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ACCESS PERMEABILITY OF IONIC CHANNELS

DEPENDENCE ON AQUEOUS JUMP DISTANCE

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The access diffusion permeability of pores with diameters comparable to the aqueous jump distance is characterized using a rate theory analysis for the aqueous diffusion process. It is found that this process gives rise to two permeability terms, one associated with bulk diffusion and the other a jump from the aqueous solution into a position where it has access to the channel. The latter term dominates for small channel diameters and vice versa for large channel diameters. The properties of access diffusion with respect to concentration polarization is shown to be different in the two limits of large and small values of the channel radius. A necessary criterion for bulk access diffusion to be rate limiting is given in terms of measured channel conductance G, aqueous jump distance λ and aqueous resistivity ρ, $G > \pi\lambda/\rho$, which does not require a knowledge of channel geometry.

1. Introduction

The measured permeability of an ionic channel contains a contribution from the diffusion of ions in the aqueous phases, the magnitude of which depends on the geometry of the system. It has recently been suggested [1] that this, so-called access diffusion process, actually constitutes the rate-limiting step in cation transport through gramicidin A channels, partly because the selectivity pattern is similar to that of the aqueous diffusion coefficients for the permeant ions and partly because the current-voltage relationship is saturating at low concentrations.

The behavior of access diffusion limitation in relation to ionic channels has been dealt with by several authors [2–5] and their approach has essentially been a straightforward application of the Nernst-Planck equations to a spherical region extending from the channel opening to infinity. With such a geometry an expression for the access permeability may be derived [5]:

$$P = 2\pi D R_0 \tag{1}$$

where D is the diffusion coefficient and R_0 the capture radius of the pore. The value of R_0 depends on the geometry of the pore and may be as small as the difference between the pore radius and the ion radius. However, if the interaction between ions and the channel extends into the solution, the effective capture radius could be considerably larger.

Eq. 1 predicts that the permeability decreases linearly with decreasing capture radius and, for a given intrinsic channel permeability, the access diffusion should therefore become rate limiting for sufficiently small capture radii. However, the derivation of eq. 1 is based on the use of macroscopic flux equations and, it has been suggested [6] that the predictions will be different when the capture radius becomes smaller than the jump distance, λ, in water ($\lambda = 0.3$ nm for water mole-

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cules [7]). In the extreme case, namely, when the capture radius is much smaller than the jump distance, the probability for a particle to find the channel opening should become proportional to the channel cross-section. The access permeability would then show a square dependence rather than a linear dependence on the capture radius. It is therefore necessary to use a different theoretical approach than has previously been used in order to describe the properties of access diffusion for very small capture radii.

It will be shown that, by using a rate theory analysis for the aqueous diffusion, the access permeability will not depend linearly on the capture radius but will, in fact, consist of two terms where one describes the bulk diffusion part and the other describes the rate of crossing the last aqueous barrier before entering the channel. The theory shows that the latter becomes slower than the first when the capture radius is smaller than the jump distance λ. The theory also gives an experimental criterion for deciding whether the system is bulk diffusion limited, and from which, for example, it is concluded that ion transport through gramicidin A channels cannot be bulk diffusion rate limited.

2. Theory

2.1. Model

It is assumed that particles diffuse through the aqueous phase by a mechanism similar to that for water molecules, namely, by a jumping motion. Such a mechanism accounts among other things for neutron scattering data [8], and a model for the self-diffusion of water has been developed according to which the water molecules exhibit vibrational motion in equilibrium positions followed by rapid diffusion over longer distances. If the average distance travelled during the rapid part is labelled λ, and if ν is the frequency with which the particle leaves the equilibrium position, the diffusion coefficient D is given by

$$D = \tfrac{1}{6}\nu\lambda^2 \qquad (2)$$

It is further assumed in the present treatment that this diffusive motion takes place throughout the aqueous phase extending from infinity up to a spherical boundary of radius R_0 which has its center at the channel entrance.

When a particle crosses the hemispherical area of radius R_0 it has access to the channel and may then continue its motion along the channel interior. Outside this capture boundary the particle therefore diffuses freely with the same statistical properties as everywhere in the aqueous phases, and once it moves across the capture boundary it interacts with the channel. The transition between aqueous free diffusion and restricted diffusion in the channel will accordingly be treated as a sharp boundary, and by making this sharp distinction between aqueous diffusion and channel transport it is possible to describe the particular properties of access diffusion and to obtain criteria for determining whether it is rate limiting.

The dynamic processes by which a particle moves through the system are illustrated schematically in fig. 1, where the motion in the aqueous phases is described by a random walk process (in three dimensions) and where, in reaching the capture boundary, the particle moves through the channel. Since the treatment focuses on the process by which particles enter the channel the simplest model for the channel will be used, namely a single barrier model.

In applying a rate theory analysis to the processes shown in fig. 1 it is further assumed that

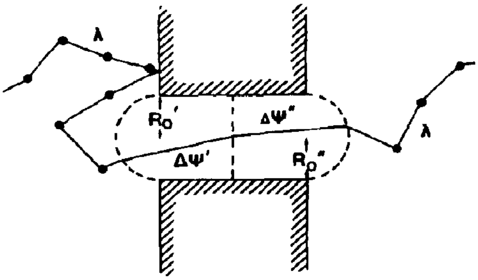

Fig. 1. The path by which an ion moves through the system (consisting of a single barrier channel with hemispherical capture boundaries) is illustrated with a solid line. The aqueous diffusion process is described by a random walk process with a jump distance λ and the channel crossing is shown as a single jump across a channel barrier. $\Delta\psi'$ and $\Delta\psi''$ are the potential differences between the capture boundaries and the barrier peak.

the thermal cloud in the aqueous equilibrium positions is very small compared to the jump distance, so that the particle does not interact with the channel until it is virtually at the capture boundary. This is clearly necessary if the capture radius is allowed to be smaller than the jump distance and is also consistent with the model for aqueous diffusion [8].

2.2. Flux equations

The flux equations for the particles will be derived assuming no electric field to be present in the aqueous solutions (for instance, in the presence of excess supporting electrolyte).

The transport equations applying to particle motion in the aqueous compartments are derived with the help of fig. 2. The number of particles leaving a volume element dV per unit of time is equal to

$$\nu C(r) dV$$

where ν is the collision frequency and $C(r)$ the concentration of particles which is a function of r (assuming spherical symmetry). The fraction of particles leaving dV and passing through dA is equal to

$$\nu C(r) dV \cdot \frac{dA}{4\pi x^3} \cdot \cos\theta$$

Taking into account the spherical symmetry, the contributions from all volume elements at equal distance x and angle θ with respect to dA becomes

$$\nu C(r) 2\pi x^2 \sin\theta dx d\theta \cdot \frac{dA}{4\pi x^2} \cdot \cos\theta$$

The integration is now carried out over the range

$$0 \leqslant \theta \leqslant \pi \qquad 0 \leqslant x \leqslant \lambda \qquad 0 \leqslant dA \leqslant 2\pi R^2$$

and gives the net flow, J, of particles crossing the hemispherical area of radius R

$$J = \pi\nu R^2 \int_0^{\lambda} \int_0^{\pi} C(r) \sin\theta \cos\theta d\theta dx \tag{3a}$$

Introducing the substitution $y = \cos\theta$, eq. 3a can be rewritten as follows

$$J = \pi\nu R^2 \int_0^{\lambda} \int_{-1}^{1} C(r) y dy dx \tag{3b}$$

where, according to fig. 2, r can be expressed in terms of R, x and y

$$r = \left[R^2 + x^2 + 2Rxy\right]^{1/2} \tag{3c}$$

Eq. 3 is a microscopic equation and the solution is valid for all r, even for values less than λ.

The general solution is given by

$$C(r) = C_{\infty} + \frac{a}{r} \tag{4a}$$

This is verified by inserting eq. 4a in eq. 3b and integrating with respect to y and x. The result is

$$J = \pi\nu R^2 \left(-\frac{1}{3} \cdot \frac{a\lambda^2}{R^2}\right) = -2\pi D a \tag{4b}$$

This result applies, depending on the sign of J, either to the left or to the right aqueous compartment. If it is assumed that positive flow is in direction from left to right, the constant a may be written for the two sides as

$$a = \pm \frac{J}{2\pi D} \tag{4c}$$

where the plus and minus signs refer to the right and left compartments, respectively.

It should be emphasized that the result derived from eq. 3 is entirely based on microscopic considerations. Although, however, eq. 4 turns out to be the same expression for the concentration profile as that derived from macroscopic theory, the dif-

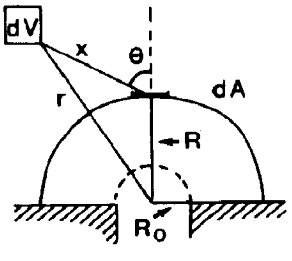

Fig. 2. Geometry of model for calculating the fraction of particles which leave the volume element dV and travel along a path which crosses the area element dA.

ference between microscopic and macroscopic theory will appear in the expression for the flow of particles across the capture boundaries. These flow equations will consist of differences between inflow from the aqueous phases and outflow across the capture boundaries

$$J = \nu\pi R_0'^2 \int_0^\lambda \int_0^1 C(r') y \,\mathrm{d}y \,\mathrm{d}x - \bar{\nu}' N' \tag{5a}$$

$$J = \bar{\nu}'' N'' - \nu\pi R_0''^2 \int_0^\lambda \int_0^1 C(r'') y \,\mathrm{d}y \,\mathrm{d}x \tag{5b}$$

N' and N'' are the probabilities of finding the particles at the capture boundaries on the two sides, respectively, and $\bar{\nu}'$ and $\bar{\nu}''$ the frequencies with which particles enter the aqueous solutions from the capture boundaries.

The difference in integration limits between eqs. 3 and 5 is due to the fact that the former contain contributions in both directions from the aqueous diffusion process whereas the latter only contain a unidirectional contribution from this process.

The complete set of flow equations is finally obtained by adding the transport equation describing the flow through the channel (assumed in this case to consist of a single barrier).

$$J = k'N' \exp(-z\Delta\psi') - k''N'' \exp(z\Delta\psi'') \tag{6}$$

where $\Delta\psi'$ and $\Delta\psi''$ are the potential differences (normalized with respect to kT/e) between the two capture boundaries, respectively, and the channel barrier peak and k' and k'' the jump rates across the barrier from either side. These rate constants are subject to the microscopic reversibility condition, which can also be used to define a quantity kR_0^2.

$$\frac{\bar{\nu}'}{k'R_0'^2} = \frac{\bar{\nu}''}{k''R_0''^2} = \frac{\nu}{kR_0^2} \tag{7}$$

According to this definition k is a rate constant which, for a symmetrical channel ($R_0' = R_0'' = R_0$), is equivalent to a jump over the barrier peak from an aqueous well.

By eliminating N' and N'' from eqs. 5 and 6 and taking into account eq. 7 the following expression for J is obtained

$$\left[1 + \frac{kR_0^2}{\nu}\left(\frac{\exp(-z\Delta\psi')}{R_0'^2} + \frac{\exp(z\Delta\psi'')}{R_0''^2}\right)\right] J = \pi k R_0^2 \int_0^\lambda \int_0^1 [C(r') \exp(-z\Delta\psi') - C(r'') \exp(z\Delta\psi'')]\, y \,\mathrm{d}y \,\mathrm{d}x \tag{8}$$

The concentrations $C'(r)$ and $C''(r)$ appearing in eq. 8 are given by eqs. 4. Inserting these in eq. 8 gives:

$$\left[1 + \frac{kR_0^2}{\nu}\left(\frac{\exp(-z\Delta\psi')}{R_0'^2} + \frac{\exp(z\Delta\psi'')}{R_0''^2}\right)\right] J = \pi k R_0^2 \frac{\lambda}{2} [C'_\infty \exp(-z\Delta\psi') - C''_\infty \exp(z\Delta\psi'')] - \frac{JkR_0^2}{2D} \int_0^\lambda \int_0^1 \left(\frac{\exp(-z\Delta\psi')}{r'} + \frac{\exp(z\Delta\psi'')}{r''}\right) \times y \,\mathrm{d}y \,\mathrm{d}x \tag{9}$$

and when divided by $JkR_0^2 \frac{\lambda}{2}$ this expression is reduced to

$$\frac{C'_\infty \exp(-z\Delta\psi') - C''_\infty \exp(z\Delta\psi'')}{J} = \frac{1}{\pi k R_0^2 \frac{\lambda}{2}} + \frac{\exp(-z\Delta\psi')}{P'} + \frac{\exp(z\Delta\psi'')}{P''} \tag{10a}$$

where

$$\frac{1}{P'} = \frac{1}{\pi\nu R_0'^2 \frac{\lambda}{2}} + \frac{f(R_0', \lambda)}{\pi\lambda D} \tag{10b}$$

$$\frac{1}{P''} = \frac{1}{\pi\nu R_0''^2 \frac{\lambda}{2}} + \frac{f(R_0'', \lambda)}{\pi\lambda D} \tag{10c}$$

and

$$f(R_0, \lambda) = \int_0^\lambda \int_0^1 \frac{y}{r} \,\mathrm{d}y \,\mathrm{d}x = \frac{1}{2} \ln\left(\frac{\lambda + \sqrt{\lambda^2 + R_0^2}}{R_0}\right) - \frac{\lambda}{6}\left[\frac{2}{R_0 + \sqrt{\lambda^2 + R_0^2}} - \frac{1}{\lambda + \sqrt{\lambda^2 + R_0^2}}\right] \tag{10d}$$

Eq. 10 is now the integrated flux equation which relates the ion flow to driving forces, channel properties and aqueous diffusion parameters. The total membrane permeability is seen from eq. 10a to be composed of three different terms. The first term expresses the intrinsic channel permeability and is seen to be determined by the transition rate k across the channel barrier and by a volume term, $v = \pi R_0^2 \lambda/2$, which is determined by the capture radius and the jump distance λ. This volume term generally appears in barrier models of ionic channels [5]. P' and P'' in eq. 10a are the access permeability terms which are seen from eqs. 10b and 10c to be split into two parts. The first part consists of the last aqueous jump, i.e., a jump from an aqueous equilibrium position bringing the particle in contact with the capture boundary. It is therefore also dependent on the volume term v. This volume term may be interpreted as the decrease in entropy resulting from the entry of particles into a small compartment with dimensions given by v.

The second parts of eqs. 10b and 10 c are the true bulk diffusion terms and they converge to eq. 1 for the macroscopic limit, i.e., when $R_0 \gg \lambda$. The total aqueous contribution to the permeability is obtained from the sum of eqs. 10b and 10c

$$\frac{1}{P} = \frac{1}{P'} + \frac{1}{P''} = \frac{1}{P_C} + \frac{1}{P_B} \tag{11a}$$

P_C is the term arising from the capture process

$$\frac{1}{P_C} = \frac{1}{v\pi R_0'^2} + \frac{1}{v\pi R_0''^2} = \frac{\lambda}{3\pi D}\left(\frac{1}{R_0'^2} + \frac{1}{R_0''^2}\right) \tag{11b}$$

and P_B is the total contribution from the bulk diffusion process

$$\frac{1}{P_B} = \frac{1}{\pi\lambda D}\left(f(R_0', \lambda) + f(R_0'', \lambda)\right) \tag{11c}$$

Fig. 3 shows the two aqueous diffusion parts, P_c and P_B plotted as functions of the ratio between the capture radius and aqueous jump distance for a symmetrical channel ($R_0' = R_0'' = R_0$). It is seen that the bulk diffusion term approaches the macroscopic behavior (dotted line) as the ratio R_0/λ increases, but when the jump distance becomes larger than the capture radius, this term is higher than predicted from macroscopic theory. This is due to the fact that access diffusion becomes split into a bulk process and a final jump process and

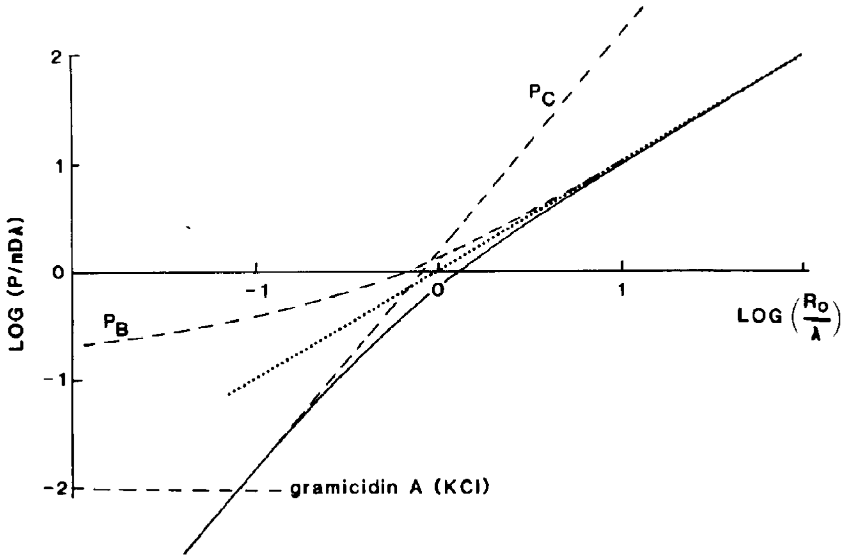

Fig. 3. The permeability terms given by eqs. 11b and 11c are normalized with respect to $\pi D\lambda$ and plotted as functions of R_0/λ (— — —) together with the total permeability P is given by eq. 11a (————). (· · · · · ·) Macroscopic limit. The experimental value of $P/\pi D\lambda$ for gramicidin A channels in the presence of K^+ ($\lambda = 0.3$ nm, $D = 2 \times 10^{-5}$ cm^2 s^{-1}) is indicated in the figure.

that the bulk process ends at a distance from the capture boundary where the particle can jump into the channel. It is also clear from fig. 3 that for small ratios of R_0/λ, the 'capture permeability' is smaller than the bulk permeability and it is therefore concluded that the latter can never be rate limiting when $R_0 < \lambda$. Since it has been suggested that transport through gramicidin A channels could be rate limited by access diffusion [1], an experimental value has been inserted in fig. 3, namely the potassium permeability of gramicidin calculated as G/C $(= 100\ \mathrm{pS/M})$, where G is the conductance at zero current at the concentration C. The aqueous diffusion coefficient for potassium is used $(D = 2 \times 10^{-5}\ \mathrm{cm^2/s})$ and the jump distance λ in water is set equal to 0.3 nm. At this calculated value of $P/\pi\lambda D$ it is seen that $P_c < P_B$ by at least an order of magnitude, from which it is concluded that if the transfer of ions is rate determined by transport in the aqueous phases it is not the bulk diffusion part, but rather the last aqueous jump bringing the ion contact with the capture boundary which constitutes the rate-limiting step. Although the term describing the last aqueous jump prior to entering the channel will contain only aqueous parameters in addition to the capture radius, it behaves as if two external barriers were added to the channel barriers. The effects of these additional barriers will also tend to eliminate the concentration polarization of the access diffusion process as will be shown below.

2.3. Concentration polarization

The presence of aqueous diffusion limitation generally causes a build-up of concentration gradients in the aqueous compartments, which is referred to as aqueous concentration polarization. The difference in concentration polarization between the two limits of R_0/λ can be calculated from eqs. 4 and 10 (fig. 4). The magnitude of polarization is expressed as

$$\frac{\Delta C}{C_\infty} = \frac{C_\infty - C(R_0)}{C_\infty} = \pm \frac{J}{4\pi D R_0 C_\infty} \tag{12}$$

and the maximum value (when $\Delta\psi \to \infty$) of this quantity is calculated from eq. 10 by assuming k to be much larger than ν. The shift in concentration polarization is seen to occur around the point when $\lambda = R_0$. Therefore, in spite of a completely diffusion-limited flow $(k \gg \nu)$, the aqueous concentration polarization is eliminated when $R_0 \ll \lambda$ and the system behaves as if it were membrane limited with respect to the permeability.

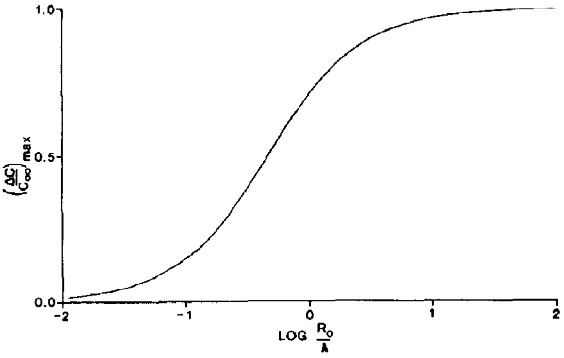

Fig. 4. Concentration polarization in the aqueous phases. The magnitude of maximum concentration polarization ($\Delta\psi \to \infty$) is calculated from eq. 11 and plotted as a function of $\log(R_0/\lambda)$.

3. Discussion

It has been shown that the access permeability is the result of two successive processes a bulk diffusion process and a subsequent jump from an aqueous equilibrium position into a position where it becomes accessible to the channel. When the capture radius is much smaller than the jump distance λ, the jump across the last barrier becomes the rate-limiting step. Fig. 4 shows that in this limit the behavior becomes indistinguishable from that of a channel with a pair of additional barriers, in other words, the system behaves like a pure barrier system with a finite number of barriers.

The bulk diffusion term, on the other hand, is associated with concentration polarization and may therefore under some circumstances give rise to $1/f$ noise [4].

It seems therefore justified to identify the bulk diffusion term with true access diffusion and the most useful distinction is therefore between that of bulk access diffusion and that of pure barrier diffusion, whether internally or externally located barriers. The expressions for the bulk access diffusion terms is given by the last term in eqs. 10b and 10c which converge to eq. 1 for the macroscopic limit. This definition of access diffusion limitation also leads to a useful experimental criterion for determining whether it forms the rate-limiting step in the overall permeation process. Bulk access diffusion limitation can occur only if the second terms in eqs. 10b and 10c are larger than the corresponding first terms and which is seen from fig. 3 to occur when

$$P/\pi D\lambda > 1 \tag{12}$$

where P is the experimentally determined membrane permeability. This criterion can also be written in terms of other physical parameters, namely, as

$$G > \pi\lambda/\rho \tag{13}$$

where G is the channel conductance and ρ the resistivity of the aqueous solutions. This inequality is useful, since it does not require a knowledge of the capture radius, and if λ is taken to be 0.3 nm and ρ is set to 100 Ω cm then G must be larger than 1 nS in order to have a possibility for bulk access diffusion to be rate limiting. This is not the case for most ionic channels which have been studied. If the above necessary condition is fulfilled, however, other properties of the system must be measured in order to decide on the possibility for bulk access diffusion to be the rate-limiting step. Such properties have been described in the paper by Läuger [5] and have also been described in this paper, for instance, the presence of concentration polarization.

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