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ION TRANSPORT THROUGH PORES: A RATE-THEORY ANALYSIS

P. LÄUGER

Department of Biology, University of Konstanz, 775 Konstanz (Germany)

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SUMMARY

This paper gives a treatment of ion transport through pores based on the theory of reaction rates. According to Eyring, the pore is considered as a sequence of energy barriers over which the ion has to jump. The present analysis differs from previous treatments in that it allows for saturation effects in the pore, *i.e.* it is assumed that for electrostatic reasons the pore may contain no more than one ion. The saturation phenomena are described by introducing the equilibrium constant for the occupancy of the pore. The theory leads to expressions which relate experimentally accessible quantities such as electrical conductivity, tracer permeability and biionic potential to the microscopic properties of the pore. It is shown that certain information on the single rate constants may be obtained from stationary conductance measurements. The specificity of the pore for different ions depends on thermodynamic quantities (equilibrium constants) as well as on kinetic parameters (rate constants). The experimental results with the gramicidin A pore are compared with the theory. The jump rates of K^+ and Na^+ in the gramicidin A pore are estimated to be of the order of $1 \cdot 10^8$–$1 \cdot 10^9$ s^{-1}.

In the last years, evidence has been accumulated that the ion permeability of biological membranes is not a diffuse property of the whole membrane, but is localized in special structures[1]. This is consistent with the view that the cell membrane is a mosaic of relatively impermeable lipid bilayer regions and built-in proteins[2]. Although as yet no single biological ion transport mechanism has been clarified in detail, it is reasonable to assume that at least in some cases the transport occurs through pores[3–6]. The term "pore" is used here in a rather general sense. A pore may be a relatively wide opening in a membrane, through which ions are able to move together with their primary hydration shells. Alternatively, a pore may consist of a chain of successive binding or coordination sites, for instance an array of peptide carbonyl groups. In the latter case the binding site may replace one or several water molecules of the inner hydration shell of the ion.

Recently, interest in pore mechanisms has been stimulated by the finding that certain peptides, such as gramicidin A, are able to create pore-like ion channels in artificial lipid bilayer membranes[7–14]. It has been proposed that gramicidin A is present in a helical conformation in the membrane and that by head-to-head association of two such helices a 4 Å wide, oxygen-lined pore is formed which bridges the entire thickness of the membrane[15–18].

An efficient pore mechanism should combine selectivity with high transport rates. For instance, the sodium channel of the squid axon membrane exhibits a $Na^+:K^+$ selectivity ratio of 20:1 and, at the same time, shows a transport rate of the order of 10^8–10^9 Na^+ per s (ref. 1). It is an important goal of the theoretical analysis of ion channels to explain how these seemingly conflicting requirements of selectivity and transport rate may be achieved. A closely related problem is the question to what extent the selectivity of a pore is determined by thermodynamic properties (equilibrium constants) and to what extent by kinetic parameters (rate constants).

The aim of this paper is to develop a simple, but straightforward description of ion transport through pores and to relate certain experimentally accessible quantities, such as electrical conductivity, tracer permeability or biionic potential, to the microscopic properties of the pore. A convenient starting point for such an attempt is to consider the pore a sequence of activation energy barriers over which the ion has to jump[19]. The physical basis of this approach has been worked out by Eyring and co-workers[20–23] in the so-called rate-theory analysis of transport processes. The present treatment, however, is more general than the Eyring analysis in so far as it allows for the phenomenon of saturation. An electrically neutral pore which is specific for, say, cations, such as the gramicidin A channel is unlikely to contain more than one ion[12]. The reason is the strong mutual repulsion of equally charged ions in a medium of low dielectric constant, *i.e.* the energy which is required to bring a second ion into the pore is many times the mean thermal energy. At high electrolyte concentrations the pore therefore saturates, and the electrical conductance reaches a limiting value. This behaviour has been observed by Hladky and Haydon[12] in their study of the gramicidin A channel.

With the restriction to pores which are either empty or single-occupied, coupling between the fluxes of different ion species is excluded from the present treatment. The phenomenon of flux coupling in narrow pores in which neighbouring ions are unable to exchange their positions[24] has been treated extensively by Heckmann[25–27], Heckmann and Vollmerhaus[28], Hladky[29,30], Yermishkin *et al.*[31–33] and others.

Description of the model

Following Eyring and co-workers[20–22], we represent the pore as a sequence of $n+1$ activation energy barriers (Fig. 1). The potential minima may be imagined as the places where the ion is in an energetically favourable position with respect to one or

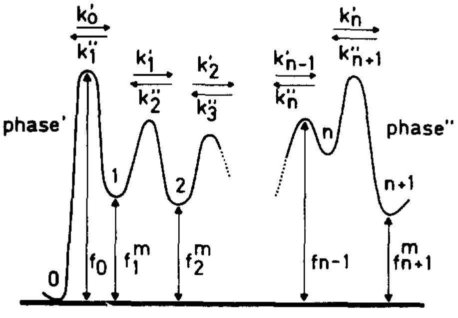

Fig. 1. Energy potential profile of the pore.

several coordinating ligands. The activation barrier between two minima may be of purely electrostatic origin, or may contain an additional term arising from the change in the conformational energy of the pore-forming macromolecule, which is associated with the passage of the ion from one site to the next. The rate constants for the jump from the ν-th minimum to the right and to the left are denoted by k_ν' and k_ν'', respectively (Fig. 1). If f_ν and f_ν^{m} are the reduced free energies of the ion (free energies divided by kT) in the ν-th minimum and at the top of the barrier to the right of the ν-th minimum (Fig. 1), then, according to the rate theory[35],

$$k'_\nu = \frac{kT}{h}\,\mathrm{e}^{-(f_\nu - f_\nu^{\mathrm{m}})} \qquad (\nu=0,1,\cdots,n) \tag{1}$$

$$k''_\nu = \frac{kT}{h}\,\mathrm{e}^{-(f_{\nu-1} - f_\nu^{\mathrm{m}})} \qquad (\nu=1,2,\cdots,n+1) \tag{2}$$

where $\boldsymbol{k}$ is the Boltzmann constant, T the absolute temperature and h Planck's constant ($kT/h \approx 6.2\cdot 10^{12}$ s^{-1} at $T=298$ °K). As usual, the transmission coefficient has been set approximately equal to one. As indicated in Fig. 1, the reference state for the free energy is chosen as the state of the ion in the left-hand aqueous solution. It is assumed that minima 0 and $(n+1)$ belong energetically to the aqueous phases, so that in the equilibrium state, *i.e.* in the absence of an external voltage, the relation $f_0^{\mathrm{m}} = f_{n+1}^{\mathrm{m}} = 0$ holds. In the non-equilibrium case, however, f_{n+1}^{m} is not equal to zero.

The model is further characterized by the following assumptions: (a) The membrane contains N_{P} identical pores per cm^2. The pores do not interact with each other, *i.e.* the probability of finding an ion in a given pore is independent of the occupancy of neighbouring pores. (b) Only ions of one valency type are able to enter the pore, for instance, univalent cations or univalent anions. The valency is denoted by z. (c) For electrostatic reasons the pore can contain no more than one ion; the pore is either empty or single-occupied. (It will be shown later that the results are easily generalized to the case that the pore can take up an unrestricted number of ions.)

The conditions at the pore mouths are described by introducing the concentration N of ions per cm^2 which are located in the outer minima (0 and $n+1$) in front of the pore. N is proportional to the number N_{P} of pores per cm^2 and to the ion concentration c in the aqueous phase*:

$$N = vcN_{\mathrm{P}} \tag{3}$$

The proportionality coefficient v has the dimension of a volume. $N/N_{\mathrm{P}} = vc$ is then the probability to find an ion in the outer minimum in front of the pore. On the other hand, the probability that a given small volume in a dilute solution of concentration c is occupied by an ion is equal to the volume times the concentration c. As we have assumed that the outer minima belong to the aqueous phases, v is equal to the volume from which the ion may enter the pore in a single jump, or approximately equal to the product of the pore cross-section σ times the "jump length" l:

$$v \approx \sigma l \tag{4}$$

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* The proportionality between N and c is consistent with the assumption that the outer minima belong to the aqueous phase and therefore do not saturate.

l is the distance between the first energy barrier in the aqueous solution and the entrance barrier of the membrane.

In general we consider the case that two different permeable ion species A and B are present in the aqueous phase. The concentrations in the left-hand and right-hand solutions are denoted by c_A', c_B' and c_A'', c_B'', respectively. According to Eqn 3, the concentrations of A and B in the outer minima are then given by $N_0^A = \nu_A c_A' N_P$, $N_0^B = \nu_B c_B' N_P$, $N_{n+1}^A = \nu_A c_A'' N_P$, $N_{n+1}^B = \nu_B c_B'' N_P$. If N_ν^A and N_ν^B are the concentrations of A and B (referred to one square cm of the membrane), in the ν-th potential minimum, then the number of pores per cm^2 which are occupied by an ion is obtained by summation of $(N_\nu^A + N_\nu^B)$ over all internal minima. Accordingly, the probability P that a pore is occupied is given by

$$P = \frac{1}{N_P} \sum_0^n (N_\nu^A + N_\nu^B) \tag{5}$$

The net flux of A over the first barrier is equal to the partial flux from left to right, $k_0^{A'} N_0^A (1-P)$, *minus* the partial flux from right to left, $k_1^{A''} N_1^A$. The factor $(1-P)$ which denotes the probability that the pore is empty has been introduced in order to account for the assumption that an ion may jump from the aqueous solutions into the pore only if the pore is not already occupied by another ion. The rate constants $k_\nu^{A'}$ and $k_\nu^{A''}$ depend, in general, on the voltage which is applied to the membrane, but are independent of ion concentrations. In the stationary state the net flux ϕ_A (moles/cm^{2}s) is the same for each barrier. This gives the following set of equations:

$$\begin{aligned}
\Phi_A &= k_0^{A'} N_0^A (1-P) - k_1^{A''} N_1^A \\
\Phi_A &= k_1^{A'} N_1^A - k_2^{A''} N_2^A \\
\Phi_A &= k_2^{A'} N_2^A - k_3^{A''} N_3^A \\
&\vdots \\
\Phi_A &= k_{n-1}^{A'} N_{n-1}^A - k_n^{A''} N_n^A \\
\Phi_A &= k_n^{A'} N_n^A - k_{n+1}^{A''} N_{n+1}^A (1-P)
\end{aligned} \tag{6}$$

A similar set of equations describes the flux of species B. Both sets are coupled *via* Eqn 5. In the following sections we apply these equations to special experimental situations.

Equilibrium state of the pore

We assume that only one permeable ion species is present in equal concentrations on both sides ($c_A' = c_A'' = c$, $c_B' = c_B'' = 0$, $N_\nu^B = 0$) and that the voltage across the membrane vanishes. The system is then in equilibrium, so that $\Phi_A = 0$. In the following, we drop the index A for simplicity and denote the equilibrium values of the rate constants and ion concentrations by $\bar{k}_\nu'$, $\bar{k}_\nu''$ and $\bar{N}_\nu$.

With $\Phi_A = 0$, Eqns 6 (together with Eqn 5) may be solved by successive elimination of the unknowns $N_\nu^A = \bar{N}_\nu$. At this point it is useful to introduce the following abbreviations:

$$R_\nu \equiv \frac{k'_0 k'_1 \cdots k'_{\nu-1}}{k''_1 k''_2 \cdots k''_\nu} = e^{-f_\nu^m} \tag{10}$$

$$\bar{R}_\nu \equiv \frac{\bar{k}'_0 \bar{k}'_1 \cdots \bar{k}'_{\nu-1}}{\bar{k}''_1 \bar{k}''_2 \cdots \bar{k}''_\nu} = e^{-\bar{f}_\nu^{\mathrm{m}}} \tag{11}$$

$$S_\nu \equiv \frac{k''_1 k''_2 \cdots k''_\nu}{k'_1 k'_2 \cdots k'_\nu} = e^{f_\nu - f_0} \tag{12}$$

$$\bar{S}_\nu \equiv \frac{\bar{k}''_1 \bar{k}''_2 \cdots \bar{k}''_\nu}{\bar{k}'_1 \bar{k}'_2 \cdots \bar{k}'_\nu} = e^{\bar{f}_\nu - \bar{f}_0} \tag{13}$$

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

The second part of these relations follows from Eqns 1 and 2, $\bar{f}_\nu$ and $\bar{f}_\nu^{\mathrm{m}}$ denoting the equilibrium values of f_ν and f_ν^{m}. With $N = vcN_{\mathrm{P}}$ (Eqn 3), the solution may be written as

$$\bar{N}_\nu = N_{\mathrm{P}} \frac{cv\bar{R}_\nu}{1 + cv \sum_1^n \bar{R}_\mu} \tag{14}$$

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

Eqn 14 expresses the saturation properties of the pore: with increasing ion concentration c the probability $\bar{N}_\nu / N_{\mathrm{P}}$ that the ν-th minimum is occupied reaches a constant limiting value

$$\frac{\bar{N}_\nu}{N_{\mathrm{P}}} \approx \frac{\bar{R}_\nu}{\sum_1^n \bar{R}_\mu} \qquad (c \to \infty) \tag{15}$$

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

If all n minima are identical, $\bar{N}_\nu / N_{\mathrm{p}}$ becomes equal to $1/n$, as expected.

Under the condition $\Phi = 0$ the $n+1$ Eqns 6 contain n unknowns $N_1, N_2, \cdots, N_n$. This means that the equilibrium rate constants $\bar{k}_\nu'$, $\bar{k}_\nu''$ are not independent from one another, in accordance with the principle of microscopic reversibility. The complete solution of Eqns 6 in the equilibrium case is then given by Eqn 14 together with the additional relation

$$\frac{\bar{k}'_0 \bar{k}'_1 \cdots \bar{k}'_n}{\bar{k}''_1 \bar{k}''_2 \cdots \bar{k}''_{n+1}} = 1 \tag{16}$$

For the description of saturation effects it is convenient to introduce an equilibrium constant K for the occupancy of the pore, which is defined by

$$K = \frac{N_{\mathrm{ip}}}{c(N_{\mathrm{P}} - N_{\mathrm{ip}})} \tag{17}$$

N_{ip} is the concentration (per cm^2) of pores which are occupied by an ion; accordingly, $(N_{\mathrm{P}} - N_{\mathrm{ip}})$ is the concentration of empty pores. With

$$N_{\mathrm{ip}} = \sum_1^n \bar{N}_\nu \tag{18}$$

and using Eqn 5, the expression 17 may be written as

$$cK = \frac{\bar{P}}{1-\bar{P}} \tag{19}$$

where $\bar{P}$ is the equilibrium value of P. Using Eqns 14, 17, and 18, the following expression is obtained for the equilibrium constant:

$$K = v \sum_{1}^{n} \bar{R}_\nu = v \sum_{1}^{n} \mathrm{e}^{-\bar{f}_\nu^{m}} \tag{20}$$

This expression has the form of a partition function and means that the higher the value of K becomes, the larger the number and the lower the energies of the potential minima are.

Ion flux as a function of concentrations and voltage

We again assume that only one permeable ion species is present in the aqueous solutions ($c_B' = c_B'' = 0$), the concentrations being denoted by $c_A' = c'$, $c_A'' = c''$. Furthermore, we allow for the presence of an electrical potential difference between the aqueous phases. Using the method of Parlin and Eyring[22], the ion flux Φ_A may be directly obtained from Eqns 5 and 6 by successive elimination of the unknowns N_ν^A. Dropping the index A, the solution may be expressed by

$$\Phi = N_P k'_0 v \frac{c' - \kappa c''}{1 + \sum_{1}^{n} S_\nu + v c' Q' + v c'' \kappa Q''} \tag{21}$$

$$\kappa \equiv \frac{k''_1 k''_2 \cdots k''_{n+1}}{k'_0 k'_1 \cdots k'_n} = \mathrm{e}^{f_{n+1}^{m}} \tag{22}$$

$$Q' \equiv \sum_{\nu=1}^{n} \left(S_\nu \sum_{\mu=1}^{\nu} R_\mu \right) \tag{23}$$

$$Q'' \equiv \sum_{1}^{n} R_\nu + \sum_{\nu=2}^{n} \left(R_\nu \sum_{\mu=1}^{\nu-1} S_\mu \right) \tag{24}$$

For $n = 1$, Q'' is defined as $Q'' = R_1$. Eqn 21 describes the ion flux as a function of concentrations c' and c'' and of voltage. The voltage is implicitly contained in the rate constants which in general depend on the electrical field strength in the pore (see below). In the limit of small ion concentrations ($c' \to 0$, $c'' \to 0$), Eqn 21 reduces to the flux equation which has been derived by Zwolinski *et al.*[20]:

$$\Phi \approx N_P k'_0 v \frac{c' - \kappa c''}{1 + \sum_{1}^{n} S_\nu} \tag{25}$$

This equation is not only valid in the case that most pores are empty (c', $c'' \to 0$), but also at arbitrary concentrations provided that the additional assumption is introduced that the entry of an ion does not depend on the occupancy of the pore and that the ions do not interact within the pore. This follows directly from Eqns 6:

if the factor $(1-P)$ which accounts for the saturation is omitted, the solution leads directly to Eqn 25.

Electrical conductance

We apply Eqn 21 to the case that both ion concentrations are the same ($c'=c''=c$). Under the influence of an external voltage $V=\psi'-\psi''$, a current $J=ze_0\Phi$ flows through the membrane (ψ' and ψ'' are the electrical potentials of the left-hand and right-hand aqueous phases, respectively, and e_0 is the elementary charge). According to Eqn 22 the quantity κ is then given by

$$\kappa=\mathrm{e}^{f^{\mathrm{m}}_{n+1}}=\mathrm{e}^{-zu} \tag{26}$$

$$u\equiv\frac{V}{kT/e_0} \tag{27}$$

For the moment we restrict ourselves to small voltages ($|zV|\ll 25$ mV, or $|zu|\ll 1$). In this limit Eqn 26 reduces to $\kappa\approx 1-zu$; furthermore, the rate constants may be replaced by their equilibrium values: $k_\nu'\approx\bar{k}_\nu'$, $k_\nu''\approx\bar{k}_\nu''$. The ohmic conductivity λ_0 of one square centimeter of the membrane is defined by

$$\lambda_0=\left(\frac{J}{V}\right)^{V\approx 0} \tag{28}$$

Using the identity

$$Q'+Q''=\sum_1^n R_\nu\left(1+\sum_1^n S_\nu\right) \tag{29}$$

together with Eqn 20, the ohmic conductivity is obtained from Eqn 21 in the form

$$\lambda_0=N_{\mathrm{P}}\,\frac{z^2{e_0}^2}{kT}\cdot\frac{vc}{1+cK}\cdot\frac{\bar{k}'_0}{1+\sum\limits_1^n\bar{S}_\nu} \tag{30}$$

$$=N_{\mathrm{P}}\,\frac{z^2{e_0}^2}{h}\cdot\frac{vc}{1+cK}\cdot\frac{1}{\sum\limits_0^n\mathrm{e}^{\bar{f}_\nu}} \tag{30a}$$

With increasing ion concentration c, λ_0 reaches asymptotically the maximum value

$$\lambda_{0,\max}=N_{\mathrm{P}}\,\frac{z^2{e_0}^2}{kT}\,\frac{v\,\bar{k}_0/K}{1+\sum\limits_1^n\bar{S}_\nu} \tag{31}$$

Eqn 30 may therefore be written as

$$\lambda_0=\frac{cK}{1+cK}\,\lambda_{0,\max} \tag{32}$$

According to Eqn 32, the half-maximum value of the conductance is reached at an ion concentration $c = 1/K$. The equilibrium constant K may therefore be obtained by measuring λ_0 as a function of c. As seen from Eqn 19, $c = 1/K$ is the concentration at which just half of the pores is occupied by an ion.

It may be instructive to write Eqn 30 in a somewhat different form by introducing the equilibrium concentrations $\bar{N}_\nu$ from Eqns 14 and 20. Then the following result is obtained in the limit $c \approx 0$ (with the notation $\bar{N}_0 = \bar{N}_{n+1} = cvN_P$):

$$\frac{1}{\lambda_0} = \frac{kT}{2z^2 e_0^2}\left(\sum_0^n \frac{1}{\bar{k}'_\nu \bar{N}_\nu} + \sum_1^{n+1} \frac{1}{\bar{k}''_\nu \bar{N}_\nu}\right) \tag{30b}$$

In this equation the membrane resistance $1/\lambda_0$ is represented as a sum of "barrier resistances".

Tracer permeability

The tracer permeability of the membrane is measured under the condition that the ions A and B are isotopes. This means that the rate constants for the transport of A and B are nearly equal ($k_\nu^{A'} \approx k_\nu^{B'} = k_\nu'$, $k_\nu^{A''} \approx k_\nu^{B''} = k_\nu''$). We further assume that the total ion concentrations on both sides are the same: $c_A' + c_B' = c_A'' + c_B'' = c$ and that no external voltage is applied to the membrane. As the membrane does not discriminate between A and B, we may therefore replace the factor $(1-P)$ in Eqns 6 by its equilibrium value $(1-\bar{P}) = 1/(1+cK)$, according to Eqn 19. Correspondingly, k_ν' and k_ν'' may be replaced by $\bar{k}_\nu'$ and $\bar{k}_\nu''$, respectively. Eqns 6 are then easily solved to yield an expression for the flux Φ_A of isotope A as a function of c_A' and c_A''. The tracer permeability coefficient p which is defined by the equation

$$\Phi_A = p(c'_A - c''_A) \tag{33}$$

may then be calculated using the expression for Φ_A. The result reads

$$p = \frac{N_P v}{1+cK} \frac{\bar{k}'_0}{1 + \sum_1^n \bar{S}_n} \tag{34}$$

The tracer permeability coefficient is a function of the total ion concentration c and goes to zero as the pore becomes saturated ($c \gg 1/K$). Comparison with Eqn 30 shows that the tracer permeability coefficient p and the ohmic conductance λ_0 are connected by the relation

$$\frac{\lambda_0}{p} = \frac{z^2 e_0^2}{kT} c \tag{35}$$

Thus, a measurement of p in addition to λ_0 yields no new information about the rate constants; it may be used, however, as a test of the consistency of the model. It should be emphasized that the connection between p and λ_0, as described by Eqn 35, is not a general property of any ion transport system, but depends on the special assumptions underlying the model. For instance, for a carrier model a more complicated relation between p and λ_0 is obtained[36].

Maximum transport rate of a single pore

An absolute upper limit of the transport rate is reached if every ion that strikes the mouth of the pore passes through[1]. The transport is then determined essentially by the diffusion of the ion in the aqueous phase. In many cases, however, the transport rate is limited by the internal properties of the pore. In analogy to the turnover number of a carrier[37], the maximum transport rate of a pore may be defined in the following way. We assume that both aqueous phases are electrically short-circuited ($V=0$) and that the concentration of permeable ions on one side of the membrane is zero. The ion flux Φ is then a function of the concentration c of the permeable ion on the other side. With increasing concentration c, the flux reaches a limiting value Φ_{max}, as the pore becomes saturated. The maximum transport rate ρ, *i.e.* the maximum number of ions per s which may pass through a single pore, is then given by Φ_{max}, divided by the pore number N_{P}. As the pore is asymmetric in general, there exist two different values ρ' and ρ'' of the maximum transport rate, depending whether the permeable ion species is present in the left-hand or on the right-hand aqueous phase:

$$\rho' \equiv \left(\frac{\Phi}{N_{\mathrm{P}}}\right)_{c'\to\infty,\, c''=0} \tag{36}$$

$$\rho'' \equiv -\left(\frac{\Phi}{N_{\mathrm{P}}}\right)_{c'=0,\, c''\to\infty} \tag{37}$$

ρ' and ρ'' are obtained from Eqn 21, if the conditions $V=0$ and $c'\to\infty$ or $c''\to\infty$ are introduced:

$$\frac{1}{\rho'} = \frac{1}{\bar{k}'_0} \sum_{\nu=1}^{n} \left(\bar{S}_\nu \sum_{\mu=1}^{\nu} \bar{R}_\mu\right) \tag{38}$$

$$\frac{1}{\rho''} = \frac{1}{\bar{k}'_0} \sum_{1}^{n} \bar{R}_\nu + \frac{1}{\bar{k}'_0} \sum_{\mu=2}^{n} \left(\bar{R}_\nu \sum_{\mu=1}^{\nu-1} \bar{S}_\nu\right) \qquad (n>2) \tag{39}$$

$$(\rho'')_{n=1} = \bar{k}''_1 \tag{39a}$$

The meaning of ρ' and ρ'' may be illustrated by the following examples:

$n=1$

$$\rho' = \bar{k}'_1$$

$$\rho'' = \bar{k}''_1$$

$n=2$

$$\rho' = \left(\frac{1}{\bar{k}'_1} + \frac{1}{\bar{k}'_2} + \frac{\bar{k}''_2}{\bar{k}'_1 \bar{k}'_2}\right)^{-1}$$

$$\rho'' = \left(\frac{}{\bar{k}''_1} + \frac{1}{\bar{k}''_2} + \frac{\bar{k}'_2}{\bar{k}''_1 \bar{k}''_2}\right)^{-1}$$

The quantities ρ' and ρ'' fulfill the symmetry requirement that the simultaneous transformations ($'\leftrightarrow''$), ($0\leftrightarrow n+1$), ($1\leftrightarrow n$), ($2\leftrightarrow n-1$), ...transform ρ' into ρ'' and ρ'' into ρ'. This is not immediately apparent from Eqns 38 and 39, but may be verified from the above examples.

Biionic potential

If both permeable ion species A and B are present in both aqueous solutions in unequal concentrations, an electrical potential difference is built up across the membrane under zero-current conditions. The magnitude of this so-called biionic potential depends on the ion specificity of the membrane. For a calculation of the biionic potential, the ion fluxes Φ_A and Φ_B have to be evaluated from Eqns 6 for arbitrary concentrations c_A', c_B', c_A'', c_B''. The solution reads (with $\kappa = e^{-zu}$):

$$\Phi_A = N_P k_0^{A'} v_A (1-P) \frac{c'_A - \kappa c''_A}{+\sum_{1}^{n} S_v^A} \tag{40}$$

$$\Phi_B = N_P k_0^{B'} v_B (1-P) \frac{c'_B - \kappa c''_B}{1+\sum_{1}^{n} S_v^B} \tag{41}$$

S_v^A, S_v^B are defined in analogy to Eqns 12 and 22 replacing k_v', k_v'' by $k_v^{A'}$, $k_v^{A''}$ or by $k_v^{B'}$, $k_v^{B''}$.

The probability P is a function of all ion concentrations, but has not to be evaluated explicitly, since the factor $(1-P)$ cancels in the final expression. As the total charge transport vanishes, the relation

$$\Phi_A + \Phi_B = 0 \tag{42}$$

holds. This gives, together with Eqns 40 and 41, the following implicit expression for the biionic potential:

$$k_0^{A'} v_A \frac{c'_A - \kappa c''_A}{1+\sum_{1}^{n} S_v^A} + k_0^{B'} v_B \frac{c'_B - \kappa c''_B}{1+\sum_{1}^{n} S_v^B} = 0 \tag{43}$$

This equation has the important property that it remains unchanged, if all concentrations are multiplied by the same factor. This means that the biionic potential (which is implicitly contained in the rate constants) is not influenced by saturation effects. The application of Eqn 43 is discussed later.

Specialization to a simple pore model

In certain cases the energy profile of the pore may be simplified considerably. If the pore is lined by chemically identical groups in regular sequence, then all internal barriers become equal (if edge effects are neglected). Furthermore, we assume in the following that the barriers at the pore mouths are identical, but, in general, different from the internal barriers. This leads to a symmetrical energy profile which is represented in Fig. 2. The ion transport may then be described by the rate constant k_i for the jump over an internal barrier, and by rate constants k_{ap} and k_{pa} for the jump from the aqueous phase into the pore and from the pore into the aqueous phase (Fig. 2). For regularly spaced barriers and for constant field strength in the pore the contribution of the external voltage V to the energy at the top of the barrier is approximately equal to $ze_0v/2(n+1)$. The rate constants k_v' and k_v'' (Eqns 1 and 2)

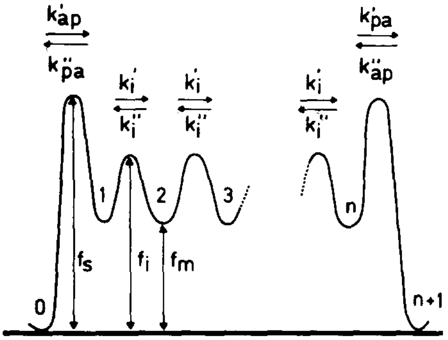

Fig. 2. Symmetrical pore model with identical internal barriers.

may then be replaced by (with $ze_0V/2(n+1)kT \equiv w$):

$$k'_1 = k'_{ap} = k_{ap}e^{w} \tag{44}$$

$$k''_2 = k''_{pa} = k_{pa}e^{-w} \tag{45}$$

$$k'_n = k'_{pa} = k_{pa}e^{w} \tag{46}$$

$$k''_{n+1} = k''_{ap} = k_{ap}e^{-w} \tag{47}$$

$$k'_\nu = k'_i = k_ie^{w} \qquad (\nu = 1,2,\cdots,n-1) \tag{48}$$

$$k''_\nu = k''_i = k_ie^{-w} \qquad (\nu = 2,3,\cdots,n) \tag{49}$$

k_i, k_{pa}, k_{ap} are the rate constants at zero voltage:

$$k_i = \frac{kT}{h}e^{-(f_i - f_m)} \tag{50}$$

$$k_{pa} = \frac{kT}{h}e^{-(f_s - f_m)} \tag{51}$$

$$k_{ap} = \frac{kT}{h}e^{-f_s} \tag{52}$$

(compare Fig. 2). According to Eqn 20, the equilibrium constant for the occupancy of the pore then assumes the form

$$K = vn\frac{k_{ap}}{k_{pa}} = vne^{-f_m} \tag{53}$$

As expected, the equilibrium constant is proportional to the number n of internal minima.

The expressions for the maximal transport rate ρ of the single pore and for the ohmic conductance λ_0 become

$$\rho' = \rho'' \equiv \rho = \frac{2}{n}\cdot\frac{k_ik_{pa}}{2k_i + (n-1)k_{pa}} \tag{54}$$

$$\lambda_0 = N_p \rho \frac{z^2 e_0^{\,2}}{2kT} \cdot \frac{cK}{1+cK} \tag{55}$$

For a discussion of the current–voltage characteristic of the membrane it is convenient to introduce the ratio λ/λ_0, where $\lambda = z e_0 \Phi / V$ is the membrane conductance and λ_0 the ohmic limit of λ. In the following we restrict ourselves to the limiting cases of small and large ion concentrations $c' = c'' \equiv c$. Introducing the abbreviation

$$q \equiv \mathrm{e}^{zu/(n+1)} \tag{56}$$

the following expressions are obtained from Eqn 21:

$$\frac{\lambda}{\lambda_0} = \frac{q^{\frac{1}{2}}(q-1)(q^n - 1/q)\,[2k_{\mathrm{i}} + (n-1)k_{\mathrm{pa}}]/zu}{(q-1)(q^n+1)k_{\mathrm{i}} + (q^n - q)k_{\mathrm{pa}}} \tag{57}$$

$$(c \approx 0)$$

$$\frac{\lambda}{\lambda_0} = \frac{n q^{\frac{1}{2}}(q-1/q)(q^n - 1/q)\,[2k_{\mathrm{i}} + (n-1)k_{\mathrm{pa}}]/zu}{(q+1)(q^n-1)k_{\mathrm{i}} + [(n-1)q(q^n - 1/q) - (q^n - q)]k_{\mathrm{pa}}} \tag{58}$$

$$(c \to \infty)$$

It is easily shown that for $k_{\mathrm{i}} = k_{\mathrm{pa}}$ both expressions reduce to

$$\frac{\lambda}{\lambda_0} = \frac{2(n+1)}{zu} \sinh\left(\frac{zu}{2(n+1)}\right) \tag{59}$$

In the limit of small voltages ($|zu| \ll 1$), Eqn 57 may be expanded to give

$$\frac{\lambda}{\lambda_0} \approx 1 - \frac{1}{6} \cdot \frac{(4n^2 - 4n - 2)k_{\mathrm{i}} - (4u^2 - 3n - 1)k_{\mathrm{pa}}^{\mathrm{p}}}{2k_{\mathrm{i}} + (n-1)k_{\mathrm{pa}}} \left(\frac{zu}{2n+2}\right)^2 \tag{60}$$

$$(zu \approx 0,\ c \approx 0)$$

This means that

$$\lambda \geqq \lambda_0 \text{ for } \frac{k_{\mathrm{i}}}{k_{\mathrm{pa}}} = \frac{\mathrm{e}^{f_{\mathrm{s}}}}{\mathrm{e}^{f_{\mathrm{i}}}} < y$$

$$\lambda \leqq \lambda_0 \text{ for } \frac{k_{\mathrm{i}}}{k_{\mathrm{pa}}} = \frac{\mathrm{e}^{f_{\mathrm{s}}}}{\mathrm{e}^{f_{\mathrm{i}}}} > y$$

$$y \equiv \frac{4n^2 - 3n - 1}{4n^2 - 4n - 2} > 1 \qquad (n \geqslant 2)$$

In other words, for $c \approx 0$ the current increases faster than the voltage if the surface barriers are lower than the internal barriers. On the other hand, if the surface barriers are sufficiently high compared with the internal barriers, a sublinear current–voltage characteristic is observed in the vicinity of $V = 0$. Current–voltage measurements may therefore be used to obtain information on the energy profile of the pore.

In Fig. 3 the ratio λ/λ_0 is plotted as a function of voltage for $n = 9$ and different values of the parameter $k_{\mathrm{i}}/k_{\mathrm{pa}}$. It is seen that an almost ohmic behavior ($\lambda/\lambda_0 \simeq 1$) is observed for saturating ion concentrations ($c \to \infty$), whereas much larger non-

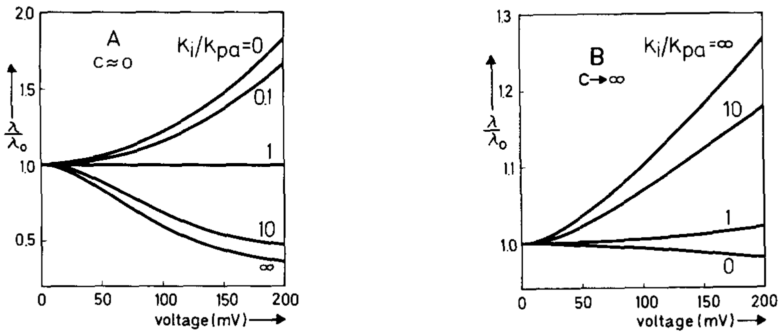

Fig. 3. Ratio of the membrane conductance λ, divided by the ohmic conductance λ_0, as a function of voltage ($n=9$, $z=1$). (A) in the limit of low ion concentration; (B) in the limit of saturating ion concentration. Note the different ordinate scales in A and B.

linearities may occur for $c \to 0$ (note the different ordinate scales in Figs 3A and 3B).

The biionic potential is usually measured under the condition that the left-hand aqueous phase contains a solution of ion species A and the right-hand phase a solution of ion species B of the same concentration ($c_A' = c_B'' = c$, $c_B' = c_A'' = 0$). In this case Eqn 43 (together with Eqn 53) yields the implicit relation

$$q^{n+1} = \frac{K_B}{K_A} \cdot \frac{\dfrac{1}{k_i^A}(q^n - q) + \dfrac{1}{k_{pa}^A}(q-1)(q^n+1)}{\dfrac{1}{k_i^B}(q^n - q) + \dfrac{1}{k_{pa}^B}(q-1)(q^n+1)} \tag{61}$$

For the interpretation of biionic potentials the Goldman–Hodgkin–Katz equation[38,39] is frequently used which under the above conditions assumes the form

$$q^{n+1} = e^{zu} = \frac{p_B^*}{p_A^*} \tag{62}$$

This equation follows from a Nernst–Planck electrodiffusion model under the assumption of constant field strength in the membrane. The "permeabilities" p_A^* and p_B^* are independent of voltage. It is interesting to note that Eqn 61 is not of the form of the Goldman–Hodgkin–Katz equation. Only in certain special cases Eqn 61 reduces to an equation of the Goldman–Hodgkin–Katz type:

a) $k_i^A / k_{pa}^A = k_i^B / k_{pa}^B$:

$$e^{zu} = \frac{K_B}{K_A} \cdot \frac{k_i^B}{k_i^A} = \frac{K_B}{K_A} \cdot \frac{k_{pa}^B}{k_{pa}^A} \tag{63}$$

b) $k_{pa}^A \ll k_i^A$, $\quad k_{pa}^B \ll k_i^B$:

$$e^{zu} \approx \frac{K_B}{K_A} \cdot \frac{k_{pa}^B}{k_{pa}^A} \tag{64}$$

c) $k_{pa}^A \gg k_i^A$, $\quad k_{pa}^B \gg k_i^B$:

$$e^{zu} \approx \frac{K_B}{K_A} \cdot \frac{k_i^B}{k_i^A} \tag{65}$$

In the general case, however, the interpretation of $\exp(zu)$ as a ratio of "ion permeabilities" has to be considered with caution.

Comparison with gramicidin A experiments

Haydon and co-workers[12,13] studied the conductance of the single gramicidin A pore in artificial lipid membranes as a function of ion concentration and voltage. According to Urry[15,18], the gramicidin pore is lined with the oxygen atoms of the amide groups, which form a more or less regular sequence of coordination sites for univalent cations. For an approximate analysis of the experimental data, we may therefore adopt the simplified model of the preceding section. The choice of the number n of barriers depends on the assumptions about the geometry of the coordination site. If a 6-fold coordination of the cation by the carbonyl oxygens is assumed for the $\beta_{3,3}{}^{6}$-helix of gramicidin A[15,18], the number of barriers becomes $n+1=6$. In the following, we shall adopt a value of $n=5$; however, the choice of n is not critical for a raw estimate of the microscopic parameters of the pore.

Hladky and Haydon[12] observed that the single-channel conductance $\Lambda=\lambda_0/N_p$ increases proportional to the ion concentration c at low values of c, but approaches a limiting value Λ_{max} at high ion concentrations. This behaviour has been explained by Hladky and Haydon by the assumption that the pore can contain no more than one cation. According to Eqn 32, we may calculate the equilibrium constant K for the occupancy of the pore from the ion concentration c at which Λ assumes the half-maximal value. The values of K for Na^+ and K^+ which are obtained in this way are given in Table I. Eqn 53 may then be used (with $n=5$) to calculate from K the free energy $F_m=RTf_m$ of the ion in the pore, *i.e.* the change in free energy associated with the transfer of the ion from the aqueous phase into a potential minimum of the pore. The volume v in Eqn 53 may be calculated approximately from Eqn 4, if the cross-section σ of the pore is taken to be 10 $Å^2$ and the jump length l to be 10 Å; this gives $v=100$ $Å^3$/ion, or 60 cm^3/mole. The values of F_m which are calculated in this way are rather small, about -2 kcal/mole[12]; the negative sign of F_m means that the entry of the ion into the pore is energetically favourable. The sign of F_m is consistent with the fact that the solvation enthalpy of alkali ions is more negative in amide solvents as compared with water[40].

The experimental current–voltage characteristic of the single channel is slightly sublinear at low ion concentrations and linear or slightly superlinear at saturating ion concentrations[12]. As Fig. 3 shows this behaviour is also found with the simplified pore model, provided that $k_i/k_{pa}>1$. The experimental value of λ/λ_0 at low ion concentration is about 0.7 for both KCl and NaCl at $V=200$ mV. Using this value, one obtains $k_i/k_{pa}\simeq 3.4$ from Eqn 57. If in turn $k_i/k_{pa}=3.4$ is inserted into Eqn 58, a value of $\lambda/\lambda_0=1.2$ is calculated for $c\to\infty$ and $V=200$ mV which agrees fairly well with the experimental results $\lambda/\lambda_0\approx 1.1$ (K^+) and $\lambda/\lambda_0\approx 1.0$ (Na^+).

If the ratio k_i/k_{pa} is known, the single rate constants may be evaluated from the maximum single-channel conductance which, according to Eqns 54 and 55, is given by

$$\Lambda_{max}=\frac{\lambda_{0,max}}{N_p}=\frac{1}{n}\cdot\frac{z^2 e_0{}^2/kT}{(2/k_{pa})+(n-1)/k_i} \tag{66}$$

The values of k_i and k_{pa} which are obtained in this way (using $k_i/k_{pa}=3.4$) are given in Table I. These values depend on the assumption that the internal barriers of the pore are of identical height; they have therefore to be considered as crude estimates. k_i and k_{pa} are of the order of 10^8–10^9 s^{-1}, corresponding (with $kT/h = 6 \cdot 10^{12}$ s^{-1}) to an activation energy of approx. 6 kcal/mole. For comparison: the jump rate k^* of an ion in free solution is approximately given by the Einstein relation $k^* = 2D/l^2$, where D is the diffusion coefficient of the ion in solution and l the distance between neighbouring equilibrium positions of the ion in the "lattice" of water molecules. With $D \approx 10^{-5}$ cm^2s^{-1} and $l \approx 10$ Å, k^* becomes $2 \cdot 10^9$ s^{-1}, only one order of magnitude larger than the jump rate in the pore.

With the numerical values of K, k_i and k_{pa} which have been determined from conductance measurements it is possible to calculate the biionic potential from Eqn 61. As the ratio k_i/k_{pa} is the same for Na^+ and K^+, we may apply Eqn 61 in the simplified form of Eqn 63. This gives $V = 20$ mV. Myers and Haydon[13] found that the biionic potential of the pair Na^+/K^+ was nearly independent of ion concentration c between $c = 10^{-3}$ M and $c = 10^{-1}$ M; they obtained values of 30–31 mV for glycerol monooleate/*n*-decane membranes and values of 31–34 mV for glyceryl monooleate/*n*-hexadecane membranes. The agreement with the predicted potential of 20 mV is rather poor; besides this, the theory does not account for the experimentally observed increase of the biionic potential at ion concentrations above 10^{-1} M.

The maximal transport rate ρ of the single pore may be directly obtained from Λ_{max} using the relation

$$\rho = \frac{2kT}{z^2 e_0^2} \cdot \Lambda_{max} \tag{67}$$

(compare Eqn 55). This gives values of ρ of the order of 10^7 s^{-1} (see Table I), about three orders of magnitude higher than the maximal transport rates of macrocyclic carriers such as valinomycin[37].

TABLE I

PROPERTIES OF THE GRAMICIDIN A PORE

Λ_{max}, single-channel conductance in the limit of high ion concentrations ($\Lambda_{max} = \lambda_{0,max}/N_P$); K, equilibrium constant for the occupancy of the pore (Eqn 17); F_m, free energy of the ion in the pore (Eqn 53), with respect to the aqueous solution ($F_m = RTf_m$, R = gas constant, T = absolute temperature); k_i, rate constant for the jump over an internal barrier of the pore; k_{pa}, rate constant for the jump from the pore into the aqueous solution; ρ, maximum transport rate of a single pore (Eqn 36).

Ion	Λ_{max} (Ω^{-1})	K (M^{-1})	F_m (*kcal/mole*)	k_i (s^{-1})	k_{pa} (s^{-1})	ρ (s^{-1})
Na^+	$3 \cdot 10^{-11}$	3.3	−1.4	$3 \cdot 10^8$	$0.8 \cdot 10^8$	$1.0 \cdot 10^7$
K^+	$5 \cdot 10^{-11}$	4.3	−1.6	$4 \cdot 10^8$	$1.3 \cdot 10^8$	$1.6 \cdot 10^7$

Specificity

The specificity of the pore may be expressed by the ratio λ_0^A/λ_0^B of the ohmic conductivities which are separately measured with two ion species A and B under otherwise identical conditions ($c_A = c_B = c$). This ratio is given by (compare Eqns 54 and 55):

$$\frac{\lambda_0^A}{\lambda_0^B} = \frac{K_A/(1+cK_A)}{K_B/(1+cK_B)} \cdot \frac{k_i^A k_{pa}^A/[2k_i^A+(n-1)k_{pa}^A]}{k_i^B k_{pa}^B/[2k_i^B+(n-1)k_{pa}^B]} \tag{68}$$

Thus, the conductance ratio depends in general on ion concentration; this has indeed been observed with gramicidin A[12].

According to Eqn 35, the conductance ratio is equal to the ratio of tracer permeabilities:

$$\frac{\lambda_0^A}{\lambda_0^B} = \frac{p_A}{p_B} \tag{69}$$

It has been shown previously that under certain conditions the biionic potential may be interpreted as the logarithm of the ratio p_A^*/p_B^* of generalized permeabilities (Eqns 62–65). By comparison with Eqns 68 and 69 it is seen that in the cases in which Eqns 63–65 become valid, the following relation holds

$$\frac{p_A^*}{p_B^*} = \frac{1+cK_A}{1+cK_B} \cdot \frac{p_A}{p_B} \tag{70}$$

This means that even in the special situation where the biionic potential is described by an equation of the Goldman–Hodgkin–Katz type, the ratio of the generalized permeabilities is not identical with the ratio of the tracer permeabilities.

As shown by Eqn 68, the ion specificity of the pore may be represented as the product of a "thermodynamic" term containing only the equilibrium constants K_A and K_B and a "kinetic" term which depends on the single rate constants k_i^A, k_i^B, k^A_{pa}, k^B_{pa}. A similar statement applies to the specificity of a carrier, but in carrier-mediated ion transport there exists a wide range of conditions under which the specificity is determined by equilibrium parameters alone[36,41]. The specificity of a pore, however, is always influenced by kinetic constants, as Eqn 68 shows. At saturating ion concentrations ($cK_A \gg 1$, $cK_B \gg 1$), the equilibrium term in Eqn 68 becomes unity, so that in this limit the conductance ratio is solely determined by transport rates.

As a simple example, we may consider a pore in which all barriers are of equal height ($k_i^A = k^A_{pa}$, $k_i^B = k^B_{pa}$). Then, in the limit $c \approx 0$, Eqns 68 and 70 simplify to

$$\frac{\lambda_0^A}{\lambda_0^B} = \frac{p_A}{p_B} = \frac{p_A^*}{p_B^*} = \frac{K_A}{K_B} \cdot \frac{k_i^A}{k_i^B} \tag{71}$$

Introducing the reduced free energies f_m^A and f_m^B of ions A and B in the potential minima with respect to water, as well as the reduced activation energies $\delta_A \equiv f_i^A - f_m^A$ and $\delta_B \equiv f_i^B - f_m^B$ (Fig. 2 and Eqn 50), Eqn 71 may be written as

$$\frac{\lambda_0^A}{\lambda_0^B} = e^{-(f_m^A - f_m^B)} \cdot e^{-(\delta_A - \delta_B)} \tag{72}$$

Again, the first term contains only equilibrium parameters, whereas the second term describes the kinetic properties of the pore.

For a numerical evaluation of Eqn 72, the pore may be considered as a sequence of coordination sites. The magnitude of the equilibrium term may then be estimated from the difference of the calculated or measured free energies of hydration and solvation in a suitable model solvent, *e.g.* a carbonyl compound[40,42]. Likewise, the kinetic term may be calculated if the energy profile of the pore is known. To illustrate this, we assume that the pore consists in an infinite array of regularly spaced dipoles of charge $\pm\theta e_0$ with which the ion interacts electrostatically (Fig. 4). If $w(x)$ is the electrostatic energy (in units of kT) of the ion at point x, the activation energy δ is then equal to $[w(\text{o}) - w(a/2)]$. We further assume that the conformation of the pore can accommodate to the size of the ion[15], so that the distances b_+ and b_- (Fig. 4) vary linearly with the ion radius. Straightforward calculation then leads to the result

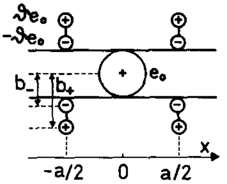

Fig. 4. Univalent cation interacting with an infinite array of dipoles of charge $\pm\theta e_0$, which are located at points $x = \pm a/2$, $\pm 3a/2$, $\pm 5a/2$, – – –

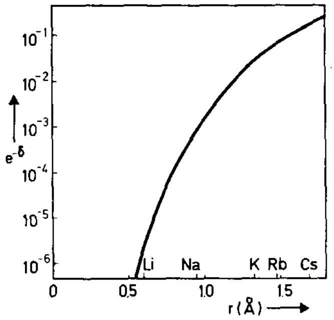

Fig. 5. Numerical evaluation of the model shown in Fig. 4, according to Eqn 73, with $\theta = 0.44$, $\varepsilon = 4$, $n_c = 4$, $a = 4$ Å, $b_+ = r + 2.7$ Å, $b_- = r + 1.4$ Å. δ is the activation energy (in units of kT) for the jump of the ion over the electrostatic barrier, and r is the ion radius.

$$\delta = \frac{\theta n_c a_0}{\varepsilon a}\left[\frac{a}{2b_-} - \frac{a}{2b_+} + 2\sum_{\nu=1}^{\infty}(-1)^{\nu} C_{\nu}\right] \tag{73}$$

$$C_{\nu} = \frac{1}{\sqrt{(\nu^2 + 4b_-^2/a^2)}} - \frac{1}{\sqrt{(\nu^2 + 4b_+^2/a^2)}}$$

$$a_0 \equiv \frac{2e_0^{\,2}}{kT} = 1121\,\text{Å} \qquad (25\,^{\circ}\text{C})$$

n_c is the number of dipoles per coordination site and ε the effective dielectric constant which accounts for the mean polarizability of the pore; for the meaning of a, b_+, b_-, see Fig. 4. A numerical example is represented in Fig. 5. The calculation is based on the molecular parameters of the carbonyl group as given by Krasne and Eisenman[40]: $\theta = 0.44$, $b_+ = r + 2.7$ Å, $b_- = r + 1.4$ Å; furthermore, the spacing of the coordination sites along the pore axis is taken to be $a = 4$ Å. It is seen from Fig. 5 that the jump rates of the different ions, which are proportional to $\exp(-\delta)$, strongly depend on ion radius r. This means that the permeability sequence of ions may be completely different from the sequence of the corresponding equilibrium constants (compare Eqns 71 and 72).

For the calculation of the activation energy in Fig. 5 it has been assumed that the diameter of the pore accommodates to the size of the ion so that there is always an optimal electrostatic interaction between ion and coordinating sites. If, on the other hand, the pore structure is rigid, the ion is asymmetrically surrounded by coordination sites, and the dependence of $\exp(-\delta)$ on r (Fig. 5) becomes less steep. A third possibility would be that the movement of the ion is more or less restricted to the central axis of a rigid pore. This may occur if the ion enters the pore together with part of its hydration shell. In this case the activation energy arising from the electrostatic interaction of the ion with the dipoles of the ligand groups becomes independent of ion radius. Possibly this is an explanation for the low selectivity of the gramicidin A pore[12,13].

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