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DIFFUSION-LIMITED ION FLOW THROUGH PORES

P. LÄUGER

Department of Biology, University of Konstanz, D-775-Konstanz (G.F.R.)

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SUMMARY

If the permeability of an ion channel is high, the overall transport becomes ultimately limited by the rate with which ions from the aqueous phase arrive at the mouth of the channel. In this study a theoretical treatment of diffusion-limited ion transport through pores is given which takes both concentration and electric potential gradients into account as driving forces for the flow of ions. The current-voltage characteristic of a diffusion-limited channel is found to be strongly saturating. Addition of an impermeable electrolyte decreases the saturation current. This ionic strength effect results from a reduction in the electric field strength near the mouth of the channel. At low voltages the permeability P of the pore is obtained as $1/P = (1/P_i)+(1/P'_c)+(1/P''_c)$ where P_i is the intrinsic permeability and P'_c, P''_c are the left-hand and right-hand convergence permeabilities, respectively, which are proportional to the aqueous diffusion coefficient of the permeable ion and to the effective capture radius of the pore.

INTRODUCTION

The ion permeability of biological membranes seems to depend, at least in some cases, on the presence of pores. A pore may consist in a built-in protein molecule or in some other fixed structural element that offers to the ion a hydrophilic pathway through the apolar core of the membrane. A pore mechanism has been postulated, for instance, for the gated sodium transport system in excitable membranes [1]. The formation of pore-like ion channels by hydrophobic peptides such as gramicidin or alamethicin has been demonstrated in experiments with artificial lipid bilayer membranes [2–4].

If the permeability of a pore is high, the ion flow becomes ultimately limited by the rate by which ions from the aqueous solution arrive at the mouth of the pore. This extrinsic limitation of ion flow is usually analyzed in either of two ways. The first method is derived from the theory of homogeneous conductors and consists in calculating the so-called convergence (or access) resistance of the pore [5–7]. In the second approach the pore mouth is treated as a hemispherical sink toward which the permeable ion species diffuses according to Fick's law [5, 6, 8]. Neither of these approaches is entirely satisfactory. In the calculation of the convergence resistance the presence of concentration gradients near the entrance of the pore is neglected. In the method based on Fick's law only the concentration gradient is considered as a driving force for the flow of ions, whereas the effect of electric field strength is omitted. In general, however, both concentration and electrical potential gradients are present on the aqueous side of the pore mouth. Ion flow through the pore leads to depletion of the permeable ion species at one end of the pore and to accumulation at the other end; these changes in the concentration and the electrical conductance of the aqueous solution in turn influence the potential drop between the mouth of the pore and the bulk solution.

In the following a more general and more rigorous analysis of diffusion-limited ion flow is given which is based on the Nernst-Planck (or electrodiffusion) treatment of ion transport in solution.

THEORY

Basic flux equations

At the mouth of the pore the ion transport mechanism changes from free three-dimensional diffusion in the aqueous phase to constrained diffusion within the pore. For the analysis of the overall transport process the use of proper boundary conditions at the pore mouth is therefore critical. An exact solution of this problem would require information on the geometry of the pore entrance, on the interaction between ion and adjacent parts of the membrane (including ion-dipole interactions and electric image forces), on the water structure near the surface of the membrane and so forth. As long as such detailed information is missing, the use of a simple geometrical model seems appropriate. In the following the boundary between pore and solution is treated as a hemispherical surface (Fig. 1). Ion transport in the solution outside the hemisphere is described as an electrodiffusion process in a continuous medium. The electrical potential and the concentrations of the different ion species are considered to be constant on the surface of the hemisphere. These assumptions are similar to those introduced in the treatment of diffusion-controlled chemical reactions in solution [9]. The radius of the hemispherical boundary is denoted by r_0. r_0 may be as small as the difference between the radius of the pore and the radius of the permeable ion (Andersen, O. S., personal communication), but larger values are also possible, for instance, if an ion hitting the membrane in the proximity of the pore is able to

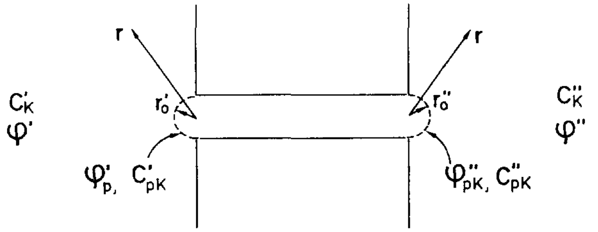

Fig. 1. Geometrical model of the pore. The boundaries between the interior of the pore and the external solutions are represented by hemispherical surfaces of radius r'_0 and r''_0. ϕ'_p and $c'_{p,k}$ are the reduced electrical potential and the concentration of ion species k at the left-hand boundary; ϕ' and c'_k are the potential and the concentration at infinite distance from the pore in the left-hand solution. ϕ''_p, $c''_{p,k}$, ϕ and c''_k are the corresponding quantities in the right-hand solution.

reach the pore mouth by surface diffusion. We therefore avoid a purely geometrical interpretation and consider r_0 as an effective capture radius [16]. For sake of generality we allow different values r'_0 and r''_0 at the left-hand and right-hand entrance of the pore (Fig. 1). In addition, we shall use the following assumptions: (a) The system is in the stationary state. (b) The density of pores in the plane of the membrane is sufficiently low so that interactions between pores may be neglected (this problem is further discussed in a later section). (c) There is no convection in the aqueous solution near the pore mouth. As most of the concentration change produced by ion flow occurs in a hemispherical zone of less than 100 nm in diameter (see below), this assumption is always fulfilled. (d) The solution outside the pore is electrically neutral. This is only true if there are no fixed charges at the pore mouth. (e) The pore is far from saturation; this means that the rate of entry of ions into the pore is proportional to the ion concentration at the mouth of the pore. We specify the boundary conditions in the aqueous solutions far from the membrane by introducing large hemispherical electrodes which are concentric with the pore openings. The electrical potential ϕ and the concentration c_k of ion species k are held constant at the electrode surfaces, ϕ', c'_k and ϕ'', c''_k being the values at the left-hand and right-hand electrode, respectively. We now assume that the radius of the hemispherical electrodes is increased to infinity. This then corresponds to the experimental situation where a voltage $\phi' - \phi''$ is applied across the aqueous solutions and where the bulk concentrations in the two solutions are c'_k and c''_k.

Under these conditions the electrical potential and the concentrations are functions of a single coordinate, the radial distance r (Fig. 1). The dependence on r of the ion concentrations in the left-hand and right-hand solutions are described by functions $C'(r)$ and $C''(r)$. We further introduce dimensionless electrical potentials $V'(r)$ and $V''(r)$ which are expressed in units of $RT/F \simeq 25$ mV (R is the gas constant, T the absolute temperature, and F Faraday's constant. If J_k is the stationary flux* of ion species k through the pore, then the flux density $J_k/2\pi r^2$ is given by the Nernst-Planck equations:

$$\frac{J_k}{2\pi r^2} = D_k \left(\frac{dC'_k}{dr} + z_k C'_k \frac{dV'}{dr}\right) \tag{1}$$

$$\frac{J_k}{2\pi r^2} = -D_k \left(\frac{dC''_k}{dr} + z_k C''_k \frac{dV''}{dr}\right) \tag{2}$$

$(k = 1, 2, \ldots, N)$

D_k is the diffusion coefficient of ion species k in the aqueous phase and z_k its valency; J_k is positive if the flux is directed from phase $'$ to phase $''$. The boundary conditions for the concentrations and the electrical potential are (compare Fig. 1):

$$C'_k(r'_0) = c'_{p,k}; \qquad C''_k(r''_0) = c''_{p,k} \tag{3}$$

$$C'_k(\infty) = c'_k; \qquad C''_k(\infty) = c''_k \tag{4}$$

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* It should be noted that for a spherical source or sink the time-dependent diffusion equation has a stationary solution with non-vanishing flow [8, 9]. This is no longer true for a membrane with homogeneously distributed permeability [10].

$$V'(r'_0) = \phi'_p; \qquad V''(r''_0) = \phi''_p \tag{5}$$

$$V'(\infty) = \phi'; \qquad V''(\infty) = \phi'' \tag{6}$$

The assumption that the external solutions are electrically neutral yields the following additional relations:

$$\sum_1^N z_k C'_k = 0 \tag{7}$$

$$\sum_1^N z_k C''_k = 0 \tag{8}$$

Finally, we write down the expression which relates the flux J_k to the concentrations $c'_{p,k}$ and $c''_{p,k}$ of ion species k at the pore openings and to the potential drop $u_p = \phi'_p - \phi''_p$ across the pore:

$$J_k = P_{i,k} \frac{z_k u_p/2}{\sinh(z_k u_p/2)} [c'_{p,k} \exp(z_k u_p/2) - c''_{p,k} \exp(-z_k u_p/2)] \tag{9}$$

$P_{i,k}$ is the intrinsic permeability coefficient of the pore for ion species k. Eqn. 9 which follows from the rate theory analysis of ion transport through pores [11, 12] has the form of Goldman's flux equation [13, 14]. In the Goldman equation, however, the permeability coefficient is regarded to be independent of voltage, whereas in Eqn. 9 $P_{i,k}$ is in general a function of u_p. It is only under certain assumptions about the potential-energy profile of the pore that $P_{i,k}$ becomes a constant (see Appendix A).

Eqns. 1, 2 and 7–9 represent a system of $3N+2$ equations from which the $3N+2$ unknown quantities $C'_k(r)$, $C''_k(r)$, $V'(r)$, $V''(r)$ and J_k may be calculated in principle. A solution in closed form, however, is obtained only under special conditions. In the following, we treat a number of simple and relevant cases in which the effects of diffusion polarisation associated with ion flow through the pore may be analysed.

For the presentation of the results it is useful to introduce quantities $P'_{c,k}$ and $P''_{c,k}$ which may be interpreted (see below) as left-hand and right-hand side convergence permeabilities of the pore with respect to ion species k:

$$P'_{c,k} = 2\pi r'_0 D_k; \qquad P''_{c,k} = 2\pi r''_0 D_k \tag{10}$$

Likewise, under conditions where the concentrations of ion species k are the same on both sides of the membrane ($c'_k = c''_k = c_k$) it is useful to define single-channel convergence conductances $\Lambda'_{c,k}$ and $\Lambda''_{c,k}$ and an intrinsic single-channel conductance $\Lambda_{i,k}$:

$$\Lambda'_{c,k} = (z_k^2 F^2/RT) c_k P'_{c,k}; \qquad \Lambda''_{c,k} = (z_k^2 F^2/RT) c_k P''_{c,k} \tag{11}$$

$$\Lambda_{i,k} = (z_k^2 F^2/RT) c_k P_{i,k} \tag{12}$$

One ion-species permeable

Specifically, we assume here that the solutions contain a 1 : 1 electrolyte of which only the cation is permeable and, in addition, a second, impermeable 1 : 1 electrolyte with common anion. Thus,

$$z_1 = z_3 = 1; \qquad z_2 = -1$$

$$P_{i,2} = P_{i,3} = 0$$

The inert electrolyte is introduced here in order to provide the possibility of independent variation of the ionic strength at a given concentration of the permeable ion.

The general solution for this case can be obtained only in implicit form and is given in Appendix B (Eqns. B4–B6). For a discussion of the solution we first consider a system with symmetrical outer phases in the ohmic limit:

$$c_1' = c_1'' = c_1, \qquad c_3' = c_3'' = c_3$$

$$c_2' = c_2'' = c_1 + c_3$$

$$|u| = |\phi' - \phi''| \ll 1$$

Under these conditions the ion flux $J = J_1$ and the concentration drop $c''_{p,1} - c'_{p,1}$ across the pore are given by (with the notation $P_{i,1} \equiv P_i$, $P'_{c,1} \equiv P'_c$, $P''_{c,1} \equiv P''_c$):

$$J \approx \frac{c_1 u}{(1/P_i) + (1/P_c') + (1/P_c'')} \tag{13}$$

$$c''_{p,1} - c'_{p,1} \approx \frac{u c_1 (c_1 + 2c_3)}{2(c_1 + c_3)} \cdot \frac{(1/P_c') + (1/P_c'')}{(1/P_i) + (1/P_c') + (1/P_c'')} \tag{14}$$

(see Appendix B). Eqn. 13 may be interpreted in the following way. In the absence of any diffusion limitation in the aqueous phases the ion flux would be simply given by $J = P_i c_1 u$ (this follows from Eqn. 9 with $u_p = u \approx 0$ and $c'_{p,1} = c''_{p,1} = c_1$). In the presence of diffusion polarisation this form of the flux equation may be retained if an overall permeability coefficient P is introduced:

$$J = P c_1 u \tag{15}$$

$$\frac{1}{P} = \frac{1}{P_i} + \frac{1}{P_c'} + \frac{1}{P_c''} \tag{16}$$

Thus, the experimentally accessible permeation resistance $1/P$ is the sum of the intrinsic permeation resistance $1/P_i$ and two terms $(1/P'_c)$ and $(1/P''_c)$ which allow for the diffusion resistance in the aqueous phases (Eqn. 10). In analogy to the convergence resistance of a pore, P'_c and P''_c may be called convergence permeability coefficients.

It is seen from Eqn. 13 that the flux of the permeable ion is independent of any addition of inert electrolyte; this, however, is only true in the ohmic limit (see below). On the other hand, the concentration difference $c''_{p,1} - c'_{p,1}$ of the permeable ion which builds up across the pore as a result of ion flow depends on the concentration c_3 of the inert electrolyte; Eqn. 14 shows that $c''_{p,1} - c'_{p,1}$ increases by a factor of two if c_3 is increased from zero to the limit $c_3 \gg c_1$. Conversely, the absolute value of the electrical potential drop between pore entrance and bulk solution is a decreasing function of c_3 (compare Eqn. B7).

An ion flow of magnitude J corresponds to a current $I = FJ$ through the single pore which may be written as

$$I = \Lambda V_m = \Lambda \frac{RT}{F} u \tag{17}$$

Λ is the single-channel conductance and V_m the applied voltage in volts. Comparison with Eqn. 15 shows that $\Lambda = (F^2/RT)c_1P$. We may therefore use Eqns. 11 and 12 and write

$$\frac{1}{\Lambda} = \frac{1}{\Lambda_i} + \frac{1}{\Lambda'_c} + \frac{1}{\Lambda''_c} \tag{18}$$

Λ_i is the intrinsic conductance which the pore would assume in the absence of diffusion polarisation; $\Lambda'_c = 2\pi z^2F^2cr'_0D/RT$ and $\Lambda''_c = 2\pi z^2F^2cr''_0D/RT$ are the convergence conductances of the pore for the permeable ion species. Eqn. 18 shows that in the limit $|u| \ll 1$ the pore resistance $1/\Lambda$ may be represented as the sum of an intrinsic pore resistance $1/\Lambda_i$ and left-hand and right-hand convergence resistances $1/\Lambda'_c$ and $1/\Lambda''_c$. It should be noted that $1/\Lambda'_c$ and $1/\Lambda''_c$ contain only the contribution of the permeable ions, whereas the convergence resistance R_c, as usually defined, is proportional to the overall specific resistance of the solution. R_c is thus a lower limit to $(1/\Lambda'_c)+(1/\Lambda''_c)$.

Next, we consider the case of a symmetrical membrane and symmetrical aqueous solutions without addition of an inert electrolyte at arbitrary voltage u:

$$c'_1 = c''_1 = c'_2 = c''_2 = c_1$$

$$c'_3 = c''_3 = 0$$

$$P'_c = P''_c = P_c$$

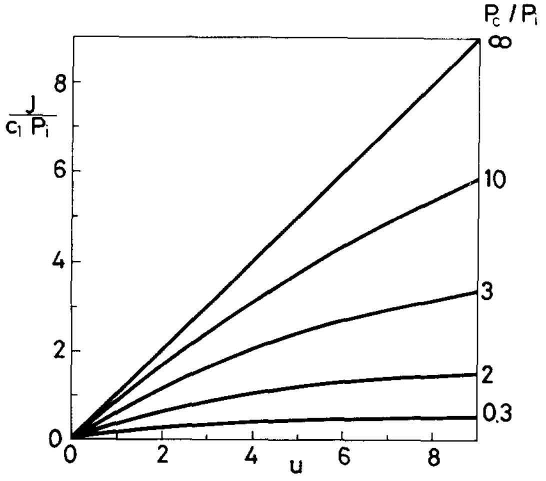

Fig. 2. Ion flux J through a single pore, divided by c_1P_i as a function of reduced voltage u (voltage divided by $RT/F \simeq 25$ mV), according to Eqns. B11 and B12. c_1 is the concentration of the permeable ion (valency $z_1 = 1$) in the aqueous solutions, P_i is the intrinsic permeability coefficient of the pore, and P_c the convergence permeability coefficient. J/c_1P_i is plotted for different values of the ratio P_c/P_i. The solutions contain only the permeable ion species and the counterion ($c_3 = 0$).

Here the solution is obtained again in implicit form (Eqns. B11 and B12) and has to be evaluated numerically. The result is represented in Fig. 2 in which the ion flux J (divided by $c_1 P_i$) is plotted as a function of voltage u for different values of the ratio P_c/P_i. For the calculation of J it has been implicitly assumed that the intrinsic permeability coefficient P_i is voltage independent. As discussed in Appendix A, P_i becomes independent of u if the pore can be represented by a sequence of many identical energy barriers. As shown in Fig. 2, the reduced flux $J/c_1 P_i$ is equal to the reduced voltage u in the limit $P_c \gg P_i$ (diffusion in the aqueous phase fast compared with transport through the pore). On the other hand, diffusion limitation becomes noticeable even if P_c is still several times as large as P_i.

If the intrinsic permeability of a pore increases to very high values, the ion flux approaches a maximum value J_{max} which is exclusively determined by diffusion in the aqueous phase. Under the condition $c'_3 = c''_3 = 0$ (no inert electrolyte added), the diffusion-limited ion flux is obtained as (Eqn. B16):

$$J_{max} = 2 \frac{\sqrt{c'_1}\,e^{u/4} - \sqrt{c''_1}\,e^{-u/4}}{(e^{u/4}/P'_c\sqrt{c'_1}) + (e^{-u/4}/P''_c\sqrt{c''_1})} \tag{19}$$

J_{max} represents an absolute upper limit of ion flow through a pore under the given conditions. For a symmetrical system ($P'_c = P''_c = P_c$, $c'_1 = c''_1 = c_1$) Eqn. 19 reduces to

$$J_{max} = 2 P_c c_1 \tanh(u/4) \tag{20}$$

This means that in the diffusion-limited case the ion flux saturates at voltages of the order of $u \simeq 4$, or $V_m \simeq 100$ mV (Fig. 3).

On the other hand, in the presence of an excess of inert electrolyte: $c'_3 = c''_3 = c_3 \gg c'_1, c''_1$ the diffusion-limited ion flux is given by (Eqn. B20):

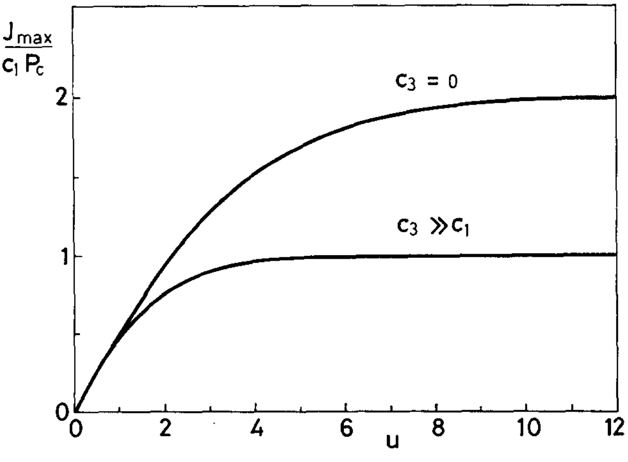

Fig. 3. Diffusion-limited ion flow as a function of the reduced voltage u (Eqns. 20 and 22). J_{max} is the ion flow through a single pore in the limit of infinite intrinsic permeability. c_1 is the aqueous concentration of the permeable ion and P_c the convergence permeability coefficient of the pore. Upper curve, no inert electrolyte present; lower curve, addition of an inert electrolyte in high concentration.

$$J_{\max} = \frac{c_1' e^{u/2} - c_1'' e^{-u/2}}{(e^{u/2}/P_c') + (e^{-u/2}/P_c'')} \tag{21}$$

In the case of a symmetrical system ($P_c' = P''_c = P_c$, $c'_1 = c''_1 = c_1$) this equation simplifies to

$$J_{\max} = P_c c_1 \tanh(u/2) \tag{22}$$

Comparison with Eqn. 20 shows that the presence of an inert electrolyte in high concentration has the effect of reducing the saturation value of $J_{\max}$ by a factor of two (Fig. 3); at the same time the saturation occurs at a lower voltage ($u \simeq 2$, or $V_m \simeq 50$ mV). This difference may be explained in the following way. If an excess of inert electrolyte is added, the electric field strength in the aqueous phase at the pore openings is strongly reduced; this is seen from a comparison of Eqns. B17 and B12. Thus, in the absence of inert electrolyte both a concentration gradient and an electric potential gradient act as driving forces for the flow of ions from the solution into the pore, whereas with an excess of inert salt only the concentration gradient remains.

In the limit of high positive voltages u where $\tanh(u/4) \approx \tanh(u/2) \approx 1$, the concentrations of the permeable ion at the pore openings become $C'_1(r_0) \approx 0$, $C''_1(r_0) \approx 2c_1$ (Eqns. B19 and B22), irrespective whether $c_3 = 0$ or $c_3 \gg c_1$. In this limit the pore acts as an ideal sink on the left side and as a source on the right side. The corresponding saturation values of the ion fluxes in the symmetrical system (Eqns. 20 and 22) are given by

$$J_{\max}(u \to \infty) = 2P_c c_1 = 4\pi D_1 r_0 c_1 \qquad (c_3 = 0) \tag{23}$$

$$J_{\max}(u \to \infty) = P_c c_1 = 2\pi D_1 r_0 c_1 \qquad (c_3 \gg c_1) \tag{24}$$

In the later case ($c_3 \gg c_1$) the flux agrees with the flux which is estimated from a simple source-sink model of the pore neglecting the effect of electric field strength [6]. In the former case ($c_3 = 0$), however, the flux calculated on the basis of the Nernst-Planck equation is twice as large as the flux obtained from the simple diffusion model.

Single electrolyte, both ions permeable

We assume that the aqueous phases contain a single electrolyte (one cation species, one anion species) and that cations and anions are permeable. The general solution for the symmetrical system ($c'_1 = c''_1 = c_1$, $c'_2 = c''_2 = c_2$; $r'_0 = r''_0 = r_0$; $P'_{c,1} = P''_{c,1} = P_{c,1}$, $P'_{c,2} = P''_{c,2} = P_{c,2}$) is given in Appendix C. In the following we consider the special case $P_{i,1} \to \infty$, $P_{i,2} \to \infty$; under this condition the solution reads (compare Appendix C):

$$C_1'(r) = C_1''(r) \equiv c_1; \qquad C_2'(r) = C_2''(r) \equiv c_2 \tag{25}$$

$$V''(r) - \phi'' = -[V'(r) - \phi'] = \frac{u}{2}\frac{r_0}{r} \tag{26}$$

$$J_{1,\max} = \tfrac{1}{2} P_{c,1} z_1 c_1 u; \qquad J_{2,\max} = \tfrac{1}{2} P_{c,2} z_2 c_2 u \tag{27}$$

It is easy to show that the same solution is obtained in the case of ideally stirred aqueous phases if the condition $C'_1(r) = C''_1(r) \equiv c_1$, $C'_2(r) = C''_2(r) \equiv c_2$ is directly introduced into Eqns. 1 and 2. The electric current through the pore in the limit of infinite intrinsic permeability is given by

$$I_{\max} = \Lambda \frac{RT}{F} u = F(z_1 J_{1,\max} + z_2 J_{2,\max}) \tag{28}$$

Together with Eqns. 10 and 27, the single channel conductance Λ is obtained as

$$\Lambda_{\max} = \frac{\pi r_0 F^2}{RT} (z_1^2 c_1 D_1 + z_2^2 c_2 D_2) \tag{29}$$

Introducing the specific resistance ρ of the aqueous phase:

$$\rho = \frac{RT/F^2}{z_1^2 c_1 D_1 + z_2^2 c_2 D_2} \tag{30}$$

Eqn. 29 may be written as

$$\Lambda_{\max} = \frac{\pi r_0}{\rho} = \frac{1}{2R_c} \tag{31}$$

$R_c = \rho/2\pi r_0$ is the well-known expression for the one-sided convergence resistance of a circular pore [6]. In the above case where both ion species which are present in the solution contribute to the electric current, the limiting conductance of the pore is directly related to the convergence resistance R_c.

Interaction between pores; time evolution towards the stationary state

The preceding analysis is valid for a single, isolated pore. In this section we discuss in more detail under what conditions the interactions between neighbouring pores may be neglected. The situation is simplest if the pores form a cluster of finite size. As shown in Appendix B, the concentration perturbation resulting from ion flow through a pore decays with reciprocal distance r from the pore mouth. This is evident, for instance, from Eqns. B19 and B22 which both may be written in the form

$$C_1(r) - c_1 = [C_1(r_0) - c_1] \frac{r_0}{r} \tag{32}$$

where C_1 may stand for C'_1 or C''_1. A similar dependence on r is found for the electrical potential. Thus, if the distance a between nearest neighbours in the cluster is much larger than the effective pore radius r_0, interaction between the individual pores may be neglected. As r_0 is of the order of 0.1 nm, a can be as small as 10 nm before overlap of the concentration and potential profiles becomes serious. A case in which electrical coupling between neighbouring pores occurs has been considered recently by von der Heydt and von der Heydt [17]. The situation becomes more complicated if an infinite array of pores is regarded. If there are N_p pores per unit area, and if r^* is the distance in the plane of the membrane from an arbitrarily selected reference pore, $N_p \cdot 2\pi r^* \mathrm{d}r^*$ pores act upon the reference pore from a distance r^*. The effect of these pores on the concentration at $r^* = 0$ is proportional to $1/r^*$, whereas their number is proportional to r^*, and thus the summation over the whole area diverges. This means that an infinite array of pores have no stationary state with non-vanishing flux. Under many conditions, however, a quasistationary state is reached, because the characteristic time τ_p which is required for the establishment of the hemispherical concentration profile at the pore mouth is very short. For a symmetrical system with a single permeable ion species 1 in the presence of an excess of inert electrolyte ($z_1 = 1$, $c'_1 = c''_1 = c_1$, $P'_c = P''_c = P_c$, $r'_0 = r''_0 = r_0$) the time course of the ion flux through a single pore in the limit $P_i \gg P_c$ is given by [8, 9]:

$$J(t) = P_c c_1 \tanh\left(\frac{u}{2}\right)\left[1 + \frac{r_0}{\sqrt{\pi D_1 t}}\right] \tag{33}$$

(For $t \to \infty$ this expression reduces to Eqn. 22).

In the derivation of Eqn. 33 it has been assumed that the pore is in equilibrium at times $t < 0$ and that a voltage u is applied to the membrane at $t = 0$; furthermore, it has been assumed that all transport steps within the pore are fast as compared with τ_p. According to Eqn. 33 the characteristic time τ_p is of the order of

$$\tau_p \simeq \frac{r_0^2}{\pi D_1} \tag{34}$$

With $r_0 = 0.2$ nm and $D_1 = 10^{-5}\ \mathrm{cm}^2 \cdot \mathrm{s}^{-1}$ this gives $\tau_p \simeq 10^{-11}$ s. This time is much too short to be directly observable. On the other hand, in an infinite array of pores where interactions over large distances play a role the very fast initial drop of the ion flow J is followed by a second, slower decay of J which results from a gradual change of ion concentration at the membrane surface. If the pores are widely separated so that the overall ion permeability of the membrane is small, the second phase of $J(t)$ is slow and a quasi-stationary state is established. In order to obtain a crude estimate for the slow decay of $J(t)$, we assume the ion permeability to be homogeneously distributed in the plane of the membrane. For the symmetric system considered above, the time course (for small times) of the ion flow per unit area, J_m, after a voltage jump at $t = 0$ is given by [10]:

$$J_m(t) \approx J_m(0)\left[1 - \frac{2}{\alpha}\sqrt{\frac{D_1 t}{\pi}}\right] \tag{35}$$

$$\alpha = D_1 c_1 \frac{\tanh(u/2)}{J_m(0)}; \qquad (t \ll \alpha^2/D_1)$$

Introducing the number N_p of pores per unit area and the conductance Λ of a single pore, $J_m(0)$ may be written as $J_m(0) = N_p \Lambda u RT/F^2$. If we require that the relative change of ion flow during the experimental time t is small, say, less than 1 %, then the relation

$$\frac{2}{\alpha}\sqrt{\frac{D_1 t}{\pi}} = \frac{N_p \Lambda RT}{c_1 F^2} \cdot \frac{2u}{\tanh(u/2)}\sqrt{\frac{t}{\pi D_1}} < 10^{-2} \tag{36}$$

must hold. With $\Lambda = 100$ pS, $c_1 = 1$ M, $u = 4$ ($V_m \simeq 0.1$ V), $D_1 = 10^{-5}\ \mathrm{cm}^2 \cdot \mathrm{s}^{-1}$, $t = 10$ s, Eqn. 36 means that N_p has to be smaller than 10^8 pores per cm^2. Thus, if the mean distance is larger than $1/\sqrt{10^8 \mathrm{cm}^{-2}} = 10^3$ nm, interactions between pores are negligible under the above conditions.

DISCUSSION

The extent to which diffusion in the aqueous phases contributes to the overall permeation resistance of a pore depends on the ratio of the convergence permeability P_c to the intrinsic permeability P_i of the pore. For a primitive geometrical model in which the pore is considered as a cylinder of length l and spherical cross-section πr_p^2, which is filled with a solution of the permeable ion of the same concentration as the external aqueous phase, this ratio becomes equal to $P_c/P_i = 2\pi r_0 D_l/(\pi r_0^2 D_l/l) = 2l/r_0$. (In this model the capture radius r_0 is equal to the difference $r_p - r_{ion}$.) As long as $l > r_0$, diffusion in the aqueous phases would not be a limiting factor in the overall transport. In a real pore, however, the partitioning of ions between water and the interior of the pore may be strongly in favour of the pore, so that P_c/P_i may become considerably smaller than $1/r_0$.

For $r_0 = 0.1$ nm and $D = 10^{-5}\ \mathrm{cm}^2 \cdot \mathrm{s}^{-1}$, P_c is approx. $6 \cdot 10^{-13}\ \mathrm{cm}^3 \cdot \mathrm{s}^{-1}$. The corresponding value of the convergence conductance Λ_c at one-molar concentration c of the permeable ion (valency $z = \pm 1$) would be $\Lambda_c = (F^2/RT)cP_c \simeq 3 \cdot 10^{-9}\ \Omega^{-1} = 3$ nS. Accordingly, the range of single-channel conductances Λ for which the diffusion resistance in the aqueous phases is negligible is given by $\Lambda \ll \Lambda_c$, or

$$\frac{\Lambda}{c} \ll 3\ \mathrm{nS/M} \tag{37}$$

This relation applies to the case that the pore is permeable to only one ion species. For most ion channels studied so far Λ/c lies below 3 nS/M. For instance, recent estimates for the density of sodium channels in squid axon yield $\Lambda/c \simeq 5$ pS/M [18]. Values of Λ/c between 50 and 200 pS/M are reported for the ionic channel opened by acetylcholine at the neuromuscular junction [19, 20]. Direct determinations of the single-channel conductance of gramicidin A in lipid bilayer membranes give Λ/c values of the order of 50 pS/M for the alkali ions [2, 21]. Much higher values ($\Lambda/c \simeq 5$ nS/M) have been observed with alamethicin channels in lipid bilayer membranes [22]. In this case, however, the capture radius of the pore is likely to be larger than assumed in Eqn. 37. On the other hand, as discussed earlier, the effective capture radius r_0 may become smaller than 0.1 nm if the ion radius is close to the pore radius.

At present, a direct experimental test whether diffusion in the aqueous phase influences the conductance Λ of a given type of channel seems difficult. Conclusions about the magnitude of the ratio Λ/Λ_c depend on the estimated values of the effective capture radius r_0. An indirect test for the presence of diffusion limitation consists in measuring the current-voltage characteristic of the single channel [2]. If the transport of ions through the channel is affected by aqueous diffusion, the current tends to saturate with increasing voltage; for an exact interpretation of the current-voltage characteristic, however, the voltage dependence of P_i has to be taken into account.

APPENDIX A

A rate theory expression for the permeability coefficient $P_{i,k}$ *(Eqn. 9)*

$P_{i,k}$ may be calculated from rate theory for a pore having n internal potential energy minima [13]. Dropping the subscript k the result reads (compare Eqn. 21 of ref. 12):

$$P_i = \frac{1-\exp(-zu_p)}{zu_p} \cdot \frac{k'_0 v}{1+\sum_1^N S_v} \tag{A1}$$

$$S_v = \frac{k''_1 k''_2 \dots k''_v}{k'_1 k'_2 \dots k'_v} \tag{A2}$$

k'_v is the rate constant for jumps from the ν-th to the $(\nu+1)$-th minimum, k''_v the rate constant for jumps from the ν-th to the $(\nu-1)$-th minimum; the energy minima with $\nu = 0$ and $\nu = n+1$ correspond to the left-hand and right-hand boundary, respectively, of the pore. v is a constant with the dimension of a volume.

For a pore with $n+1$ identical, regularly spaced energy barriers the relations

$$k'_0 = k_{ap}e^{w}; \qquad k''_{n+1} = k_{ap}e^{-w} \tag{A3}$$

$$k'_v = k_i e^{w}; \qquad k''_v = k_i e^{-w} \tag{A4}$$

$$(\nu = 1, 2, \dots, n)$$

$$w = zu_p/2(n+1) \tag{A5}$$

hold [12]. k_{ap} and k_i are the rate constants at zero voltage for jumps from the aqueous phase into the pore and for jumps across the internal barriers, respectively. Introducing Eqns. A3–A5 into Eqns. A1 and A2, P_i is obtained in the form

$$P_i = \frac{2vk_{ap}}{zu_p} \sinh\left(\frac{zu_p/2}{n+1}\right) \tag{A6}$$

If n is large, P_i becomes independent of voltage:

$$P_i \approx \frac{vk_{ap}}{n+1} \tag{A7}$$

It is useful to write this equation in a slightly different form, introducing the equilibrium constant $K = vnk_{ap}/k_i$ for the occupancy of the pore [12] and the diffusion coefficient $D_p = k_i d^2/(n+1)^2$ of the ion within the pore [15] (d is the pore length). This gives (with $n \gg 1$):

$$P_i \approx \frac{KD_p}{d^2} \tag{A8}$$

This is the continuum approximation of the permeability coefficient. If the membrane contains N_p pores per unit area and if the single pore can be occupied by only one ion at a time, the number of occupied pores per unit area in the limit of small ion concentration c is equal to cKN_p [12]. The quantity KN_p/d can therefore be identified with the partition coefficient γ of the ion between membrane and aqueous phase. The overall permeability coefficient P_m which is referred to unit area of the membrane is then obtained in the familiar form

$$P_m = N_p P_i = \frac{\gamma D_p}{d} \tag{A9}$$

APPENDIX B

Solution of the flux equations in the case $z_1 = z_3 = 1, z_2 = -1, P_{i,2} = P_{i,3} = 0$

If the aqueous phases contain only three different kinds of ion and if only ion species 1 is permeable ($J_2 = J_3 = 0$) then Eqn. 1 may be integrated for ion species 2 and 3 to give

$$C'_k(r) = c'_k \exp\left[-z_k(V' - \phi')\right] \qquad \text{(B1)}$$

$$(k = 2, 3)$$

Introduction of Eqn. B1 into Eqn. 7 yields, with $z_1 = z_3 = 1, z_2 = -1$:

$$C'_1(r) = c'_2 \exp(V' - \phi') - c'_3 \exp\left[-(V' - \phi')\right] \qquad \text{(B2)}$$

After substitution of $C'_1(r)$ from Eqn. B2, Eqn. 1 for ion species 1 may be integrated to give (with $J_1 \equiv J$ and $P'_{c,1} \equiv P'_c$):

$$\exp\left[V'(r) - \phi'\right] = 1 - \frac{J}{2c'_2 P'_c} \frac{r'_0}{r} \qquad \text{(B3)}$$

P'_c is defined by Eqn. 10. Applying Eqn. B3 to $r = r_0$ one obtains [with $y' \equiv \exp(\phi'_p - \phi'), y'' \equiv \exp(\phi''_p - \phi'')$]:

$$J = -2P'_c c'_2 (y' - 1) \qquad \text{(B4)}$$

In a completely analogous way, integration of Eqn. 2 yields ($P''_{c,1} \equiv P''_c$):

$$J = 2P''_c c''_2 (y'' - 1) \qquad \text{(B5)}$$

Furthermore, introduction of $c'_{p,1} = C'_1(r'_0)$ from Eqn. B2 and of $c''_{p,1} = C''_1(r''_0)$ into Eqn. 9 gives (with $P_{i,1} \equiv P_i$ and $u \equiv \phi' - \phi''$):

$$J = P_i\left[u + \ln(y'/y'')\right] \cdot \frac{e^{u/2}\sqrt{y'/y''}(c'_2 y' - c'_3/y') - e^{-u/2}\sqrt{y''/y'}(c''_2 y'' - c''_3/y'')}{e^{u/2}\sqrt{y'/y''} - e^{-u/2}\sqrt{y''/y'}} \qquad \text{(B6)}$$

Eqns. B4–B6 are implicit equations for the three unknown quantities y', y'', and J.

Case 1

Under the condition

$$c'_1 = c''_1 = c_1, \qquad c'_3 = c''_3 = c_3$$

$$c'_2 = c''_2 = c_1 + c_3; \qquad |u| \ll 1$$

where the approximations $e^u \approx 1 + u$, $y' \approx 1 + \phi'_p - \phi'$, $y'' \approx 1 + \phi''_p - \phi''$ hold, Eqns. B2–B6 can be solved in closed form to give

$$V''(r) - \phi'' = -\left[V'(r) - \phi'\right] = \frac{c_1}{c_1 + c_3} \cdot \frac{uP}{4\pi D_1 r} \qquad \text{(B7)}$$

$$C''_1(r) - c_1 = -\left[C'_1(r) - c_1\right] = \frac{c_1 + 2c_3}{c_1 + c_3} \cdot \frac{c_1 u P}{4\pi D_1 r} \qquad \text{(B8)}$$

$$\frac{1}{P} = \frac{1}{P_i} + \frac{1}{P'_c} + \frac{1}{P''_c} \tag{B9}$$

From Eqns. B7 and B8 the flux J:

$$J \approx (c_1+c_3)[P''_c(\phi''_p-\phi'')-P'_c(\phi'_p-\phi')] \tag{B10}$$

and the concentration difference $c''_{p,1}-c'_{p,1}$ may be calculated; the results are given in Eqns. 13 and 14.

Case 2

For a symmetrical membrane and symmetrical solutions without added inert electrolyte:

$$c'_1 = c''_1 = c'_2 = c''_2 = c_1$$

$$c'_3 = c''_3 = 0$$

$$P'_c = P''_c = P_c$$

at arbitrary voltage u, Eqns. B4–B6 simplify to ($q \equiv P_c/P_i$):

$$\frac{J}{c_1 P_i} = 2q(1-y') \tag{B11}$$

$$2q = \left(u+\ln\frac{y'}{2-y'}\right)\frac{y'^2(e^u-1)+4(y'-1)}{(y'-1)[2-y'(e^u+1)]} \tag{B12}$$

The numerical evaluation of these two equations is represented in Fig. 2

Case 3

Under the condition $P_i \to \infty$ (intrinsic permeability much higher than the convergence permeability), the ratio J/P_i approches zero so that the denominator on the right side of Eqn. B16 vanishes (it is easily shown that, in general, $u \neq \ln(y'/y'')$). Together with Eqns. B4 and B5 this yields two equations from which y' and y'' may be determined. Introduction of the result into Eqn. B4 then gives

$$J = 2P'_c c'_2(1-A+\sqrt{A^2+B}) \tag{B13}$$

$$A = \frac{\alpha(1+\alpha)c''_2}{\alpha^2 c''_2 - c'_2 e^u}\ ; \qquad \alpha = \frac{P'_c c'_2}{P''_c c''_2} \tag{B14}$$

$$B = \frac{c''_3 - c'_3 e^u - (1+\alpha)^2 c''_2}{\alpha^2 c''_2 - c'_2 e^u} \tag{B15}$$

Case 3a

In the absence of inert electrolyte ($c'_3 = c''_3 = 0$). Eqns. B13–B15 yield

$$J = 2\frac{\sqrt{c'_1}e^{u/4} - \sqrt{c''_1}e^{-u/4}}{(e^{u/4}/P'_c\sqrt{c'_1})+(e^{-u/4}/P'_c\sqrt{c''_1})} \tag{B16}$$

The dependence of concentration and electrical potential on distance r is obtained from Eqns. B2 and B3 (and the analogous equations for phase $''$). For a completely symmetric system ($c'_1 = c''_1 = c'_2 = c''_2 = c_1$, $r'_0 = r''_0 = r_0$, $P'_c = P''_c = P_c$) the result reads

$$V'(r) - \phi' = \ln\left[1 - \frac{r_0}{r}\tanh(u/4)\right] \tag{B17}$$

$$V''(r) - \phi'' = \ln\left[1 + \frac{r_0}{r}\tanh(u/4)\right] \tag{B18}$$

$$C''_1(r) - c_1 = -[C'_1(r) - c_1] = c_1\frac{r_0}{r}\tanh(u/4) \tag{B19}$$

Case 3b

If an excess of inert electrolyte is present ($c'_3 = c''_3 = c_3 \gg c'_1, c''_1$), the ion flux, as obtained from Eqns. B13–15, is given by

$$J = \frac{c'_1 e^{u/2} - c''_1 e^{-u/2}}{(e^{u/2}/P'_c) + (e^{-u/2}/P''_c)} \tag{B20}$$

Again, Eqns. B2, B3 and B20 may be used to calculate the dependence of concentration and potential on r. For a symmetric system ($c'_1 = c''_1 = c_1$, $r'_0 = r''_0 = r_0$, $P'_c = P''_c = P_c$) the following result is obtained:

$$V''(r) - \phi'' = -[V'(r) - \phi'] = \frac{r_0}{2r}\frac{c_1}{c_3}\tanh\left(\frac{u}{2}\right) \approx 0 \tag{B21}$$

$$C''_1(r) - c_1 = -[C'_1(r) - c_1] = c_1\frac{r_0}{r}\tanh\left(\frac{u}{2}\right) \tag{B22}$$

Eqn. B21 shows that in the presence of inert electrolyte in high concentration ($c_1/c_3 \rightarrow 0$) the electric field strength at the pore mouth vanishes, as expected. We may use this fact to derive a generalized version of Eqn. B20 which is valid at arbitrary P_i. For this purpose we introduce $\mathrm{d}V'/\mathrm{d}r = \mathrm{d}V''/\mathrm{d}r = 0$ into Eqns. 1 and 2 and $u_p = u$ into Eqn. 9. This gives

$$J = \frac{c'_1 e^{u/2} - c''_1 e^{-u/2}}{\dfrac{\sinh(u/2)}{u/2}\cdot\dfrac{1}{P_i} + \dfrac{e^{u/2}}{P'_c} + \dfrac{e^{-u/2}}{P''_c}} \tag{B23}$$

This expression agrees with an equation derived by Neumcke (ref. 8, Eqn. 30). It should be noted here that P_i, in general, is a function of voltage u.

APPENDIX C

Solution of the flux equations in the case $P_{i,1} \neq 0$, $P_{i,2} \neq 0$ (single electrolyte)

We assume that the system contains only two ion species 1 and 2, both of which are permeable. In the completely symmetrical case:

$$c'_k = c''_k = c_k \qquad (k = 1, 2)$$

$$r'_0 = r''_0 = r_0$$

$$P'_{c,k} = P''_{c,k} = P_{c,k}$$

The implicit solution of Eqns. 1–9 reads:

$$C'_1(r) - c_1 = -[C''_1(r) - c_1] = \frac{z_2}{z_1 - z_2}\left(\frac{J_1}{P_{c,1}} + \frac{J_2}{P_{c,2}}\right)\frac{r_0}{r} \tag{C1}$$

$$V'(r) - \phi' = A \ln \frac{r}{r+b}; \qquad V''(r) - \phi'' = A \ln \frac{r}{r-b} \tag{C2}$$

$$b = \frac{z_1 z_2 r_0}{z_1^2 c_1 + z_2^2 c_2}\left(\frac{J_1}{P_{c,1}} + \frac{J_2}{P_{c,2}}\right) \tag{C3}$$

$$A = \frac{1}{z_1 z_2} \cdot \frac{(z_1 J_1/P_{c,1}) + (z_2 J_2/P_{c,1})}{(J_1/P_{c,1}) + (J_2/P_{c,2})} \tag{C4}$$

$$\begin{aligned} \frac{J_1}{P_{i,1}} = \frac{z_1 u_p/2}{\sinh(z_1 u_p/2)} \cdot \Bigg\{ & \left[c_1 + \frac{z_2}{z_1 + z_2}\left(\frac{J_1}{P_{c,1}} + \frac{J_2}{P_{c,2}}\right)\right] \exp(z_1 u_p/2) \\ & - \left[c_1 - \frac{z_2}{z_1 - z_2}\left(\frac{J_1}{P_{c,1}} + \frac{J_2}{P_{c,2}}\right)\right] \exp(-z_1 u_p/2) \Bigg\} \end{aligned} \tag{C5}$$

$$u_p = u + A \ln \frac{r_0 - b}{r_0 + b} \tag{C6}$$

Relations analogous to Eqns. C1 and C5 are obtained for ion species 2 by interchanging the subscripts 1 and 2. In order to derive upper limits for J_1 and J_2 we consider the special case $P_{i,1} \rightarrow \infty$, $P_{i,2} \rightarrow \infty$. Under this condition $J_1/P_{i,1}$ in Eqn. C5 vanishes. As for $|u_p| < \infty$ the first term $(z_1 u_p/2)/\sinh(z_1 u_p/2)$ on the right side of Eqn. C5 differs from zero, the second term must vanish. The same argument applies to the equation for $J_2/P_{i,2}$. It is then easy to show that the only possible solution is

$$u_p = 0; \qquad \frac{J_1}{P_{c,1}} = -\frac{J_2}{P_{c,2}} \tag{C7}$$

According to Eqn. C1 (and the analogous equation for ion species 2) this means that

$$C'_1(r) = C''_1(r) \equiv c_1 \tag{C8}$$

$$C'_2(r) = C''_2(r) \equiv c_2 \tag{C9}$$

With these relations Eqns. 1 and 2 are easily integrated to give

$$V''(r) - \phi'' = -[V'(r) - \phi'] = \frac{J_1}{z_1 c_1 P_{c,1}}\frac{r_0}{r} = \frac{J_2}{z_2 c_2 P_{c,2}}\frac{r_0}{r} \tag{C10}$$

Eqn. C10 further yields

$$u_p = [V'(r_0) - V''(r_0)] = u - \frac{J_1}{z_1 c_1 P_{c,1}} - \frac{J_2}{z_2 c_2 P_{c,2}} = 0 \tag{C11}$$

The final result is obtained by combination of Eqns. C10 and C11:

$$J_1 = J_{1,\max} = \tfrac{1}{2} z_1 c_1 P_{c,1} u \tag{C12}$$

$$J_2 = J_{2,\max} = \tfrac{1}{2} z_2 c_2 P_{c,2} u \tag{C13}$$

$$V''(r) - \phi'' = -[V'(r) - \phi'] = \frac{u}{2} \frac{r_0}{r} \tag{C14}$$

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