) and $K^+$ channels (Hille, 1973).

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[12]Urry et al. (1980) finds evidence for multiple $Na^+$ binding using $^{23}Na^+$ NMR to probe the equilibrium and kinetic aspects of ion binding into gramicidin A channels in lysophosphatidylcholine micelles. The relation between these results and those obtained in hydrocarbon containing planar bilayers is unclear, however, primarily because the rate constants obtained from the NMR studies predict that the flux-ratio exponents should deviate significantly from 1.0 (estimates for the maximal flux ratio exponents vary between 1.2 and 2.0). This was not observed; the tracer flux measurements in DPPC membranes indicate that the flux ratio exponent for $Na^+$ is 1.0 at attainable salt concentrations (Procopio and Andersen, 1979). The reason for the discrepancy between the two sets of results is not understood. It may reflect that the properties of the gramicidin A channel are much more dependent on the lipid environment that is commonly believed (see also Fröhlich, 1979 *a*, *b*).

There is, however, a serious problem with the selective exclusion schemes. The cross sections of the selectivity filters are generally believed to be very close to the cross sections of the most permeable ions (give or take a water molecule or two). This implies that the ions must hit the selectivity filter fairly precisely: the capture radius should be small. By analogy with the results presented here for gramicidin A channels, one would therefore expect that serious diffusion limitations would occur. Why, then, do the permeability characteristics of voltage-gated channels seem to be impervious to aqueous diffusion limitations? The channel cross section at the selectivity filter must somehow be irrelevant for ion entry into the channel. This will be the case if the channel entrances are much wider than indicated by the estimated dimensions of the selectivity filter.

There is considerably pharmacological evidence that the inner and outer entrances of $K^+$ and $Na^+$ channels have larger cross-sectional areas than the intermediary part of the channels (Hille, 1967; Armstrong, 1971; Armstrong and Hille, 1972; Hille, 1975 *c*; Yeh and Armstrong, 1978). These "antechambers" have primarily been utilized for pharmacological dissection of different currents, but their existence may also have significance for the normal permeability characteristics of the channels, see Fig. 7.

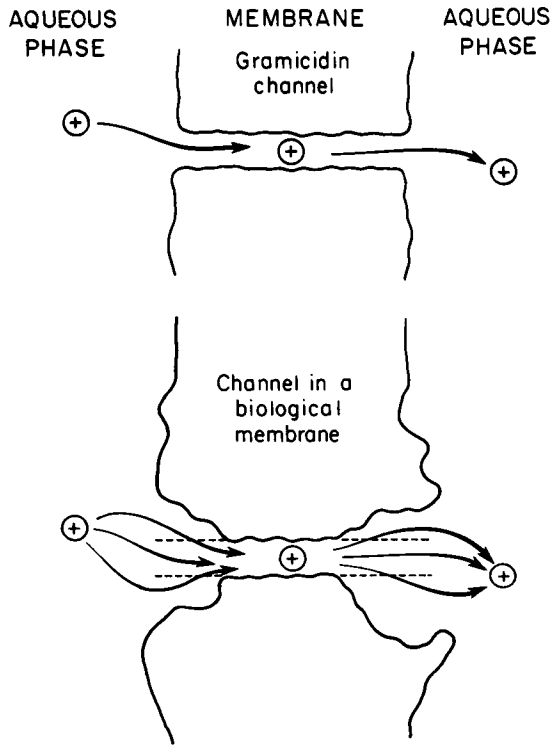


FIGURE 7 Top, schematic illustration of a gramicidin A channel in a membrane. Note the fairly restricted access to the channel itself. Bottom, a possible structure for a voltage-gated channel in a biological membrane. The interrupted lines indicate the outlines of the gramicidin A channel. Note how the presence of the antechambers makes the channel entrance much more accessible.

The top half of the figure depicts a gramicidin A channel, the bottom half an idealized channel in a biological membrane. Note, how access will be much better for the channel with the antechambers than for the one with the gramicidin A channel.

The simplicity of the proposed scheme is somewhat misleading, however. The recessed location of the entrance to the selectivity filter will by itself produce additional radial constraints on ion entry into the selectivity filter (Samson and Deutch, 1978) and thus decrease the diffusion-controlled association rate constant into this critical part of the channel. These kinetic limitations will be overcome, however, if it is energetically favorable for the permeant ions to reside in the antechambers, because there is a potential difference between the bulk aqueous phase and the entrance to the selectivity filter, due to an applied potential or to the existence of fixed negative charges in the antechamber, or because the walls of the antechamber are lined by groups that solvate the ions well. These latter groups may also help to increase the rate constant for dehydration of the incoming ion and thus decrease the kinetic barrier associated with the dehydration-solvation reactions. The antechambers will thus act as ion binding sites and the channel will have multiple ion binding sites, and could be a multiply-occupied, possibly single-filing structure.

Whether this scheme has any relevance for understanding the selectivity properties of channels in biological membranes in terms of their structure remains to be seen. But it should be noted that that the rate constant for entry of tetraethanolammonium into the inner antechamber of the $K^+$ channel of the squid giant axon is voltage-independent and decreases appropriately when the aqueous viscosity is increased (French et al., 1981). This argues strongly that entry of tetraethanolammonium is diffusion controlled and suggests aqueous diffusion limitations would be a problem for the $K^+$ ion, unless some mechanism exists to increase the aqueous access permeability.

## APPENDIX A

### Explicit Calculations of *f*, the Geometric Factor in Eq. 6

In general, these calculations are extremely difficult. A considerable simplication is, however, possible by noting that there exits a general relation (e.g. Smythe, 1968),

$$R = \rho \cdot \epsilon_0 \cdot C \qquad \text{(A1)}$$

which relates the resistance to current flow between two bodies, $R$, to their mutual capacitance in vacuo, $C$, the specific resistance of the conducting medium, $\rho$ and the specific capacitance in vacuo, $\epsilon_0$. This relation was first used by Hall (1975) for the calculation of access resistances.

Four different geometries are of particular interest in the present situation. The channel entrance may be regarded as a hemisphere protruding from the plane of the membrane, a circle in the plane of the membrane, a prolate ellipsoid protruding from the plane of the membrane, or an ellipse in the plane of the membrane. The reason for

addressing the latter two possibilities is that the shape of the entrance to the gramicidin A channel must be distorted from that of an idealized circle or sphere due to the abrupt termination of the helix at the COOH-terminal end.

The calculation of the access resistance is accomplished by calculating the capacitance of a conducting surface of appropriate shape to an electrode of infinite extent infinitely far away. The capacitance of a conducting hemi-ellipsoidal surface can then be expressed as an elliptic integral (Smythe, 1968; section 5.02):

$$C = 4 \cdot \pi \cdot \epsilon \cdot \left\{ \int_0^\infty [(a^2 + \theta)(b^2 + \theta)(c^2 + \theta)]^{-0.5} \, d\theta \right\}^{-1} \quad \text{(A2)}$$

where $a$ is the length of the long semi-axis of revolution, $b$ is the length of the short semi-axis, and $d$ is either equal to $b$, for the protuding surfaces, or equal to 0 for the plane surfaces. The convergence resistance $R_c$ is obtained as

$$R_c = \rho/(4 \cdot \pi) \cdot \int_0^\infty [(a^2 + \theta)(b^2 + \theta)(c^2 + \theta)]^{-0.5} \, d\theta. \quad \text{(A3)}$$

When the entrance is half a prolate ellipsoid the integral in Eq. A3 reduces to

$$\int_0^\infty [(b^2 + \theta) \cdot \sqrt{a + \theta}]^{-1} \, d\theta =$$

$$(1/\sqrt{a^2 - b^2}) \cdot \ln [(a + \sqrt{a^2 - b^2})/(a - \sqrt{a^2 - b^2})] \quad \text{(A4)}$$

or because

$$\rho = kT/(F \cdot e \cdot D \cdot c) \quad \text{(A5)}$$

one finds that

$$g_a = F \cdot e \cdot 4 \cdot \pi \cdot D \cdot c \cdot \sqrt{a^2 - b^2}/$$

$$\{kT \cdot \ln[(a + \sqrt{a^2 - b^2})/(a - \sqrt{a^2 - b^2})]\}. \quad \text{(A6)}$$

It is convenient to use $a$ as the "radius," in which case

$$f = 4 \cdot \pi \cdot \sqrt{1 - b^2/a^2}$$

$$/\ln[(1 + \sqrt{1 - b^2/a^2})/(1 - \sqrt{1 - b/a^2})]. \quad \text{(A7)}$$

$f$ is a comparatively weak function of $b/a$: when the surface is a hemisphere (in the limit when $b/a = 1$), $f = 2 \cdot \pi$; when $b/a = 0.75$, $f = 5.7$; and when $b/a = 0.25$, $f = 4.1$. Variations in the "capture radius" of the channel will primarily reflect variations of the long axis of the ellipsoid.

When the entrance is an elliptic disk the integral can be expressed as complete elliptic integral (of the first kind). Using the transformation, $\theta = t^2$ the integral can be transformed into the standard form, and one finds that

$$g_a = F \cdot e \cdot 2 \cdot \pi \cdot a \cdot D \cdot c \Big/ \left[ kT \cdot F\left(\frac{\pi}{2} \middle\backslash x\right) \right] \quad \text{(A8)}$$

where $\alpha = \cos^{-1}(b/a)$. The function $F(\pi/2 \backslash \alpha)$ is tabulated in Abramowitz and Stegun (1965). The factor $f$ is, using $a$ as the radius,

$$f = 2 \cdot \pi / F\left(\frac{\pi}{2} \middle\backslash x\right) \quad \text{(A9)}$$

which, again, is a fairly weak function of $b/a$: when the surface is a circle in the plane of the membrane (when $b/a = 1$), $f = 4$; when $b/a = 0.75$, $f = 3.5$; and when $b/a = 0.25$, $f = 2.3$). Once again the capture radius is therefore primarily determined by the long axis of the ellipse.

The magnitude of the capture radius that is calculated from a known $p_a$ will certainly depend upon the assumptions made in the evaluation of $f$. For any reasonable dimensions of channels and ion one may, nevertheless, conclude that variations in $p_a$ reflect a corresponding change in the dimensions of the surface that the ion must hit to enter the channel. The fundamental question; "what is the appropriate geometry and how do the dimensions of the entrance relate to the dimensions of the channel and ion?", is not resolved, however. It thus seems most reasonable to use the simplest possible assumption: the channel entrance is a hemisphere, because this leads to very signficant simplifications of other parts of the theory of diffusion-controlled ion movement.

## APPENDIX B

## Single-Channel Current-Voltage Characteristics in the Presence of Aqueous Diffusion Limitations

The model is similar to that used in Andersen (1983 *a*, *b*). It is assumed that the channel contains two major free energy minima (the ion binding sites) and that these are separated from each other and from the aqueous phases by energy barriers. It is further assumed that ion movement through the channel can be subdivided into three distinct steps:

$$I' + G \underset{k'_{-1}}{\overset{k'_1}{\rightleftharpoons}} IG \underset{l''}{\overset{l'}{\rightleftharpoons}} GI \underset{k''_1}{\overset{k'_{-1}}{\rightleftharpoons}} G + I. \quad \text{(B1)}$$

It is assumed that the bulk phase ion concentrations are equal to each other. The ion concentrations close to the channel, $c'$ and $c''$ respectively, are not, however, constant equal to $c$ the bulk phase concentration:

$$c' = c - J/p_a \quad \text{(B2)}$$

and

$$c'' = c + J/p_a \quad \text{(B3)}$$

where $J$ denote the flux through the channel. The single-channel current is given by

$$i(u) = \frac{e \cdot (k'_1 \cdot l' \cdot k'_{-1} \cdot c' - k''_1 \cdot l'' \cdot k''_{-1} \cdot c'')}{(k'_{-1} \cdot k''_{-1} + l' \cdot k'_{-1} + l'' \cdot k''_{-1}) + k'_1 \cdot c' \cdot (l' + l'' + k'_{-1}) + k''_1 \cdot c'' \cdot (l' + l'' + k''_{-1})} \quad \text{(B4)}$$

If it is assumed that the energy barriers are sharp such that the rate constants are exponential functions of the potential difference between an energy well for the aqueous phase) and the adjacent barrier peak then the current can be expressed as

$$i(u) = \frac{e \cdot l \cdot k_{-1} \cdot [c \cdot \sinh(u/2) - (J/p_a) \cdot \cosh(u/2)]}{K \cdot [l \cdot \cosh[(\delta_2 + \delta_3) \cdot u] + k_{-1}/2] + c \cdot l \cdot \{\cosh[(\delta_1 + \delta_3) \cdot u] + \cosh[(\delta_1 - \delta_3) \cdot u] + k_{-1} \cdot \cosh[(\delta_1 + \delta_2) \cdot u] - J/p_a \cdot (l \cdot \{\sinh[(\delta_1 + \delta_3) \cdot u] + \sinh[(\delta_1 - \delta_3) \cdot u]\} + k_{-1} \cdot \sinh[(\delta_1 + \delta_2] \cdot u])} \quad \text{(B5)}$$

where $K = k_{-1}/k_1$, and $\delta_1$, $\delta_2$, and $\delta_3$ denote the fraction of $u$ that affects $k_1$, $k_{-1}$, and $l$, respectively, and Eqs. B2 and B3 have been used to eliminate the unknown $c'$ and $c''$. The explicit solution for $i(u)$ is

$$i(u) = e \cdot \left\{ \frac{[F_1(u) + F_2(u) + l \cdot k_{-1} \cdot \cosh(u/2)/p_a] - \sqrt{D}}{2 \cdot F_3(u)/p_a} \right\} \quad \text{(B6)}$$

where

$$F_1(u) = K \cdot \{l \cdot \cosh[(\delta_2 + \delta_3)u] + k_{-1}/2\} \quad \text{(B7)}$$

$$F_2(u) = c \cdot l \cdot \{\cosh[(\delta_1 + \delta_3) \cdot u] + \cosh[(\delta_1 - \delta_3) \cdot u]\} + k_{-1} \cosh[(\delta_1 + \delta_2)u] \quad \text{(B8)}$$

$$F_3(u) = l \cdot \{\sinh[(\delta_1 + \delta_3) \cdot u] + \sinh[(\delta_1 - \delta_3) \cdot u]\} + k_{-1} \cdot \sinh[(\delta_1 + \delta_2)u] \quad \text{(B9)}$$

and

$$D = (F_1(u) + F_2(u) + l \cdot k_{-1} \cdot \cosh(u/2)/p_a)^2 - 4 \cdot F_3(u) \cdot k_{-1} \cdot l \cdot c \cdot \sinh(u/2)/p_a. \quad \text{(B10)}$$

Eqs. B6–B10 are straightforward, but difficult to visualize. (Fig. 8 illustrates some representative current-voltage characteristics.) It is therefore important that there exist two limiting cases where Eq. B5 can be expressed in simple terms.

The first of these occur when $c << K$, in which case Eq. B5 reduces to

$$i(u) = e \cdot c \cdot \frac{k_1 \cdot \sinh(u/2)}{\{k_1/p_a \cdot \cosh(u/2) + \cosh[(\delta_2 + \delta_3)u] + k_{-1}/(2 \cdot l)\}}. \quad \text{(B11)}$$

Eq. B11 should be compared to Eq. 1 of Eisenman et al. (1980) to see how the presence of diffusion limitations ($k_1/p_a > 0$) will produce an additional term in the limiting current-voltage relation, which must be taken into account when analyzing shapes of single-channel current-voltage characteristics.

The presence of this extra term implies that the current-voltage characteristics may be sublinear at low salt concentrations even though $k_{-1} > l$ (where one normally would expect to see superlinear current-voltage characteristics with fairly concentration-independent shapes). The reason for this behavior is that the effective association rate constants and dissociation rate constants at low salt concentrations are given by

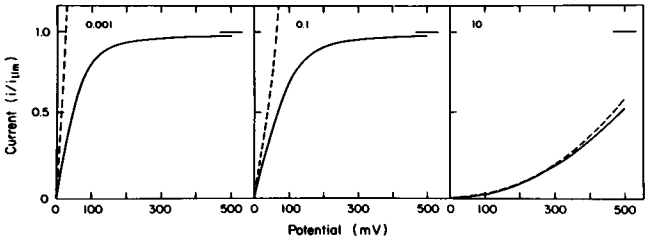


FIGURE 8 Current-voltage characteristic of a channel with aqueous diffusion-limitations. The characteristics solid curves are calculated according to Eqs. B6–B10. $K = 0.1$ M, $l = 2 \cdot 10^7$/s, $k_{-1} = 2 \cdot 10^8$/s, $k_1 = 2 \cdot 10^9$ liter/(mol · s), $p_a = 1.5 \times 10^8$ liter/(mol · s). $\delta_1 = \delta_2 = 0.08333$, $\delta_3 = 0.33334$. The permeant ion concentrations are denoted in each section of the figure. The currents are normalized by $i_{lim}$ whose magnitude is denoted by the solid horizontal lines to the right in each section. The interrupted lines indicate the currents in the absence of diffusion control ($p_a = \infty$). 25°C.

$$k_1^{eff} = k_1 \cdot p_a/(k_1 + p_a) \quad \text{(B12)}$$

and by

$$k_{-1}^{eff} = k_{-1} \cdot p_a/(k_1 + p_a), \quad \text{(B13)}$$

where the $k'$ should be primed as usual to denote any bias due to an applied potential. The usual condition for sublinearity, $k_{-1}/l << 1$, will thus be replaced by $k_{-1}^{eff}/l << 1$. But this means that the condition for sublinearity becomes

$$k_{-1}/l << (k_1 + p_a)/p_a \quad \text{(B14)}$$

which reverts to the usual crition when $k_1 << p_a$.

Eq. B11 simplifies further if either $k_{-1}/l << 1$ and $\delta_1 = 0$ when

$$i(u) = e \cdot c \cdot k_1 \cdot p_a \cdot \tanh(u/2)/(k_1 + p_a), \quad \text{(B15)}$$

or if $k_1/p_a$ simultaneously is much larger than $k_{-1}/l$ and 1.0 when

$$i(u) = e \cdot c \cdot p_a \cdot \tanh(u/2). \quad \text{(B16)}$$

This is the classic expression for a diffusion-controlled current.

The second limiting case occurs when $u$ is so low that the hyperbolic sine (sinh) terms in the denominator of Eq. B5 [$F_3(u)$] can be neglected. The small-signal conductance is then expressed as

$$g(c) = \frac{e^2}{2 \cdot kT} \cdot \frac{l \cdot k_{-1} \cdot c}{K \cdot (l + k_{-1}/2) + l \cdot k_{-1}/p_a + c \cdot (2 \cdot l + k_{-1})}. \quad \text{(B17)}$$

As usual $g(c)$ can be expressed as

$$g(c) = g_{max} \cdot c/(c + K_g) \quad \text{(B18)}$$

where

$$g_{max} = (e^2/kT) \cdot l \cdot k_{-1}/[2(2l + k_{-1})] \quad \text{(B19)}$$

but $K_g$, the activity for half-maximal conductance, is no longer equal to $K/2$ (see, for example, Andersen 1983 *b*):

$$K_g = \frac{[K \cdot (2l + k_{-1}) + 2 \cdot l \cdot k_{-1}/p_a]}{[2(2l + k_{-1})]}. \quad \text{(B20)}$$

The single-site dissociation constant is therefore

$$K = 2 \cdot K_g - 2 \cdot l \cdot k_{-1}/[(2 \cdot l + k_{-1}) \cdot p_a] \quad \text{(B21)}$$

$$= 2 \cdot [K_g - 2 \cdot g_{max} \cdot kT/(e^2 \cdot p_a)] \leq 2 \cdot K_g. \quad \text{(B22)}$$

Neglect of aqueous diffusion limitations may thus lead to considerable errors in the estimate of $K$. This is illustrated in Fig. 9, where it additionally is shown how the conductance vs. concentration relation only exhibits the simple Langmuirian behavior at low potentials. At higher potentials, the Eadie-Hofstee plots become nonlinear.

## APPENDIX C

## A Singly-Occupied Channel May Exhibit Concentration-Dependent Permeability Ratios When Aqueous Diffusion Limitations are Significant

It is assumed that the intrinsic channel permeability properties can be described by the two-sites-one-ion model used in Appendix B. The

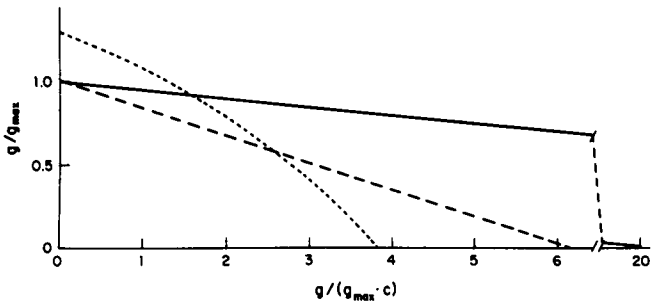


FIGURE 9 Eadie-Hofstee plots of conductance vs. activity in the absence and presence of aqueous diffusion limitations. The curves are generated according to Eqs. B6–B10 after converting to conductances. $K = 0.1$ M, $l = 2 \cdot 10^7$/s, $k_{-1} = 2 \cdot 10^8$/s, $k_1 = 2 \cdot 10^8$ liter/(mol · s), $p_a = 1.5 \cdot 10^8$ liter/(mol · s). The solid line is drawn with $p_a = \infty$ (no diffusion limitation). The interrupted line is for $V = 0$ mV, the stippled line is for $V = 100$ mV. 25°C.

aqueous phase to the left contains permeant ion X at concentration $c_x$. The aqueous phase to the right contains the permeant ion Y at concentration $c_y$. Only one permeant ion is present in each bulk aqueous phase, and the aqueous convergence permeability is assumed to be finite, such that the permeant ion concentrations at the channel entrance may differ from the bulk concentrations. The flux of X and Y are now obtained by solving the kinetic equations for the two schemes

$$X' + G \underset{k''_{-x}}{\overset{k'_x}{\rightleftharpoons}} XG \underset{l''_x}{\overset{l'_x}{\rightleftharpoons}} GX \underset{k''_{-x}}{\overset{k'_{-x}}{\rightleftharpoons}} G + X'' \qquad \text{(C1)}$$

and

$$Y' + G \underset{k''_{-y}}{\overset{k'_y}{\rightleftharpoons}} YG \underset{l''_y}{\overset{l'_y}{\rightleftharpoons}} GY \underset{k''_y}{\overset{k'_{-y}}{\rightleftharpoons}} G + Y''. \qquad \text{(C2)}$$

The two schemes are connected by the relation

$$W(G) + W(XG) + W(GX) + W(YG) + W(GY) = 1 \qquad \text{(C3)}$$

where $W(G)$, etc. denotes the probability of finding the channel in the unoccupied state, etc. The ion concentrations in Eqs. C1 and C2 are related to the bulk phase concentrations through the four relations following:

$$c'_x = c_x - J_x(u)/p_a^x \qquad \text{(C4)}$$

$$c''_x = J_x(u)/p_a^x \qquad \text{(C5)}$$

$$c''_y = c_y + J_y(u)/p_a^y \qquad \text{(C6)}$$

$$c'_y = -J_y(u)/p_a^y. \qquad \text{(C7)}$$

The biionic potential is defined by

$$J_x + J_y = 0. \qquad \text{(C8)}$$

The kinetic equations associated with Eqs. C1–C3 are solved by standard methods (e.g., Wong, 1975). This yields the flux of X and Y as a function of the unknown concentrations $c'_x$, $c''_x$, $c'_y$, and $c''_y$. These concentrations are eliminated using Eqs. C4–C7. The resulting flux expressions can then be written as

$$J_x(u) = p_x(u) \cdot [c_x \cdot \exp(u/2) - 2 \cdot J_x(u) \cdot \cosh(u/2)/p_a^x]/\mathbf{D}(u) \qquad \text{(C9)}$$

$$J_y(u) = -p_y(u) \cdot [c_y \cdot \exp(-u/2) - 2 \cdot J_y(u) \cdot \cosh(u/2)/p_a^y]/\mathbf{D}(u) \qquad \text{(C10)}$$

where

$$p_i(u) = k_i \cdot l_i/\{k_{-i} + 2 \cdot l_i \cdot \cosh[(\delta_2 + \delta_3) \cdot u]\} \qquad \text{(C11)}$$

with the appropriate subscripts for X and Y, and

$$\mathbf{D}(u) = 1 + \frac{c_x \cdot f_x(u)}{K_x} - \frac{J_x \cdot g_x(u)}{p_a^x} + \frac{c_y \cdot f_y(u)}{K_y} - \frac{J_y \cdot g_x(u)}{p_a^y} \qquad \text{(C12)}$$

where

$$f_x(u) = \frac{\exp(+\delta_1 \cdot u) \cdot [k_{-x} \cdot \exp(+\delta_2 \cdot u) + 2 \cdot l_x \cdot \cosh(\delta_3 \cdot u)]}{k_{-x} + 2 \cdot l_x \cdot \cosh[(\delta_2 + \delta_3) \cdot u]} \qquad \text{(C13)}$$

$$f_y(u) = \frac{\exp(-\delta_1 \cdot u) \cdot [k_{-y} \cdot \exp(-\delta_2 \cdot u) + 2 \cdot l_y \cdot \cosh(\delta_3 \cdot u)]}{k_{-y} + 2 \cdot l_y \cdot \cosh[(\delta_2 + \delta_3) \cdot u]} \qquad \text{(C14)}$$

$$g_x(u) = \frac{2}{K_x} \cdot \frac{l_x\{\sinh[\delta_1 + \delta_3]u + \sinh[(\delta_1 - \delta_3)u]\} + k_{-x} \cdot \sinh[(\delta_1 + \delta_2)u]}{k_{-x} + 2 \cdot l_x \cdot \cosh([\delta_2 + \delta_3] \cdot u)} \qquad \text{(C15)}$$

$$g_y(u) = \frac{2}{K_y} \cdot \frac{l_y\{\sinh[(\delta_1 + \delta_3)u] + \sinh[(\delta_1 - \delta_3)u]\} + k_{-y} \cdot \sinh[(\delta_1 + \delta_2)u]}{k_{-y} + 2 \cdot l_y \cdot \cosh[(\delta_2 + \delta_3) \cdot u]} \qquad \text{(C16)}$$

A complete solution for the biionic potential based on Eqs. C8–C16 is complex, and generally only possible by numerical methods. It is possible, however, to simplify the situation without loss of generality by assuming that the bi-ionic potentials are low ($u << 1$). The sinh terms ($g_x$ and $g_y$) will, in this situation, be negligible, and Eqs. C9 reduces to

$$J_x(u) = \frac{p_x(u) \cdot c_x \cdot \exp(u/2)}{1 + \left(\frac{c_x \cdot f_x(u)}{K_x}\right) + \left(\frac{c_y \cdot f_y(u)}{K_y}\right) + 2 \cdot \left(\frac{p_x}{p_a^x}\right) \cdot \cosh(u/2)}, \qquad \text{(C17)}$$

and Eq. C10 reduces to

$$J_y(u) = -\frac{p_y(u) \cdot c_y \cdot \exp(-u/2)}{1 + \left(\frac{c_x \cdot f_x(u)}{K_x}\right) + \left(\frac{c_y \cdot f_y(u)}{K_y}\right) + 2 \cdot \left(\frac{p_y}{p_a^y}\right) \cdot \cosh(u/2)}. \quad \text{(C18)}$$

At the biionic potential then,

$$\exp(u) = \left[\frac{p_y(u) \cdot c_y}{p_x(u) \cdot c_x}\right] \cdot \frac{1 + \left(\frac{c_x \cdot f_x(u)}{K_x}\right) + \left(\frac{c_y \cdot f_y(u)}{K_y}\right) + 2 \cdot \left(\frac{p_x}{p_a^x}\right) \cdot \cosh(u/2)}{1 + \left(\frac{c_x \cdot f_x(u)}{K_x}\right) + \left(\frac{c_y \cdot f_y(u)}{K_y}\right) + 2 \cdot \left(\frac{p_y}{p_a^y}\right) \cdot \cosh(u/2)}. \quad \text{(C19)}$$

The permeability ratio is therefore a function of the permeant ion concentrations unless $p_x/p_a^x = p_y/p_a^y\ (=0)$.

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