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MICROSCOPIC CALCULATION OF ION-TRANSPORT RATES IN MEMBRANE CHANNELS

P. LÄUGER

Department of Biology, University of Konstanz, D-7750 Konstanz, F.R.G.

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A method, based on rate theory, is described by which transport rates in ion channels can be calculated using only microscopic parameters, such as atomic coordinates, force constants and intermolecular energy parameters. The channel is treated as a system of elastically bound ligands interacting with the ion by coulombic and Lennard-Jones forces. Jump frequencies of the ion are obtained from the potential of mean force which represents a thermal average over the different configurations of the ligand system. The method is illustrated by application to a special channel model, a helical arrangement of dipolar ligands, which can be tilted toward the channel axis against a harmonic restoring force. The jump frequency is found to be a non-monotonous function of ion radius. Furthermore, the ion specificity of the channel strongly depends on whether the ligand system is 'hard' or 'soft', i.e., on the extent to which the interaction with the ion can lead to a reorientation of the ligand groups.

1. Introduction

There is now strong evidence that ion permeation through biological membranes depends on the presence of built-in proteins which provide for energetically favourable pathways through the apolar interior of the membrane. An ion moving through such a protein channel is thought to interact with polar ligands of the protein which (partly) replace the primary hydration shell of the solvated ion [1]. A well studied example is the cation-permeable channel formed by the peptide gramicidin A, which, according to the model of Urry [2], consists of a helix with 6.3 amino acids per turn. The central hole along the axis of the helix has a diameter of about 0.4 nm and is lined with the peptide carbonyl groups. Conformational energy calculations suggest that the structure lies in a broad energy minimum. Accordingly, passage of the ion through the channel probably involves a rocking motion of the carbonyl group, such that the oxygen atom moves closer to the helix axis [3]. This flexibility of the ligand system is likely to have an important influence on the ion specificity of the channel.

Detailed structural information on physiological ion channels is not available so far, but it may be anticipated that such information will be obtained in the future, for instance, from X-ray data. This study is concerned with the problem of how ion-transport rates for a given channel can be calculated, if the structure of the channel is known. A truly microsopic theory of transport rates should contain only 'microscopic' parameters of the system, i.e., atomic coordinates, particle masses, force constants and intermolecular interaction parameters, whereas 'macroscopic' parameters such as viscosity or mobility should not appear in the equations of the theory. Except for transport processes at low temperature and with the possible exception of certain H^+-transport phenomena, such a microscopic theory may be based, for all practical purposes, on the laws of classical mechanics.

A straightforward approach to the calculation of ion-transport rates in channels is given by the method of molecular dynamics in which the Newtonian equations of motion are integrated using a digital computer. This technique has been applied recently to the study of ion transport in a model

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channel [4]. While molecular dynamics simulations yield rather detailed information on the motion of ions in the channel, the expenditure of computing time for the simulation of a large ion-ligand system (such as a protein channel) is considerable [5].

An entirely different method for the treatment of transport kinetics is provided by rate theory [6]. In the rate-theoretical approach, the pathway of an ion through the channel is considered as a series of 'binding sites' separated by energy barriers, the binding sites being positions where the ion interacts in an energetically favourable way with the ligand system. Transport of the ion through the channel may then be treated as a sequence of thermally activated jumps over the barriers. In the theoretical description of ion channels, rate theory has been used so far in a rather formal way, i.e., the frequency k of transition over a given barrier has been introduced as a purely phenomenological quantity without specifying k in terms of the microscopic parameters of the channel [6–11]. (A more explicit use of rate theory has been made only in the treatment of the voltage dependence of rate constants.) In the following, a method is described by which actual values of transition frequencies can be calculated from structural data and intermolecular energy parameters of the ion-ligand system.

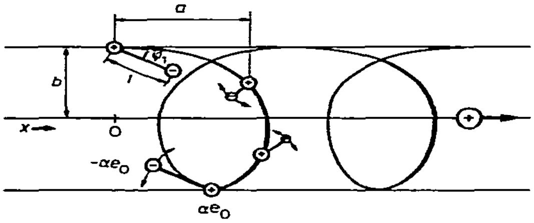

Fig. 1. Helical arrangement of dipolar ligands. Each dipole is fixed with the positive end of the helix but can be tilted with the negative end towards and away from the channel axis against a harmonic restoring force in such a way that the negative end moves in the plane through the channel axis and the positive end. The motion of the ion is restricted to the channel axis.

2. Calculation of transition frequencies from the potential $V(x)$ of mean force

The formalism we shall be using can be introduced in a rather general way, but it seems preferable to exemplify the method by referring to a specific class of ion-ligand systems. For this purpose we consider a helical arrangement of dipolar ligand groups (fig. 1). The positive end of the dipole is fixed, while the negative end can move toward and away from the channel axis against a harmonic restoring force. The cation passing through the channel is assumed to move along the channel axis. In general, the interaction energies between the ion and ligand i and between ligand i and ligand j will contain coulombic terms as well as terms arising from repulsion and van der Waal's attraction. If the channel contains n dipolar groups, the potential energy of the ion-ligand system is a function of the position x of the ion on the axis and of n tilt angles ϕ_i (Fig. 1).

In the usual version of rate theory, the jump frequency k' over a barrier from left to right is often approximated by the relationship

$$k' = \nu \exp(-E/kT) \qquad (1)$$

where ν is the oscillation frequency of the ion in the energy well to the left of the barrier, E the energy difference between the top of the barrier and the well, k Boltzmann's constant and T the absolute temperature. When the ligand system is assumed to be rigid, i.e., when all ϕ_i values in fig. 1 are fixed, the potential energy U of the ion is a time-independent function of position x. The barrier height E of eq. 1 is then simply obtained from the values of $U(x)$ taken at the positions of the barrier and the well. In reality, however, the ligands of a channel in thermal contact with its surroundings can assume many different orientations, and the potential energy fluctuates with time [12]. For each position x of the ion, the state of the channel is distributed over a spectrum of orientational substates. Thus, a meaningful potential function should represent an average over the different ligand configurations.

For the definition of a generalized potential function taking into account the existence of configurational substates, we consider the potential energy $U(x, \phi_1, \ldots, \phi_n)$ of the channel with an ion located at position x and with the n dipoles having tilt angles $\phi_1, \ldots, \phi_n$. Accordingly, $U(\infty, \phi_1, \ldots, \phi_n)$ is the potential energy of the empty channel which serves as a reference state. Under equilibrium conditions, the probability of occurrence of a given ligand configuration is proportional to the configuration integral $Q(x)$:

$$Q(x) = \int \ldots \int \exp[-U(x, \phi_1, \ldots, \phi_n)/kT] \, \mathrm{d}\phi_1 \ldots \mathrm{d}\phi_n \qquad (2)$$

We now define a generalized potential $V(x)$ by the relationship

$$V(x) = -kT \ln\left[\frac{Q(x)}{Q(\infty)}\right] \qquad (3)$$

In this definition the empty channel ($x = \infty$) is used as a reference state. As discussed in appendix A, $V(x)$ is identical with the so-called potential of mean force acting on the ion along the x-axis (the force averaged over all ligand configurations at a given temperature T). Furthermore, $V(x)$ is related to the free energy $F(x)$ of a channel having an ion in position x (see appendix A):

$$V(x) = F(x) - F(\infty) \qquad (4)$$

According to the definition of free energy, $V(x)$ contains contributions from attractive and repulsive interactions in the ion-ligand system and, at the same time, accounts for the thermal motions of the ligand groups.

Since the potential function $V(x)$ is determined entirely by equilibrium properties of the channel, it is obvious that the use of $V(x)$ in the description of non-equilibrium transport phenomena requires additional assumptions. In the following we assume that the ligand system remains always in an equilibrium state, even if ions are driven through the channel by an external force, such as a gradient of concentration or electrical potential. When an ion jumps into a binding site, the neighbouring ligands tend to reorient under the influence of coulombic (and other) interactions. The assumption introduced above requires that the relaxation time of the ligands is much shorter than the average dwell time of the ion in the binding site [12].

We now consider the case that $V(x)$ has a minimum at position x_m in the channel and that the adjacent energy barriers are situated at x' and x'' (fig. 2). The probability that an ion located near x_m will jump over the barrier to the right of x_m during the time interval $\mathrm{d}t$ is then equal to $k' \mathrm{d}t$, where k' is the transition frequency. As shown in appendix A, under the condition of fast ligand equilibration, the transition frequency is related in a simple way to $V(x)$:

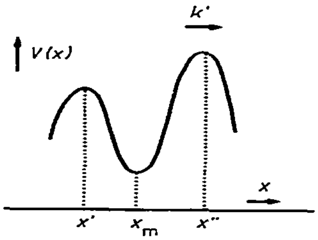

Fig. 2. Positions of potential maxima and minima along the channel axis.

$$k' = \left(\frac{kT}{2\pi m}\right)^{1/2} \frac{\exp[-V(x'')/kT]}{\int_{x'}^{x''} \exp[-V(x)/kT] \, \mathrm{d}x} \qquad (5)$$

where m is the mass of the ion. It should be noted that the right-hand side of eq. 5 contains only microscopic parameters, i.e., the ionic mass m and the intermolecular interaction parameters entering into $V(x)$ via the potential $U(x, \phi_1, \ldots, \phi_n)$. Eq. 5 therefore represents a strictly microscopic description of transport rates in a channel.

It is easy to show that eq. 5 reduces to eq. 1 in certain limiting cases. For instance, if a harmonic force acts on the ion in the vicinity of x_m, then

$$V(x) \approx V(x_m) + \frac{\theta}{2}(x - x_m)^2, \quad (x \approx x_m) \qquad (6)$$

where θ is the force constant. If we further assume that the barriers on both sides of x_m are high, only the immediate vicinity of x_m contributes to the integral in eq. 5:

$$\begin{aligned} &\int_{x'}^{x''} \exp[-V(x)/kT] \, \mathrm{d}x \\ &\quad \approx \exp[-V(x_m)/kT] \int_{-\infty}^{\infty} \exp[-\theta(x - x_m)^2/2kT] \, \mathrm{d}x \\ &\quad \approx (2\pi kT/\theta)^{1/2} \exp[-V(x_m)/kT] \end{aligned} \qquad (7)$$

Introduction of the barrier height $E = V(x'') - V(x_m)$ and of the oscillation frequency $\nu = (1/2\pi)(\theta/m)^{1/2}$ of the ion in the energy well then yields eq. 1.

3. Numerical results for a helical channel

In the following we apply the rate-theoretical method described above to the model of a helical channel shown in fig. 1. We assume that a univalent cation (charge e_0) moves along the channel axis and thereby interacts with the dipolar ligands. The total ion-ligand interaction is the sum of the coulombic interaction and additional attractive and repulsive interactions which may be described by a Lennard-Jones [6,12] potential. Thus, in the model chosen here, the ion is represented by a Lennard-Jones sphere with embedded point charge [14]. Likewise, the dipolar ligand is described by two identical Lennard-Jones spheres held at a fixed distance with embedded point charges of magnitude $\pm \alpha e_0$. In addition to coulombic and Lennard-Jones interactions, the potential energy U of the ion-ligand system contains energy terms from harmonic restoring forces which depend on the tilt angles ϕ_i of the dipoles (fig. 1). If γ is the force constant, this energy is given by $(\gamma/2)(\phi_i - \phi_i^0)^2$. (It is obvious that in the equilibrium state at $T=0$, the tilt angles are, in general, different from ϕ_i^0, since the equilibrium configuration is determined not only by the force constant γ but also by coulombic and Lennard-Jones interactions.) We denote the Lennard-Jones parameters for the ion-ligand interaction by ϵ and σ and those for the ligand-ligand interaction by ϵ_1 and σ_1 (for simplicity, the positive and negative end of the dipole are considered as identical Lennard-Jones centres). The potential energy U is then obtained as:

$$
\begin{aligned}
U = {} & \frac{\alpha e_0^2}{4\pi\epsilon_0} \sum_i \left[\frac{1}{r_i^+} - \frac{1}{r_i^-} \right] + 4\epsilon \sum_i \left[\left(\frac{\sigma}{r_i^+} \right)^{12} - \left(\frac{\sigma}{r_i^+} \right)^6 \right. \\
& \left. + \left(\frac{\sigma}{r_i^-} \right)^{12} - \left(\frac{\sigma}{r_i^-} \right)^6 \right] \\
& + \frac{\alpha^2 e_0^2}{4\pi\epsilon_0} \sum_{i<j}\sum \left[\frac{1}{r_{ij}^+} + \frac{1}{r_{ij}^-} - \frac{1}{r_{ij}} - \frac{1}{r_{ji}} \right] \\
& + 4\epsilon_1 \sum_{i<j}\sum \left[\left(\frac{\sigma_1}{r_{ij}^+} \right)^{12} - \left(\frac{\sigma_1}{r_{ij}^+} \right)^6 + \left(\frac{\sigma_1}{r_{ij}^-} \right)^{12} - \left(\frac{\sigma_1}{r_{ij}^-} \right)^6 \right. \\
& \left. + \left(\frac{\sigma_1}{r_{ij}} \right)^{12} - \left(\frac{\sigma_1}{r_{ij}} \right)^6 + \left(\frac{\sigma_1}{r_{ji}} \right)^{12} - \left(\frac{\sigma_1}{r_{ji}} \right)^6 \right] \\
& + \frac{\gamma}{2} \sum_i (\phi_i - \phi_i^0)^2 \\
& (i,j = 1,2,\ldots,n) \qquad (8)
\end{aligned}
$$

where ϵ_0 is the permittivity of free space. The first and second sum in eq. 8 describe the coulombic and Lennard-Jones interactions between ion and ligands, while the double sums account for ligand-ligand interactions. Besides, the following notation has been used where, for instance, $r(i_-, j_+) \equiv r_{ij}$ signifies the distance between the negative end of the i-th dipole and the positive end of the j-th dipole:

$r_{ij} \equiv r(i_-, j_+)$; $r_{ij}^+ \equiv r(i_+, j_+)$; $r_{ij}^- \equiv r(i_-, j_-)$

$r_i^+ \equiv r(\text{ion}, i_+)$; $r_i^- \equiv r(\text{ion}, i_-)$

Expressions for the distances r_i^+, r_{ij}, etc., as functions of the variables x and ϕ_i are given in appendix B.

According to eqs. 2 and 3, the calculation of the potential $V(x)$ which enters into the expression for the transition frequency k' requires the numerical evaluation of multiple integrals in the tilt angles ϕ_i. The computation of $V(x)$ was performed by approximating the integral in eq. 2 by a Gauss-Hermite product formula, as described in appendix C. A small ligand system was chosen consisting of six dipoles arranged regularly on a single helical turn (the angles ϕ_i of eqs. B5–B7 are therefore multiples of $2\pi/6$). For the length l and charge αe_0 of the dipole, the values $l = 0.124$ nm and $\alpha = 0.4$ of the peptide carbonyl group were used [17]. The orientation of the dipoles was assumed to be antiparallel with $\phi_1^0 = \phi_3^0 = \phi_5^0 = 20°$ and $\phi_2^0 = \phi_4^0 = \phi_6^0 = 160°$ (fig. 1) and the axial distance between neighbouring dipoles was chosen to be $a = 2l = 0.248$ nm. For the Lennard-Jones parameter σ of ion-ligand interaction, average crystallographic cation-oxygen distances were used, as given by Hille [1]: Li^+, 0.193 nm; Na^+, 0.23 nm; K^+, 0.272 nm; Rb^+, 0.286 nm; Cs^+, 0.303 nm. The ligand-ligand interaction parameter σ_1 was taken to be 0.285 nm which is equal to the mean of the oxygen-oxygen and carbon-carbon contact distances [17]. For simplicity, the same energy parameter $\epsilon = \epsilon_1$ for ion-ligand and ligand-ligand interactions was chosen, corresponding to a reduced temperature $\theta = \epsilon/k$ of 75 K (for comparison, the θ values for neon and argon are about 30 and 120 K, respectively [18]).

An important parameter in the potential energy U is the force constant γ, since ion channels probably differ in their flexibility, i.e., in their ability to adjust to the size of the permeating ion. Calculations were therefore carried out for different values of γ. For convenience, γ may be expressed by the mean thermal amplitude $\Delta\phi$ of the tilt angle of an isolated dipole:

$$\Delta\phi \equiv \left(\overline{[\phi_i - \phi_i^0]^2}\right)^{1/2} = (kT/\gamma)^{1/2} \tag{9}$$

For instance, a force constant of about 1×10^{-18} J has been estimated for the skeletal deformation vibrations in peptides [4], corresponding (at $T =$ 300 K) to $\Delta\phi \approx 0.064 \triangleq 3.7°$.

Results of numerical calculations of $V(x)$ (carried out on a Telefunken TR 440 computer) are shown in figs. 3–5. The channel radius b has been chosen such that the larger ions (Rb^+, Cs^+) come into van der Waals' contact with the negative pole of the ligands for $\phi_i \approx \phi_i^0$. In fig. 3, ligands and ion have been drawn to scale using the Lennard-Jones diameters and geometrical channel parameters given above (for convenience, the helical arrangement of the ligands has been projected onto two rows lying in the plane of the drawing). The potential $V(x)$ shown in fig. 3 corresponds to the limiting case of a completely rigid ligand system ($\phi_i \equiv \phi_i^0, \Delta\phi = 0$). $V(x)$ has three minima which are located at those positions where the ion is closest to the negative charge of the ligands. (For the geometry chosen here, coulombic attraction between ion and oxygen atom is always much larger than Lennard-Jones repulsion even at the distance of closest approach.) As expected, the potential is negative inside the channel and approaches zero at large values of $|x|$.

The effect of flexibility of the ligand system is shown in fig. 4 where $V(x)$ is represented for different values of the mean thermal amplitude of the tilt angle $\Delta\phi$. In a non-rigid ligand system ($\Delta\phi > 0$), the orientation of the dipoles depends on the position of the ion; furthermore, the dipoles

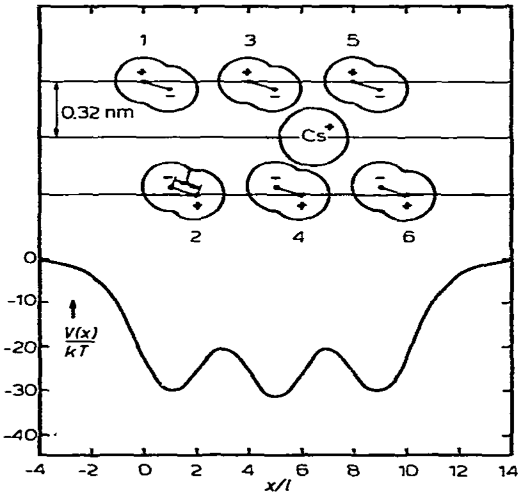

Fig. 3. Potential function $V(x)$ (in units of kT) for a completely rigid ligand system ($\phi_i \equiv \phi_i^0$, $\Delta\phi = 0$). The ligand dipoles are arranged antiparallel ($\phi_1^0 = \phi_3^0 = \phi_5^0 = 20°$, $\phi_2^0 = \phi_4^0 = \phi_6^0 =$ 160°) on a helix of radius $b = 0.32$ nm. Ligands and ion (Cs^+) are drawn to scale using the Lennard-Jones diameters and geometrical channel parameters given in the text (for convenience, the ligands are projected onto two rows lying in the plane of the drawing). $l = 0.124$ nm, $a = 2l$, $\alpha = 0.4$, $\epsilon/k = \epsilon_1 = 75$ K, $\sigma = 0.303$ nm, $\sigma_1 = 0.285$ nm, $T = 300$ K, $H = 5$.

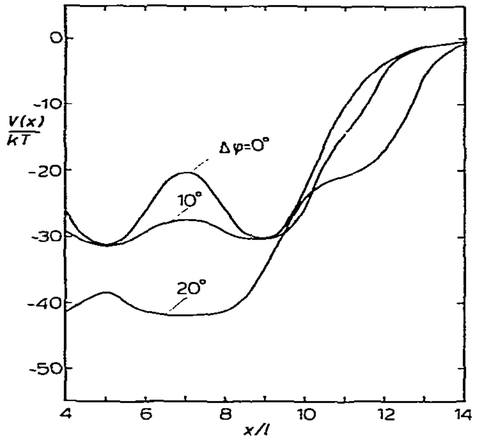

Fig. 4. Potential function $V(x)$ for Cs^- for the same parameter values as in fig. 3, but for a flexible ligand system. $\Delta\phi$ is the mean thermal amplitude of the tilt angle (eq. 9). $V(x)$ is symmetric with respect to $x/l = 5$. The computations were carried out on a Telefunken TR 440 computer with the following values of H (appendix C): $\Delta\phi = 0$, $H = 5$; $\Delta\phi = 10°$, $H = 8$; $\Delta\phi = 20°$, $H = 10$. It was checked that the use of larger values of H did not change $V(x)$ significantly. For the configuration integral $Q(\infty)$ of the empty channel (eq. 3), the following values were obtained: 0($\Delta\phi = 0$), 0.155 ($\Delta\phi = 10°$) and 43.8 ($\Delta\phi = 20°$). $Q(\infty)$ was calculated by putting the ion-ligand interaction energies equal to zero. $b = 0.32$ nm, $T = 300$ K.

carry out thermal vibrations. It is seen from fig. 4 that the shape of the potential drastically changes with increasing values of $\Delta\phi$. For small $\Delta\phi$, potential minima occur at $x/l = 5$ and $x/l = 7$ where the cation is close to the negative ends of two neighbouring dipoles. At $\Delta\phi = 10°$, however, the potential wells become less pronounced and at $\Delta\phi = 20°$ a potential maximum appears at $x/l = 5$. This interesting phenomenon is connected with the fact that $V(x)$ is the potential of mean force which is closely related to the free energy of the ion-ligand system (eq. 4) and therefore accounts also for entropy effects. If a bulky ion (such as Cs^+) is located at a position in the channel where the amplitude of thermal movement of the dipoles is large ($x/l = 5$), the vibrations of the ligand system become restricted and this results in an unfavourable contribution to the free energy. Consistent with this interpretation is the finding that for smaller ions the shape of $V(x)$ is much less sensitive to a variation of $\Delta\phi$.

The importance of ligand flexibility is further illustrated in fig. 5 where the potential functions of a small (Li^+) and a large ion (Cs^+) are compared. The energy at the top of the barrier where the ion is close to the fixed positive of ends of dipoles 3 and 4 (fig. 3) is found to be nearly the same for Li^+ and Cs^+. On the other hand, the energy wells adjacent to the barrier are much deeper for Li^+ than for Cs^+. This lowering of the energy well results from ligand reorientation which brings the negative ends of dipoles 3 and 4 close to the small Li^+.

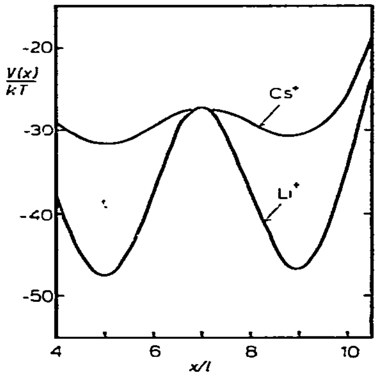

Fig. 5. Potential function $V(x)$ for Li^+ and Cs^+ at 300 K and a mean thermal vibration amplitude of $\Delta\phi = 10°$. The computations were carried out with $H=6$ (Li^+) and $H=8$ (Cs^+). Other parameters were the same as in fig. 3. $b=0.32$ nm.

For the calculation of transition rates, a somewhat larger helix diameter ($b = 0.3453$ nm) was chosen and the thermal vibration amplitude was restricted to $\Delta\phi \leq 10°$ (at 300 K). Under these conditions, the general form of the potential $V(x)$ is as shown in fig. 3 with a minimum at $x_m/l = 5$ and maxima at $x'/l = 3$ and $x''/l = 7$ (for all alkali metal ions and for temperatures between 250 and 350 K). The transition frequency k' over the barrier at x'' from left to right (fig. 2) was calculated by numerical evaluation of eq. 5, as described in appendix C. Values of k' for the alkali metal ions and for different thermal vibration amplitudes $\Delta\phi$ are given in fig. 6. It is seen that even for a rigid ligand system ($\Delta\phi = 0$) where the coulombic interaction is the same for all ions, the transition frequency is a non-monotonous function of ion radius r_+. This non-monotonous specificity sequence results from the combined effects of coulombic and Lennard-Jones interactions and the $1/m^{1/2}$ dependence of transition frequency (m,

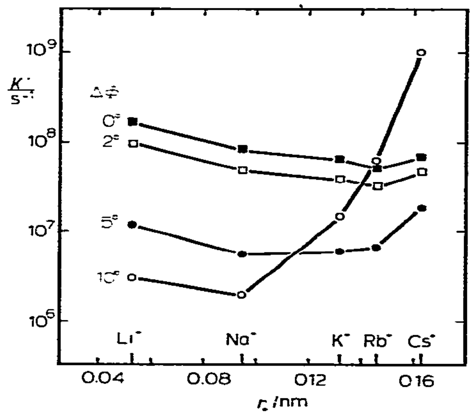

Fig. 6. Transition frequency k' over the barrier at $x''/l = 7$ from left to right (fig. 3) as a function of ion radius r_+ for different thermal vibration amplitudes $\Delta\phi$. The helix radius was $b = 0.3453$ nm; all other channel parameters were the same as in fig. 3. The calculation of k' was performed by numerical evaluation of eq. 5 with $G=8$, $H=5$ ($\Delta\phi = 0°$); $G=10$, $H=6$ ($\Delta\phi = 2°$); and $G=10$, $H=8$ ($\Delta\phi = 5°, 10°$). $T=300$ K.

mass of the ion). With increasing flexibility of the ligand system ($\Delta\phi > 0$) the jump frequency over the barrier tends to decrease and, at the same time, the dependence of k' on r_+ becomes more pronounced. The decrease in k' is connected with the fact that a smaller force constant allows a more favourable orientation of the dipoles around the ion, which lowers the energy level of the potential well and thereby increases the barrier height. At high vibration amplitudes ($\Delta\phi = 10°$), the jump frequency of Cs^+ is strongly increased, reaching a value above the level of the rigid ligand system. This behaviour presumably is a consequence of the restriction of thermal vibration by the bulky ion and of the concomitant upward shift of the energy level of the potential well.

It should be noted that the overall permeability of a channel not only depends on the transition frequencies over potential barriers but also on the probability that the channel is occupied by an ion. This probability is largely determined by the energy level of the potential wells with respect to the hydrated state of the ion in water [22]. The transition frequencies given in fig. 6 follow a sequence which shows a minimum at an intermediate value of ion radius r_+. Such 'inverted' specificity sequences have been discussed by Eisenman [23]. In any case, the calculations of k' suggest that the ion specificity of a channel depends on whether the ligand system is hard or soft, i.e., on the extent to which interaction with the ion can lead to a reorientation of the ligand groups.

It is interesting to see whether the simple expression eq. 1 can be used to calculate approximate values of the transition rate constant k', given the shape of the potential $V(x)$ of mean force. For this purpose, the oscillation frequency $\nu = (1/2\pi)(\theta/m)^{1/2}$ of the ion in the potential well at $x = x_m$ was estimated from the parabolic approximation of $V(x)$ in the vicinity of the well (eq. 6). Furthermore, the barrier height $E = V(x'') - V(x_m)$ was evaluated from the computed $V(x)$. In table 1, values of k' as obtained from ν and E using eq. 1 are compared with the results of the numerical evaluation of eq. 5. It is seen that the k' values determined by the two procedures agree closely, the maximum deviation being about 20%. This means that for an estimate of k' eq. 1 can be used once the potential $V(x)$ of mean force has been calculated. Furthermore, table 1 shows that the oscillation frequency ν is nearly the same at all temperatures (250–350 K).

The temperature dependence of transition frequency k' is represented in fig. 7 for Cs^- and

Table 1

Comparison of the transition rate constants k' obtained from the approximate relationship $k' = \nu \exp(-E/kT)$ (eq. 1) and the results of the numerical evaluation of eq. 5

The oscillation frequency $\nu = (1/2\pi)(\theta/m)^{1/2}$ of the ion in the potential well at $x = x_m$ has been determined from the computed potential $V(x)$ of mean force, using a parabolic approximation for $V(x)$ in the vicinity of the well (eq. 6). The barrier height E was obtained as $E = V(x'') - V(x_m)$. The helix diameter was $b = 0.3452$ nm, all other parameters were as indicated in the legend to fig. 3. The numerical integrations were carried out with $G = 8$, $H = 5$ (for $\Delta\phi = 2°$) and $G = 10$, $H = 8$ (for $\Delta\phi = 10°$).

Ion	$\Delta\phi$ (°)	T (K)	ν (s^{-1}) ($\times 10^{12}$)	E (kJ/mol)	k' (from ν and E) (s^{-1})	k' (from eq. 5) (s^{-1})
Li^-	2	300	2.77	25.7	9.32×10^7	1.08×10^8
	10	300	2.47	33.9	3.09×10^6	3.02×10^6
Cs^-	2	250	0.574	23.6	6.76×10^6	7.86×10^6
		300	0.573	23.6	4.51×10^7	4.58×10^7
		350	0.574	23.6	1.75×10^8	1.82×10^8
	10	250	0.499	16.2	2.10×10^8	2.08×10^8
		300	0.499	15.4	1.04×10^9	1.01×10^9
		350	0.500	14.9	2.98×10^9	2.84×10^9

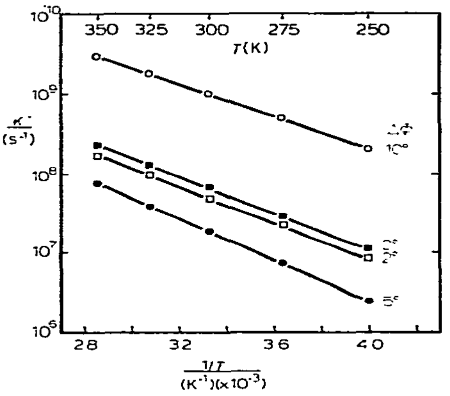

Fig. 7. Temperature dependence of transition frequency k' for Cs^+ at different thermal vibration amplitudes $\Delta\phi$. The helix radius was $b = 0.3453$ nm, the other parameters were the same as in fig. 3. The computation was carried out with $G = 8$, $H = 5$ ($\Delta\phi = 0°$, $2°$); $G = 10$, $H = 6$ ($\Delta\phi = 5°$); and $G = 10$, $H = 8$ ($\Delta\phi = 10°$). The following values of the activation energy E_a were obtained using the relationship $E_a/R = -\mathrm{d}\ln k'/\mathrm{d}(1/T)$: 19.9 kJ/mol ($\Delta\phi = 0°$), 20.6 kJ/mol ($\Delta\phi = 2°$), 23.7 kJ/mol ($\Delta\phi = 5°$), 17.0 kJ/mol ($\Delta\phi = 10°$).

different thermal vibration amplitudes $\Delta\phi$. For $\Delta\phi \leq 10°$ the values of k' fall approximately on a straight line in the Arrhenius plot of fig. 7. This means that the activation energy $E_A = -R\,\mathrm{d}\ln k'/\mathrm{d}(1/T)$ is almost temperature independent. Indeed, the barrier height E, as obtained from $V(x)$, remains constant in the temperature range 250–350 K for $\Delta\phi = 2°$ and shows only a small variation with temperature for $\Delta\phi = 10°$ (table 1). For larger vibration amplitudes, however, a more pronounced temperature dependence of E may be expected.

4. Discussion

In this study a method has been described for calculating transition frequencies in an ion channel on the basis of purely microscopic channel parameters, such as atomic coordinates and force constants. The theoretical expressions for the transition frequency have been given for a special channel geometry (a helical arrangement of dipolar ligands), but the generalization to other (and more complicated) channel models is obvious from the derivation of the equations. Once the rate constants of transition over each barrier of a channel are known, the overall permeability (and conductance) of the channel can be obtained in a straightforward way [6–9].

Of central importance in the calculation of transition frequencies is the potential $V(x)$ of mean force which has the character of a free energy and which accounts for the fact that a ligand system in thermal equilibrium with its surroundings can assume many different configurations. The shape of $V(x)$ depends not only on the geometry of the channel, but also on the forces with which the ligands are bound to their resting positions. Soft and hard ligand systems are found to differ strongly in their ability to discriminate between ions of different size. Furthermore, according to its definition, $V(x)$ depends on temperature. In the limit of low temperature ($T \rightarrow 0$), $V(x)$ approaches the so-called ‘adiabatic potential’ [4], i.e., the potential which is assumed when all ligands are in their equilibrium configuration (for a given position of the ion).

The treatment given here has essentially the same range of validity as rate theory on which it is largely based. Rate theory describes non-equilibrium phenomena (such as ion transport driven by an external force) using equilibrium properties of the system (e.g., equilibrium dsitribution functions). The justification of this approach is based on the following argument [20]. Under equilibrium conditions (i.e., for vanishing driving force), the frequency of transitions over the barrier from both sides must be equal. If the ions on the right-hand side of the barrier are suddenly removed, the flow from right to left will cease. The flow from left to right will continue, however, and it is the essential assumption of rate theory that this flow is the same as before. It is obvious that this assumption requires that the ligand system remains always in equilibrium, even for finite driving forces [21]. In other words, the time needed for the adjustment of the ligands after a jump of the ion has to be small compared with the dwelling time of the ion in the potential well. If, however, the motion of the ligands is slow, the state of the ligand system becomes dependent on the previous history of the channel (whether an ion has passed before over the barrier, for instance). Suppression of jumps from right to left would then affect transition rates from left to right. This means that channels exhibiting slow conformational transitions [12] are excluded from the present analysis. Such systems may be treated by methods of molecular dynamics [4].

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Appendix A

A.1. Derivation of eq. 5

We consider a large number N of channels of the type depicted in fig. 1, each having an ion of mass m at some position x in the interval (x', x'') on the axis. The coordinates x' and x'' are arbitrary for the moment, but will be taken later as the locations of two neighbouring barriers in the free-energy profile. The ion is assumed to move along the channel axis. The Hamiltonian $\mathcal{H}$ of the channel depends on position x and momentum p_x of the ion and on the n tilt angles ϕ_i and the n angular momenta M_i of the identical ligands:

$$\mathcal{H} = U(x, \phi_1, \ldots, \phi_n) + \frac{p_x^2}{2m} + \sum_{i=1}^{n} \frac{M_i^2}{2I} \qquad \text{(A1)}$$

U is the potential energy of the ion-ligand system and I the moment of inertia of a ligand group. Under equilibrium conditions, the average number $\mathrm{d}N$ of channels in the interval $(x,\ x+\mathrm{d}x;\ p_x,\ p_x+\mathrm{d}p_x;\ \phi_1,\ \phi_1+\mathrm{d}\phi_1; \ldots; \phi_n,\ \phi_n+\mathrm{d}\phi_n;\ M_1,\ M_1+\mathrm{d}M_1; \ldots;\ M_n,\ M_n+\mathrm{d}M_n)$ of phase space is given by:

$$\begin{aligned}\mathrm{d}N = {} & Nf(x, p_x, \phi_1, \ldots, \phi_n, M_1, \ldots, M_n) \\ & \times \mathrm{d}x\, \mathrm{d}p_x\, \mathrm{d}\phi_1 \ldots \mathrm{d}\phi_n\, \mathrm{d}M_1 \ldots \mathrm{d}M_n \end{aligned} \qquad \text{(A2)}$$

The distribution function f is connected with the Hamiltonian by the statistical-mechanical relationship [13]:

$$f = \frac{\exp(-\mathcal{H}/kT)}{Z} \qquad \text{(A3)}$$

where Z is the (classical) partition function:

$$\begin{aligned} Z &= \int \ldots \int \exp(-\mathcal{H}/kT)\, \mathrm{d}x\, \mathrm{d}p_x\, \mathrm{d}\phi_1 \ldots \mathrm{d}\phi_n\, \mathrm{d}M_1 \ldots \mathrm{d}M_n \\ &= (2\pi mkT)^{1/2} (2\pi IkT)^{n/2} \\ &\quad \times \int \ldots \int \exp[-U(x, \phi_1, \ldots, \phi_n)/kT]\, \mathrm{d}x\, \mathrm{d}\phi_1 \ldots \mathrm{d}\phi_n \end{aligned} \qquad \text{(A4)}$$

After introducing the configuration integral $Q(x)$:

$$Q(x) = \int \ldots \int \exp[-U(x, \phi_1, \ldots, \phi_n)/kT]\, \mathrm{d}\phi_1 \ldots \mathrm{d}\phi_n \qquad \text{(A5)}$$

Z may be written as

$$Z = (2\pi mkT)^{1/2} (2\pi IkT)^{n/2} \int_{x'}^{x''} Q(x)\, \mathrm{d}x \qquad \text{(A6)}$$

We now consider the situation depicted in fig. 2 where x' and x'' are the locations of barriers on either side of an energy well. In the ensemble of N channels, Φ' transitions take place per unit time over the barrier at x'' from left to right. If $\mathrm{d}\Phi'$ is the contribution of the $\mathrm{d}N$ systems defined by eq. A2 to the total ion flux Φ' over the barrier, then

$$\mathrm{d}\Phi' = \left(\frac{\mathrm{d}N}{\mathrm{d}x}\right)_{x=x''} \cdot \frac{p_x}{m} \qquad (p_x > 0) \qquad \text{(A7)}$$

Eq. A7 expresses the fact that the flux from left to right is equal to the density $\mathrm{d}N/\mathrm{d}x$ of systems times the velocity $v_x = p_x/m > 0$ in the direction of the positive x-axis. After introduction of eq. A2 into eq. A7, the total flux Φ' is obtained as

$$\begin{aligned}\Phi' = N \int \ldots \int \frac{p_x}{m} f(x'', p_x, \phi_1, \ldots, \phi_n, \\ M_1, \ldots, M_m) \mathrm{d}p_x\, \mathrm{d}\phi_1 \ldots \mathrm{d}\phi_n\, \mathrm{d}M_1 \ldots \mathrm{d}M_n \end{aligned} \qquad \text{(A8)}$$

where the integration in p_x has to be carried out between $p_x = 0$ and $p_x = \infty$. Using the relationship

$$\int_0^{\infty} \frac{p_x}{m} \exp(-p_x^2/2mkT)\, \mathrm{d}p_x = kT \qquad \text{(A9)}$$

as well as eqs. A3, A5 and A6, the result is obtained as

$$\Phi' = N \left(\frac{kT}{2\pi m}\right)^{1/2} \frac{Q(x'')}{\int_{x'}^{x''} Q(x)\, \mathrm{d}x} \qquad \text{(A10)}$$

Since N is the number of channels having an ion located between x' and x'', the transition rate constant k' is defined by the relationship

$$\Phi' = Nk' \tag{A11}$$

According to eq. 3, $Q(x)$ may be replaced by the generalized potential $V(x)$:

$$V(x) = -kT\ln\frac{Q(x)}{Q(\infty)} \tag{A12}$$

$Q(\infty)$ is the configuration integral of the empty channel which serves as a reference state. Eqs. A10–A12 then yield eq. 5:

$$k' = \left(\frac{kT}{2\pi m}\right)^{1/2}\frac{\exp[-V(x'')/kT]}{\int_{x'}^{x''}\exp[-V(x)/kT]\,\mathrm{d}x} \tag{A13}$$

In order to discuss the meaning of $V(x)$, we note that the partion function $Z^*(x)$ of a channel with an ion in position x is given by (cf. eqs. A4 and A5):

$$Z^*(x) = (2\pi mkT)^{1/2}(2\pi IkT)^{n/2}Q(x) \tag{A14}$$

This means that eq. A12 is equivalent to

$$V(x) = -kT\ln\frac{Z^*(x)}{Z^*(\infty)} \tag{A15}$$

or, since $-kT\ln Z^*(x)$ is the Helmholtz free energy $F(x)$ of a channel with an ion located at x:

$$V(x) = F(x) - F(\infty) \tag{A16}$$

$F(\infty)$ is the free energy of the empty channel.

It may further be shown that $V(x)$ is identical with the potential of mean force $\overline{W}_x$ acting on the ion along the x-axis [19]. For this purpose eq. A12 is differentiated with respect to x, using eq. A5:

$$-\frac{\mathrm{d}V}{\mathrm{d}x} = \left\{\int\cdots\int\left(-\frac{\partial U}{\partial x}\right)\exp[-U(x,\phi_1,\ldots,\phi_n)/kT]\times\mathrm{d}\phi_1\ldots\mathrm{d}\phi_n\right\}\times\left\{\int\cdots\int\exp[-U(x,\phi_1,\ldots,\phi_n)/kT]\,\mathrm{d}\phi_1\ldots\mathrm{d}\phi_n\right\}^{-1} \tag{A17}$$

Since $-\partial U/\partial x = W_x$ is the force acting on the ion for a given configuration x, $\phi_1,\ldots,\phi_n$, the right-hand side of eq. A17 is the mean force (averaged over all configurations in thermal equilibrium). Thus,

$$-\frac{\mathrm{d}V}{\mathrm{d}x} = \overline{W}_x \tag{A18}$$

Appendix B

B.1. Geometrical relationships for a helical arrangement of ligands

In order to express the distances r_i^-, r_{ij}, etc., as functions of the variables x and ϕ_i, we introduce the helix radius b (fig. 1), the dipole length l and the distance a (along the x-axis) of the fixed positive ends of neighbouring dipoles (the length of a helix with n dipoles is then equal to na). Furthermore, it is convenient to specify the location of the positive and negative ends of the i-th dipole using cylindrical coordinates x_i^+, ρ_i^+, θ_i^+ and x_i^-, ρ_i^-, θ_i^- (x_i and ρ_i are the axial and radial distances and $\theta_i^+ = \theta_i^- = \theta_i$ is the angle between the plane containing the helix axis and dipole axis and a reference plane going through the helix axis):

$$x_i^+ = (i-1)a, \quad x_i^- = x_i^+ + l\cos\phi_i \tag{B1}$$

$$\rho_i^+ = b, \quad \rho_i^- = \rho_i^+ - l\sin\phi_i \tag{B2}$$

The angles θ_i depend on the number p of ligands per turn: $\theta_i = 2\pi(i-1)/p$.
Elementary geometrical considerations then lead to the following expressions:

$$(r_i^+)^2 = (x - x_i^+)^2 + b^2 \tag{B3}$$

$$(r_i^-)^2 = (x - x_i^-)^2 + (\rho_i^-)^2 \tag{B4}$$

$$(r_{ij})^2 = (x_i^- - x_j^+)^2 + (\rho_i^-)^2 + b^2 - 2b\rho_i^-\cos(\theta_i - \theta_j) \tag{B5}$$

$$(r_{ij}^+)^2 = (x_i^+ - x_j^+)^2 + 2b^2 - 2b^2\cos(\theta_i - \theta_j) \tag{B6}$$

$$(r_{ij}^-)^2 = (x_i^- - x_j^-)^2 + (\rho_i^-)^2 + (\rho_j^-)^2 - 2\rho_i^-\rho_j^-\cos(\theta_i - \theta_j) \tag{B7}$$

Appendix C

C.1. Numerical evaluation of eqs. 2 and 5

For the approximate calculation of the multiple integral in eq. 2 we write eq. 8 in the form

$$U = U_0 + \frac{\gamma}{2}\sum_i(\phi_i - \phi_i^0)^2 \tag{C1}$$

where U_0 contains all ion-dipole and dipole-dipole interactions.

Using the notation $\exp(-U_0/kT) \equiv G$ and introducing the substitution $y_i \equiv (\gamma/2kT)^{1/2}(\phi_i -$

ϕ_i^0), one obtains

$$\exp(-U/kT)=\exp\left[-(y_1^2+\ldots+y_n^2)\right]\cdot G(x,y_1,\ldots,y_n) \tag{C2}$$

Eq. 2 then yields

$$Q(x)=\left(\frac{2kT}{\gamma}\right)^{n/2}\int_{-\infty}^{\infty}\cdots\int_{-\infty}^{\infty}\exp\left[-(y_1^2+\ldots+y_n^2)\right] \times G(x,y_1,\ldots,y_n)\,\mathrm{d}y_1\ldots\mathrm{d}y_n \tag{C3}$$

The integral in eq. C3 can be approximated by an n-fold sum using the M-point Gauss-Hermite formula [15,16]:

$$Q(x)\simeq\left(\frac{2kT}{\gamma}\right)^{n/2}\sum_{i=1}^{M}\sum_{j=1}^{M}\cdots\sum_{k=1}^{M} \times B_iB_j\ldots B_kG(x,\eta_i,\eta_j,\ldots,\eta_k) \tag{C4}$$

The coefficients B_ν and the points η_ν of the Gauss-Hermite formula are tabulated in ref. 16 for various values of M.

For the evaluation of eq. 5, we introduce eq. 3 and substitute $s\equiv(2x-x'-x'')/(x''-x')$. With the notation $Q(x)=Q^*(s)$ this yields:

$$k'=\left(\frac{kT}{2\pi m}\right)^{1/2}\frac{Q(x'')}{\int_{x'}^{x''}Q(x)\,\mathrm{d}x}=\frac{\left(\frac{2kT}{\pi m}\right)^{1/2}}{(x''-x')}\frac{Q^*(1)}{\int_{-1}^{1}Q^*(s)\,\mathrm{d}s} \tag{C5}$$

The integral in the denominator may be approximated by a mixed Gauss-Legendre-Hermite formula [15]. Introducing eq. C4 and using the notation $G(x,\eta_i,\eta_j,\ldots\eta_k)=G^*(s,\eta_i,\eta_j,\ldots\eta_k)$, one obtains

$$\int_{-1}^{1}Q^*(s)\,\mathrm{d}s\simeq\left(\frac{2kT}{\gamma}\right)^{n/2}\sum_{\nu=1}^{L}\sum_{i=1}^{M}\sum_{j=1}^{M}\cdots\sum_{k=1}^{M} \times A_\nu B_iB_j\ldots B_kG^*(\sigma_\nu,\eta_i,\eta_j,\ldots,\eta_k) \tag{C6}$$

The Gauss-Legendre coefficients A_ν and the points σ_ν are tabulated in ref. 16 for different values of L.

In order to check the computer programme, a number of numerical tests were performed. If the dipoles are widely separated ($a\to\infty$), the interaction energy U_i in eq. C1 vanishes so that G becomes unity. According to eq. C3 the configuration integral $Q(\infty)$ of the empty channel then reduces to

$$Q(\infty)=\left(\frac{2kT}{\gamma}\right)^{n/2}\int_{-\infty}^{\infty}\cdots\int_{-\infty}^{\infty}\exp\left[-(y_1^2+\ldots+y_n^2)\right] \times\mathrm{d}y_1\ldots\mathrm{d}y_n =\left(\frac{2\pi kT}{\gamma}\right)^{n/2},\quad(a\to\infty) \tag{C7}$$

The result of the computer calculation for $Q(\infty)$ was found to agree with the value of the right-hand side of eq. C7 evaluated directly.

Another limiting case is given when the force constant becomes very large ($\gamma\to\infty$), so that the channel behaves as a virtually rigid ligand system. Under this condition, $\exp(-U/kT)$ vanishes for all values of ϕ_i except for the immediate vicinity of $\phi_i=\phi_i^0$. This gives:

$$Q(x)\approx\left(\frac{2kT}{\gamma}\right)^{n/2}\int_{-\infty}^{\infty}\cdots\int_{-\infty}^{\infty}\exp\left[-(y_1^2+\ldots+y_n^2)\right] \times\exp\left[-U_0(x,\phi_1^0,\ldots,\phi_n^0)/kT\right]\mathrm{d}y_1\ldots\mathrm{d}y_n \approx\left(\frac{2\pi kT}{\gamma}\right)^{n/2}\exp\left[-U_0(x,\phi_1^0,\ldots,\phi_n^0)/kT\right]\quad(\gamma\to\infty) \tag{C8}$$

Using eq. 3 it is seen that in the limit $\gamma\to\infty$ the relationship

$$V(x)\approx U_0(x,\phi_1^0,\ldots,\phi_n^0)-U_0(\infty,\phi_1^0,\ldots\phi_n^0) \tag{C9}$$

holds. The difference $\Delta U_0(x)=U_0(x,\phi_i^0,\ldots,\phi_n^0)-U_0(\infty,\phi_1^0,\ldots,\phi_n^0)$ is the potential energy of the ion in a rigid channel which can be directly calculated for selected values of x and a number of ligand conformations (e.g., $\phi_i^0\equiv0$ or $\phi_i^0\equiv90°$). The values of $\Delta U_0(x)$ calculated in this way were identical with the results for $V(x)$ obtained from the computer programme for large values of γ (e.g., $\gamma/kT=10^8$). A further comparison was carried out between the value of $Q(\infty)$ calculated directly (according to eq. C8) and the result from the computer programme.

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