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FLUCTUATIONS OF BARRIER STRUCTURE IN IONIC CHANNELS

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Summary

In rate-theory analysis of ion transport in channels, the energy of binding sites and the height of activation barriers are usually considered to be time-independent and not influenced by the movement of the ion. The assumption of a fixed barrier structure seems questionable, however, in view of the fact that proteins may exist in a large number of conformational states and may rapidly move from one state to the other. In this study, some of the effects of multiple conformational states of a channel on ion transport are analyzed.

In the first part of the paper, the ion permeability of a channel with *n* binding sites is treated on the assumption that interconversion of channel states is much faster than ion transfer between binding sites. Under this condition, the form of the flux equation remains the same as for a channel with fixed barriers, provided that the rate constants for ion jumps are replaced by weighted averages over the rate constants for the individual conformational states.

In the second part, a channel with two (main) barriers and a single (main) binding site is considered, with the rates of conformational transitions being arbitrary. This case, in particular, includes the situation where a jump of the ion is followed by a slow transition to a more polarized state of the binding site. Under this condition, the conductance of the channel exhibits a nonlinear dependence on ion concentration which is different from a simple saturation behavior. Under non-stationary conditions damped oscillations may occur.

Introduction

The pathway of ions through protein channels may be described as a sequence of binding sites which are separated by activation-energy barriers. Using rate-theory analysis [1], the transport of ions in the channel may then be treated as a series of thermally activated jumps over the barriers [2–7]. In this treatment, the barrier heights are usually considered to be fixed, i.e., independent of time and not influenced by the movement of the ion. This description, which corresponds to an essentially static picture of protein structure, represents a useful approximation in many cases. In view of recent findings on the dynamics of protein molecules, however, the assumption of a time-independent barrier structure is not entirely satisfactory for a more detailed understanding of ion transport in channels.

A protein molecule in thermal equilibrium may exist in a large number of conformational states and may rapidly move from one state to the other [8,9]. Evidence for fluctuations of protein structure comes from X-ray diffraction studies [8,10], NMR [11,12] and optical [13] experiments and from the kinetic analysis of ligand rebinding to myoglobin after flash photolysis [14]. These studies have shown that internal motions in proteins occur in the time range from nanoseconds to seconds. It is, therefore, likely that the energy levels of barriers and ion binding sites in a channel protein are subjected to fluctuations over a wide spectrum of correlation times.

In the following, we analyze some of the effects of multiple conformational states of a channel on ion permeability. In the first part of the paper, we deal with the relatively simple case in which interconversion of channel states is much faster than ion transfer between neighboring binding sites. Under this condition, the description of channel permeability is formally similar to previous treatments based on the assumption of fixed barrier structure but with the rate constants now being composite quantities containing the contributions of the different conformational states.

In the second part of the paper, the more important case of a coupling between ion movement and conformational transitions is discussed. When an ion jumps into a binding site, the strong coulombic field around the ion tends to polarize the neighborhood by reorienting dipolar groups of the protein. This orientation shifts the energy level of the binding site and, most likely, also the height of adjacent barriers. If the rate of conformational change induced by the ion is comparable to or smaller than the jump rate, the ion may leave the binding site before the protein structure has relaxed to the polarized state [21]. Likewise, after the ion has left a binding site, a certain time is required for the channel to return to the original conformation, and the next ion may find the structure still in a partly polarized state. Similar concepts have been used in recent treatment of dielectric relaxation in solids [15] which have been based on the assumption that the dielectric behavior is governed by many-body interactions and that the screening of charges may involve a slow adjustment of the surrounding structure after a jump of the charge into a new position. Changing the ion concentration in the aqueous phase (and thus the average occupancy of the channel) may shift the distribution of conformational states of the channel and affect its permeability. As will be shown below, this behavior can lead to a nonlinear concentration dependence of permeability which is different from the simple saturation characteristic predicted for channels with fixed barrier structure and single occupancy.

Fast interconversion of conformational states

In the following, we consider a channel which has n internal binding sites separated by energy barriers (Fig. 1); the outer binding sites, 0 and $n + 1$, belong to the aqueous phase and have (in the absence of an applied voltage) identical energy levels. We assume that the aqueous ion concentration is low enough so that the channel contains no more than one ion at a time. If an ion is located in the ν-th binding site, the channel is said to be in state ν. In order to account for the fact that a channel protein can exist, in general, in many conformational states, we assign a number of substates to each state ν which are denoted by i_ν ($i_\nu = 1, 2, \ldots, m_\nu$). We further assume that interconversion of substates is much faster than ion transfer between binding sites, i.e., that the mean lifetime of a substate, i_ν, is much smaller than the average time an ion spends in binding site ν. This means that the substates of a given state ν are always in equilibrium with each other, even for non-zero ion flow through the channel. On the other hand, we require that the mean lifetime of a substate is longer than the actual transfer time over the barrier which is of the order of the reciprocal vibration frequency of the ion in the binding site (approx. 10^{-12} s). This simply means that an ion crossing the barrier finds the barrier in a well defined state.

If, at a given time, N_ν channels in the membrane are in state ν (binding site ν occupied), then a fraction $a(i_\nu)$ of these channels will be in substate i_ν:

$$N(i_\nu) = a(i_\nu) \cdot N_\nu \tag{1}$$

As we have assumed that the substates of a state ν are always in equilibrium with each other, the quantities $a(i_\nu)$ are given by a Boltzmann distribution:

$$a(i_\nu) = \frac{\exp(-U(i_\nu)/kT)}{\sum\limits_{i_\nu} \exp(-U(i_\nu)/kT)} \tag{2}$$

where $U(i_\nu)$ is the energy of the channel protein in substate i_ν (referred to an arbitrary reference state), k is the Boltzmann constant and T the absolute temperature.

For a channel which is in substate i_ν, the rate constant for a jump from binding site ν to the right (over the $(\nu + 1)$-th barrier) is, according to rate

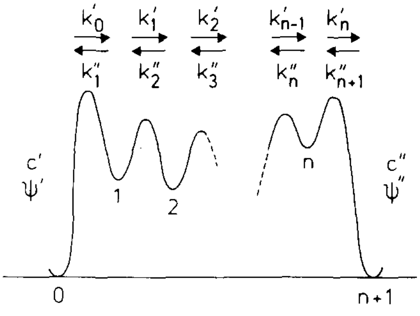


Fig. 1. Energy profile of the channel.

theory [1,16], approximately given by:

$$k'(i_\nu) = f(i_\nu) \cdot \exp(-E'(i_\nu)/kT) \tag{3}$$

where $f(i_\nu)$ is the vibration frequency of the ion in binding site ν and $E'(i_\nu)$ is the energy difference between the top of the $(\nu + 1)$-th barrier and binding site ν (Fig. 1). The total ion flow, $\Phi'_{\nu+1}$, in the membrane over the $(\nu + 1)$-th barrier from left to right is the sum of the contributions of all substates i_ν:

$$\Phi'_{\nu+1} = \sum_{i_\nu} N(i_\nu) \cdot k'(i_\nu) \tag{4}$$

Introducing the notation:

$$k'_\nu \equiv \sum_{i_\nu} a(i_\nu) \cdot k'(i_\nu) \tag{5}$$

$$k''_{\nu+1} \equiv \sum_{i_\nu} a(i_{\nu+1}) \cdot k''(i_{\nu+1}) \tag{6}$$

the net flow, $\Phi_{\nu+1}$, over the $(\nu + 1)$-th barrier is obtained in the form (using Eqn. 1):

$$\Phi_{\nu+1} = \Phi'_{\nu+1} - \Phi''_{\nu+1} = k'_\nu N_\nu - k''_{\nu+1} N_{\nu+1} \tag{7}$$

This equation is formally identical with the corresponding expression for a channel with fixed barrier structure [4]. Accordingly, for a membrane with N_t channels which is in contact with ionic solutions of concentration c' (left side) and c'' (right side), the stationary flux, Φ, in the presence of a voltage, $V_m = \psi' - \psi''$, is given by the previously derived expression [4]:

$$\Phi = N_t \beta c' \cdot \frac{1 - \exp[-z(u - u_0)]}{1 + \gamma c' + \delta c''} \tag{8}$$

$$u \equiv \frac{V_m e_0}{kT} \tag{9}$$

where z is the valency of the ion, e_0 the elementary charge and β, γ and δ are known functions of the rate constants k'_ν, k''_ν [4]. u_0 is the equilibrium voltage (in units of $kT/e_0 \simeq 25$ mV) for the permeable ion:

$$u_0 \equiv \frac{1}{z} \cdot \ln \frac{c''}{c'} \tag{10}$$

Eqn. 8 has the same form as the flux equation for a channel with fixed barrier structure [4], but the rate constants are now composite quantities (Eqns. 5 and 6). Neglecting differencies in the frequencies, $f(i_\nu)$, one obtains from Eqns. 2, 3 and 5:

$$k'_\nu \propto \sum_{i_\nu} \exp\left(-\frac{U(i_\nu) + E'(i_\nu)}{kT}\right) \tag{11}$$

This means that the contributions of the single substates to the overall transport over the barrier are weighted according to the sum of the barrier height, $E'(i_\nu)$, and the conformational energy, $U(i_\nu)$, of the substate.

Coupling between ion transport and conformational transitions

In the following, we consider the case of arbitrary interconversion rates of channel conformations. Under this condition, a flow of ions driven by a difference of electrochemical potential may create a nonequilibrium distribution of conformational substates [17,18]. While a general treatment of systems with coupling between transport and conformational transitions is necessarily complex, we restrict the analysis to the case of a channel with a single binding site where an explicit solution can be given.

An ion jumping from the aqueous phase into the binding site tends to reorient neighboring dipolar residues of the channel molecule, leading to a local polarization. This reorientation may be slow compared with the transport rate. When the ion leaves the polarized binding site, the polarization may persist for some time until the channel returns to the normal state. In general, both the energy level of the binding site as well as the height of the barriers depend on the state of the channel (Fig. 2).

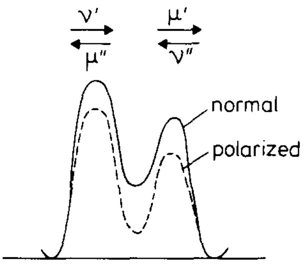
We assume that the transitions between the normal and the polarized state occur in a single step; this assumption is introduced here only for mathematical convenience and should be replaced by a more detailed description if necessary. Furthermore, there may be a fast component in the polarization which does not appear explicitly in the kinetic equations. Thus, the channel molecule may exist in four distinct substates: C (empty, normal); C^* (occupied, normal); C_p^* (occupied, polarized); C_p (empty, polarized).

The rate constants for transitions between substates are indicated in Fig. 3. Transitions between empty and occupied states may occur by exchange of an ion between either the left or the right aqueous phase. Thus (Fig. 2),

$$\nu = \nu' + \nu'' \; ; \; \mu = \mu' + \mu'' \tag{12}$$

$$\nu_p = \nu_p' + \nu_p'' \; ; \; \mu_p = \mu_p' + \mu_p'' \tag{13}$$

The jump rates ν', ν'', ν_p', ν_p'' of ions into the empty site depend on the aqueous ion concentrations, whereas μ', μ'', μ_p', μ_p'' may be assumed to be



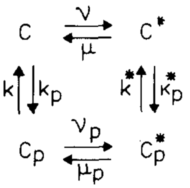


Fig. 2. Energy profile of a channel with one binding site in the 'normal' and the 'polarized' state. ν', ν'', μ', μ'' are the rate constants for transfer of an ion between binding site and external solution in the normal state.

Fig. 3. Transitions between the four different substates of a channel with one binding site.

true monomolecular rate constants. The total number, $N_{\rm t}$, of channels in the membrane is the sum of channel numbers of the different substates:

$$N_{\rm t} = N + N^* + N_{\rm p} + N_{\rm p}^* \tag{14}$$

Thus, only three of the four variables N, N^*, $N_{\rm p}$, $N_{\rm p}^*$ are independent. Choosing N, N^* and $N_{\rm p}$ as the independent variables, the dependence of N, N^*, $N_{\rm p}$ on time t is given by (compare Fig. 3):

$$\frac{{\rm d}N}{{\rm d}t} = -(\nu + k_{\rm p})\,N + \mu N^* + kN_{\rm p} \tag{15}$$

$$\frac{{\rm d}N^*}{{\rm d}t} = -(\mu + k_{\rm p}^*)\,N^* + \nu N + k^* N_{\rm p}^* \tag{16}$$

$$\frac{{\rm d}N_{\rm p}}{{\rm d}t} = -(\nu_{\rm p} + k)\,N_{\rm p} + \mu_{\rm p} N_{\rm p}^* + k_{\rm p} N \tag{17}$$

In general, all rate constants of the model may depend on voltage u. Furthermore, according to the principle of microscopic reversibility, certain relationships between the rate constants must be fulfilled; these relationships are obtained as (Appendix A):

$$\frac{\nu'\mu'}{\nu''\mu''} = \frac{\nu_{\rm p}'\mu_{\rm p}'}{\nu_{\rm p}''\mu_{\rm p}''} = \frac{\nu'\mu_{\rm p}' k k_{\rm p}^*}{\nu_{\rm p}''\mu'' k_{\rm p} k^*} = \exp[z(u - u_0)] \tag{18}$$

In the equilibrium case ($u = u_0$), Eqns. 12 and 13 together with Eqn. 18 give:

$$\bar{\nu}\bar{\mu}_{\rm p}\bar{k}_{\rm p}^*\bar{k} = \bar{\nu}_{\rm p}\bar{\mu}\bar{k}_{\rm p}\bar{k}^* \tag{19}$$

where the bar denotes the value of the rate constant at the equilibrium voltage u_0.

Steady-state transport

The net flow, Φ, in the steady state may be obtained by summing over all transitions in which an ion jumps over a given barrier. For the left-hand barrier this gives:

$$\Phi = \nu' N - \mu'' N^* + \nu_{\rm p}' N_{\rm p} - \mu_{\rm p}'' N_{\rm p}^* \tag{20}$$

An analogous expression for Φ is obtained for the right-hand barrier. For the explicit calculation of Φ it is advantageous to use a more symmetrical relationship for Φ which is obtained by combining the equations for the left and right barrier:

$$\Phi = \tfrac{1}{2}\,[(\nu' - \nu'')\,N + (\mu' - \mu'')\,N^* + (\nu_{\rm p}' - \nu_{\rm p}'')\,N_{\rm p} + (\mu_{\rm p}' - \mu_{\rm p}'')\,N_{\rm p}^*] \tag{21}$$

By solving Eqns. 14—17 under steady-state conditions (${\rm d}N/{\rm d}t = {\rm d}N^*/{\rm d}t = {\rm d}N_{\rm p}/{\rm d}t = {\rm d}N_{\rm p}^*/{\rm d}t = 0$), one finds:

$$N = \frac{N_{\rm t}}{\sigma}\,[\mu k^*(\nu_{\rm p} + k) + \mu_{\rm p} k(\mu + k_{\rm p}^*)] \tag{22}$$

$$N^* = \frac{N_{\rm t}}{\sigma}\,[\nu_{\rm p} k^*(\nu + k_{\rm p}) + \nu k(\mu_{\rm p} + k^*)] \tag{23}$$

$$N_{\mathrm{p}} = \frac{N_{\mathrm{t}}}{\sigma}\,[\mu_{\mathrm{p}} k_{\mathrm{p}}^{*}(\nu + k_{\mathrm{p}}) + \mu k_{\mathrm{p}}(\mu_{\mathrm{p}} + k^{*})] \tag{24}$$

$$N_{\mathrm{p}}^{*} = \frac{N_{\mathrm{t}}}{\sigma}\,[\nu k_{\mathrm{p}}^{*}(\nu_{\mathrm{p}} + k) + \nu_{\mathrm{p}} k_{\mathrm{p}}(\mu + k_{\mathrm{p}}^{*})] \tag{25}$$

$$\sigma \equiv (k + k_{\mathrm{p}}) \cdot (\mu\mu_{\mathrm{p}} + \mu k^{*} + \mu_{\mathrm{p}} k_{\mathrm{p}}^{*}) + (k^{*} + k_{\mathrm{p}}^{*}) \cdot (\nu\nu_{\mathrm{p}} + \nu k + \nu_{\mathrm{p}} k_{\mathrm{p}}) + \nu\mu_{\mathrm{p}}(k + k_{\mathrm{p}}^{*})$$
$$+ \nu_{\mathrm{p}}\mu(k^{*} + k_{\mathrm{p}}) \tag{26}$$

Inserting Eqns. 22—25 into Eqn. 21 yields (together with Eqn. 18):

$$\Phi = \frac{N_{\mathrm{t}}}{\sigma}\{1 - \exp[-z(u - u_0)]\} \cdot [\nu'\mu'(\nu_{\mathrm{p}} k^{*} + kk^{*} + \mu_{\mathrm{p}} k)$$
$$+ \nu_{\mathrm{p}}'\mu_{\mathrm{p}}'(\nu k_{\mathrm{p}}^{*} + k_{\mathrm{p}} k_{\mathrm{p}}^{*} + \mu k_{\mathrm{p}}) + \nu'\mu_{\mathrm{p}}' k k_{\mathrm{p}}^{*} + \nu_{\mathrm{p}}'\mu' k^{*} k_{\mathrm{p}}] \tag{27}$$

From the steady-state flux, Φ, the ohmic single-channel conductance, Λ, may be calculated which is defined by: *

$$\Lambda = \frac{1}{N_{\mathrm{t}}} \lim_{u \to 0} \left(\frac{z e_0 \Phi}{u k T / e_0}\right)_{c' = c'' = c} \tag{28}$$

In order to obtain Λ as a function of ion concentration (c) we take the concentration dependence of the rate constants ν', ν'', ν_{p}', ν_{p}'' into account:

$$\nu' = c'\rho' \;;\; \nu'' = c''\rho'' \tag{29}$$

$$\nu_{\mathrm{p}}' = c'\rho_{\mathrm{p}}' \;;\; \nu_{\mathrm{p}}'' = c''\rho_{\mathrm{p}}'' \tag{30}$$

ρ', ρ'', ρ_{p}', ρ_{p}'' are concentration-independent quantities. Eqns. 26—30 then give (with $\rho \equiv \rho' + \rho''$ and $\rho_{\mathrm{p}} \equiv \rho_{\mathrm{p}}' + \rho_{\mathrm{p}}''$):

$$\Lambda(c) = \frac{z^2 e_0^2}{kT} \cdot \frac{c(A + Bc)}{C + Dc + Ec^2} \tag{31}$$

$$A \equiv \rho'\mu' k(\mu_{\mathrm{p}} + k^{*}) + \rho_{\mathrm{p}}'\mu_{\mathrm{p}}' k_{\mathrm{p}}(\mu + k_{\mathrm{p}}^{*}) + \rho'\mu_{\mathrm{p}}' k k_{\mathrm{p}}^{*} + \rho_{\mathrm{p}}'\mu' k^{*} k_{\mathrm{p}} \tag{32}$$

$$B = \rho'\rho_{\mathrm{p}}\mu' k^{*} + \rho_{\mathrm{p}}'\rho\mu_{\mathrm{p}}' k_{\mathrm{p}}^{*} \tag{33}$$

$$C \equiv (k + k_{\mathrm{p}}) \cdot (\mu\mu_{\mathrm{p}} + \mu k^{*} + \mu_{\mathrm{p}} k_{\mathrm{p}}^{*}) \tag{34}$$

$$D \equiv (k^{*} + k_{\mathrm{p}}^{*}) \cdot (\rho k + \rho_{\mathrm{p}} k_{\mathrm{p}}) + \rho\mu_{\mathrm{p}}(k + k_{\mathrm{p}}^{*}) + \rho_{\mathrm{p}}\mu(k^{*} + k_{\mathrm{p}}) \tag{35}$$

$$E \equiv \rho\rho_{\mathrm{p}}(k^{*} + k_{\mathrm{p}}^{*}) \tag{36}$$

According to the definition of Λ, the rate constants refer to voltage $u = 0$ in these equations. It is seen from Eqn. 31 that $\Lambda(c)$ is a nonlinear function of

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* It should be noted that, by definition, Λ is the ensemble average over all channels, which may be considered equal to the time average during the observation of a single channel. This includes the possibility that in extreme cases (when the transition rate constants are small compared with the bandwidth of the recording device), fluctuations in the single-channel current resulting from conformational transitions may be directly observed. Such a limiting case, of course, is given by a channel with open-closed kinetics.

ion concentration containing terms which are quadratic in c. This behavior may be compared with the properties of a one-site channel with fixed barrier structure which always exhibits a simple saturation characteristic of the form [4]:

$$\Lambda(c) = \frac{z^2 e_0^2}{kT} \cdot \frac{\rho' \mu' c}{\mu + \rho c} \, . \tag{37}$$

As may be expected, Eqn. 31 reduces to Eqn. 37 in the limit $k_\mathrm{p} = k_\mathrm{p}^* = 0$ where the polarized state becomes inaccessible.

The peculiar nonlinear concentration dependence of Λ (Eqn. 31) is connected with the fact that the distribution of channel molecules between normal and polarized states depends on ion concentration (c) and that both states have, in general, different (concentration-dependent) conductances. An essential requirement for the occurrence of quadratic concentration terms in $\Lambda(c)$ is transition rates between normal and polarized states which are comparable to or slower than the translocation rates. In this case, ion flow through the channel induced by an external potential difference drives the channel into a nonequilibrium state with respect to the distribution between normal and polarized states. If, however, k, k_p, k^* and k_p^* are much larger than ν, ν_p, μ and μ_p, Eqn. 31 reduces to a simple saturation characteristic of the form $\Lambda \propto c/(1 + \text{constant} \cdot c)$.

For certain combinations of rate constants, the conductance (Λ) goes through a maximum with increasing ion concentration (c). From Eqn. 31 it is seen that a maximum is always assumed whenever the relationship $AE > BD$ is fulfilled which is equivalent to the condition:

$$(k^* + k_\mathrm{p}^*) \cdot \left(\frac{\mu'}{\mu} - \frac{\mu_\mathrm{p}'}{\mu_\mathrm{p}}\right)^2 > \left(\frac{1}{k} + \frac{1}{k_\mathrm{p}}\right) \cdot \left(\frac{\mu' \mu''}{\mu} k^* + \frac{\mu_\mathrm{p}' \mu_\mathrm{p}''}{\mu_\mathrm{p}} k_\mathrm{p}^*\right) . \tag{38}$$

According to Eqn. 31, the maximum of $\Lambda(c)$ occurs at the concentration:

$$c_\mathrm{max} = \frac{BC}{AE - BD} + \sqrt{\left(\frac{BC}{AE - BD}\right)^2 + \frac{AC}{AE - BD}} \tag{39}$$

For an illustration of the concentration dependence of Λ, we consider the special case of a channel which is symmetrical in the normal (unpolarized) state:

$$\rho' = \rho'' \; ; \; \mu' = \mu'' \tag{40}$$

Furthermore, in order to reduce the number of free parameters, we assume that in this specific example the transition and exchange rate obey the following relationships:

$$k_\mathrm{p}^* = k \; ; \; k^* = k_\mathrm{p} \; ; \; \rho' \rho_\mathrm{p}' = (\rho_\mathrm{p}'')^2 \tag{41}$$

In Fig. 4 the ratio, $\Lambda(c)/\Lambda(\infty)$, is plotted as a function of the dimensionless concentration $\rho c/\mu$ (according to Eqn. A3 in Appendix A, $\rho c/\mu$ is equal to the ratio, $\bar{N}^*/\bar{N}$, of the equilibrium concentrations of the occupied and free state of the unpolarized channel). The numerical evaluation has been carried out for fixed values $k_\mathrm{p}/\mu'' = 10$ and $\mu''/\mu_\mathrm{p}'' = 10$ with $\mu_\mathrm{p}''/\mu_\mathrm{p}'$ as a

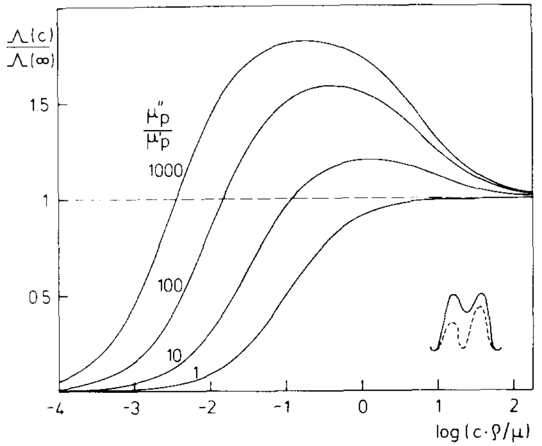

Fig. 4. Single-channel conductance, Λ, as a function of ion concentration c under the conditions, $\rho' = \rho''$, $\mu' = \mu''$, $k_p^* = k$, $k^* = k_p$, $\rho'\rho_p' = (\rho_p'')^2$, $k_p/\mu'' = 10$, $\mu''/\mu_p'' = 10$ with μ_p''/μ_p' as a parameter. The dimensionless concentration, $\rho c/\mu$, is equal to $\bar{N}^*/\bar{N}$. Inset: energy profiles giving rise to a pronounced maximum of $\Lambda(c)$; full line, normal state; dashed line, polarized state.

parameter. It is seen from Fig. 4 that a pronounced maximum of $\Lambda(c)$ occurs when μ_p''/μ_p' becomes large, i.e., when the channel is strongly asymmetric in the polarized state. Furthermore, by varying μ''/μ_p'', it is found that a large value of μ''/μ_p'' is favorable for the occurence of a sharp maximum. An energy profile which leads to such a situation ($\mu_p'' >> \mu_p'$, $\mu'' >> \mu_p''$) is depicted in the insert to Fig. 4. The condition of microscopic reversibility (Eqn. 18) requires in the above case that:

$$\frac{k}{k_p} = \sqrt{\frac{\rho_p'}{\rho'} \cdot \frac{\mu''}{\mu_p''}} \; . \tag{42}$$

Large values of μ''/μ_p'' are therefore connected with large ratios k/k_p and k_p^*/k^* (compare Eqns. 41). This means that the empty channel has to be predominantly in the normal state and the occupied channel predominantly in the polarized state in order that a pronounced maximum of $\Lambda(c)$ occurs. At low ion concentration c, the conductance Λ increases with c, as the occupancy of the channel increases. At higher concentrations, however, the channel is driven into the less conductive, polarized state so that $\Lambda(c)$ eventually decreases towards a limiting value $\Lambda(\infty)$.

In the more general case of a one-site channel with n conformational states, $\Lambda(c)$ may be shown to have the form:

$$\Lambda(c) = c\,\frac{P_{n-1}(c)}{Q_n(c)} \tag{43}$$

where P_{n-1} and Q_n are polynomials of $(n-1)$-th and n-th order, respectively. This means that the concentration dependence of channel conductance may become rather complicated.

Transient phenomena

If an external parameter such as ion concentration or voltage is suddenly changed, the system relaxes toward a new stationary state. This time behavior is described by the solution to Eqns. 15—17 which may be obtained in the form of a sum of three exponential terms. The expressions for the three relaxation times and amplitudes are rather complicated, however, and we therefore restrict the discussion to a somewhat simpler limiting case. For this purpose, we assume that in the normal (unpolarized) state the left barrier is so low that there is always equilibrium between the binding site and the left-hand aqueous phase:

$$\frac{N^*}{N} = \frac{\bar{N}^*}{\bar{N}} = \frac{\nu'}{\mu''} \equiv K \tag{44}$$

Introducing $N_0 \equiv N + N^*$ as a new variable, we may write $N = N_0/(1 + K)$ and $N^* = N_0K/(1 + K)$ so that Eqns. 15—17 reduce to:

$$\frac{\mathrm{d}N_0}{\mathrm{d}t} = k^*N_\mathrm{t} - vN_0 + sN_\mathrm{p} \tag{45}$$

$$\frac{\mathrm{d}N_\mathrm{p}}{\mathrm{d}t} = \mu_\mathrm{p}N_\mathrm{t} + rN_0 - wN_\mathrm{p} \tag{46}$$

$$r \equiv \frac{k_\mathrm{p}}{1 + K} - \mu_\mathrm{p} \tag{47}$$

$$s \equiv k - k^* \tag{48}$$

$$v \equiv k^* + \frac{k_\mathrm{p}}{1 + K} + k_\mathrm{p}^* \frac{K}{1 + K} \tag{49}$$

$$w \equiv \nu_\mathrm{p} + \mu_\mathrm{p} + k \tag{50}$$

Eqns. 45 and 46 are formally identical to the equations used to describe ion transport through a two-site channel with fixed barrier structure [19]. The solutions have the form [19]:

$$N_0(t) = N_0(\infty) + A_1e^{\lambda_1 t} + A_2e^{\lambda_2 t} \tag{51}$$

$$N_\mathrm{p}(t) = N_\mathrm{p}(\infty) + B_1e^{\lambda_1 t} + B_2e^{\lambda_2 t} \tag{52}$$

$$\lambda_{1/2} = -\tfrac{1}{2}(v + w) \pm \tfrac{1}{2}\sqrt{(v - w)^2 + 4rs} \tag{53}$$

Interestingly, for certain combinations of rate constants, i.e., for $(v - w)^2 + 4rs < 0$, the coefficients λ_1 and λ_2 become complex; for instance, for $k^* = \nu_\mathrm{p} = k_\mathrm{p}/(1 + K) \equiv \alpha$, $\mu_\mathrm{p} = k_\mathrm{p}^*K/(1 + K) \equiv 2\alpha$; $k \equiv 3\alpha$, $\lambda_{1/2}$ becomes equal to $\alpha(-5 \pm \sqrt{-1})$.

Under these conditions, the system exhibits damped oscillations of frequency $\omega = (1/2)\sqrt{|(v - w)^2 + 4rs|}$ [19]. Denoting the times where a maximum and the following minimum occur by t' and t'', respectively, the damping coefficient δ (for N_0) may be defined by

$$\delta \equiv \left| \frac{N_0(t') - N_0(\infty)}{N_0(t'') - N_0(\infty)} \right| \tag{54}$$

It can be shown [20] that for the model considered here, the relationship $\delta \geqslant \exp(\pi\sqrt{3}) \approx 231$ holds; this means that the oscillation is strongly damped. A much weaker damping, however, may be predicted for channels with more conformational states (Ref. 38 and Stephan, W. and Frehland, E., unpublished data). A specific example is discussed in Appendix B.

Discussion

In the preceding sections, we have analyzed a generalization of the barrier model of ion channels which takes fluctuations of barrier structure into account. Such fluctuations in the energy profile of a channel may be expected to arise from transitions between the different conformational states of the channel protein. In view of the complex nature of the general solution to this problem, two simple situations have been considered. In the first case, it has been assumed that the mean lifetime of a conformational state is much shorter than the average time an ion spends in a binding site. Under this condition, the form of the flux equation (for a barrier with n binding sites) remains the same as for a channel with fixed barriers, provided that the rate constants for ion jumps are replaced by weighted averages over the rate constants for the individual conformational states. Despite the formal identity of the flux equations, the nature of the transport process, however, is different, since an ion will preferably jump over the barrier when the barrier is low; this means that the jump rate now largely depends on the frequency with which conformational states with low barrier heights are assumed.

In the second case, a channel with two (main) barriers and a single (main) binding site has been considered, with the rates of conformational transitions being arbitrary. This case, in particular, includes the situation where a jump of the ion into an energy minimum is followed by a slow transition to a more polarized state of the binding site. Under this condition, where coupling between transport and conformational transitions occurs, the channel conductance (Λ) exhibits an interesting nonlinear dependence of ion concentration c. In a certain range of values of the rate constants, $\Lambda(c)$ goes through a maximum. Such a nonlinear concentration behavior is usually taken as evidence for the influence of ion-ion interactions in the channel or for the existence of regulatory ion-binding sites. A well known example for a nonlinear concentration dependence of conductance (different from a simple saturation behavior) is the gramicidin channel [22—27]. In this case, the conductance at higher ion concentrations seems to be determined, at least in part, by multiple occupancy of the channel [28]. The possibility that the behavior of the gramicidin channel is influenced by slow conformational transitions has also been discussed [29]. Indeed, it seems possible that interactions between amino acid side chains lead to the occurrence of multiple conformational states differing in the geometry of the ligand system.

It is interesting to note that the two-state channel model which has been analyzed in the preceding sections is intermediate between a 'simple' channel mechanism (i.e., a channel with fixed barrier structure) and a carrier mechanism. A carrier may be defined as a transport system with a binding site that is exposed either to the left or to the right. Thus, the two-state channel approaches a carrier when in one state the barrier to the right becomes very high and in the other state the barrier to the left becomes very high. Indeed, it may be shown that the expression for Λ (Eqns. 31—36) transforms into the corresponding expression for a carrier [37] in the limit $\mu' \approx 0$, $\rho'' \approx 0$, $\mu_p'' \approx 0$, $\rho_p' \approx 0$ (or $\mu'' \approx 0$, $\rho' \approx 0$, $\mu_p' \approx 0$, $\rho_p'' \approx 0$). The model which has been discussed here very likely also accounts for concentration-dependent permeability ratios and blocking effects in ion channels. Such phenomena have been observed in nerve membranes [30—34]. Another observation which is possibly connected with ion-induced conformational changes is the dependence of the mean lifetime of channels with open-closed kinetics on ion concentration [18,35,36].

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Appendix A

Derivation of Eqn. 18

In the following, we denote the values of the state variables N, N_p, . . . and of the rate constants k, k_p . . . at the equilibrium voltage $u = u_0$ by $\bar{N}$, $\bar{N}_p$ . . . and $\bar{k}$, $\bar{k}_p$ . . . , respectively. The equilibrium between normal and polarized states is then described by:

$$\frac{\bar{N}}{\bar{N}_p} = \frac{\bar{k}}{\bar{k}_p} \tag{A1}$$

$$\frac{\bar{N}^*}{\bar{N}_p^*} = \frac{\bar{k}^*}{\bar{k}_p^*} \tag{A2}$$

Furthermore, at equilibrium the principle of detailed balance requires that:

$$\frac{\bar{N}^*}{\bar{N}} = \frac{\bar{\nu}'}{\bar{\mu}''} = \frac{\bar{\nu}''}{\bar{\mu}'} \tag{A3}$$

$$\frac{\bar{N}_p^*}{\bar{N}_p} = \frac{\bar{\nu}_p'}{\bar{\mu}_p''} = \frac{\bar{\nu}_p''}{\bar{\mu}_p'} \tag{A4}$$

The rate constants for the transitions between empty and occupied states may be assumed to be proportional to the aqueous ion concentrations:

$$\nu' = \rho' c' \; ; \nu'' = \rho'' c'' \tag{A5}$$

where ρ' and ρ'' are concentration-independent coefficients. Eqns. A3 and A5 give, together with the Nernst equation (Eqn. 10):

$$\frac{\bar{\mu}'\bar{\rho}'}{\bar{\mu}''\bar{\rho}''} = \frac{c''}{c'} = \exp(zu_0) \tag{A6}$$

As $\bar{\mu}'$, $\bar{\mu}''$, $\bar{\rho}'$ and $\bar{\rho}''$ are independent of ion concentration, the relationship:

$$\frac{\mu'\rho'}{\mu''\rho''} = \exp(zu) \tag{A7}$$

holds at any voltage u. Using again Eqns. A5 and 10, one finds:

$$\frac{\nu'\mu'}{\nu''\mu''} = \exp[z(u - u_0)]\ . \tag{A8}$$

In an analogous way one obtains from Eqns. A1—A4:

$$\frac{\nu'_{\mathrm{p}}\mu'_{\mathrm{p}}}{\nu''_{\mathrm{p}}\mu''_{\mathrm{p}}} = \exp[z(u - u_0)] \tag{A9}$$

$$\frac{\nu'\mu'_{\mathrm{p}}kk^*_{\mathrm{p}}}{\nu''_{\mathrm{p}}\mu''k_{\mathrm{p}}k^*} = \exp[z(u - u_0)] \tag{A10}$$

This proves Eqn. 18.

Appendix B

Oscillatory behavior

In this section, we show that for a system with the full set of three variables (Eqns. 15—17) the damping coefficient may be considerably smaller than the minimum value of $\delta \approx 231$ obtained for a two-variable system. For this purpose, we solve Eqns. 14—17 for the limiting case $\mu' \approx 0$, $\nu'' \approx 0$, $\mu''_{\mathrm{p}} \approx 0$, $\nu'_{\mathrm{p}} \approx 0$, which corresponds to the 'carrier limit' of the model (see Discussion). Furthermore, in order to simplify the equations, we put:

$$\nu' = \mu'_{\mathrm{p}} = k = k^*_{\mathrm{p}} \equiv k' \tag{B1}$$

$$\nu''_{\mathrm{p}} = \mu'' = k_{\mathrm{p}} = k^* \equiv k'' \tag{B2}$$

This means that in order to fulfill the requirements of microscopic reversibility (Eqn. 18), the following relationships must hold:

$$\frac{\mu'}{\nu''} = \frac{\nu'_{\mathrm{p}}}{\mu''_{\mathrm{p}}} = \left(\frac{k'}{k''}\right)^3 = \exp[3z(u - u_0)/4] \tag{B3}$$

Under these conditions, the eigenvalues of Eqns. 15—17 are obtained as:

$$\lambda_j = k'\left[\exp\left(-i\frac{\pi j}{2}\right) - 1\right] + k''\left[\exp\left(i\frac{\pi j}{2}\right) - 1\right] \tag{B4}$$

$(j = 1, 2, 3)$

where i is the imaginary unit ($i^2 = -1$). The damping coefficient, δ_j, may be defined (as in Eqn. 54) for each of the elementary oscillations:

$$\exp(Re\lambda_j \cdot t) \cdot \cos(Im\lambda_j \cdot t) \quad , \quad \exp(Re\lambda_j \cdot t) \cdot \sin(Im\lambda_j \cdot t)$$

where Re and Im mean 'real part of' and 'imaginary part of', respectively. It is then easy to show that δ_j is given by:

$$\delta_j = \exp\left(\pi\left|\frac{Re\lambda_j}{Im\lambda_j}\right|\right), \quad (j = 1, 3) \tag{B5}$$

(for $j = 2$ the damping coefficient becomes infinite). Using Eqn. B4, the minimum damping coefficient is obtained as:

$$\delta \equiv \min(\delta_1, \delta_3) = \exp\left(\pi\left|\frac{k' + k''}{k' - k''}\right|\right) \tag{B6}$$

The smallest value of δ is assumed either for $k' << k''$ or $k'' >> k'$. This means that $\delta \geqslant \exp(\pi) \approx 23.1$, a value which is about 10-times below the limit for the two-variable system. In fact, $\delta \approx 23.1$ corresponds to the minimum damping in a general four-node cycle (Stephan, W. and Frehland, E., unpublished data). According to Eqn. B3, weak damping ($k' << k''$ or $k' >> k''$) is only possible far from equilibrium.

References

1 Glasstone, S., Laidler, K.J. and Eyring, H. (1941) The Theory of Rate Processes, McGraw-Hill, New York

2 Zwolinski, B.J., Eyring, H. and Reese, C.E. (1949) J. Phys. Chem. 53, 1426—1453

3 Woodbury, J.W. (1971) in Chemical Dynamics, Papers in Honor of Henry Eyring (Hirschfelder, J., ed.), pp. 601—617, John Wiley and Sons, New York

4 Läuger, P. (1973) Biochim. Biophys. Acta 311, 423—441

5 Hille, B. (1975) J. Gen. Physiol. 66, 535—560

6 Sandblom, J., Eisenman, G. and Neher, E. (1976) J. Membrane Biol. 31, 383—417

7 Urban, B.W. and Hladky, S.B. (1979) Biochim. Biophys. Acta 554, 410—429

8 Frauenfelder, H., Petsko, G.A. and Tsernoglu, D. (1979) Nature 280, 558—563

9 McCammon, J.A., Wolynes, P.G. and Karplus, M. (1979) Biochemistry 18, 927—942

10 Huber, R., Deisenhofer, J., Colman, P.M., Matsushima, M. and Palm, W. (1976) Nature 264, 415—420

11 Wagner, G. and Wüthrich, K. (1978) Nature 275, 247—248

12 Williams, R.J.P. (1978) Proc. R. Soc. B200, 353—389

13 Munro, I., Pecht, I. and Stryer, L. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 56—60

14 Austin, R.H., Beeson, K.W., Eisenstein, L., Frauenfelder, H. and Gunsalus, I.C. (1975) Biochemistry 14, 5355—5373

15 Jonscher, A.K. (1977) Nature 267, 673—679

16 Hill, T.L. (1960) Introduction to Statistical Thermodynamics, Chapter 11, Addison-Wesley, Reading

17 Starzak, M.E. (1973) J. Membrane Biol. 13, 37—60

18 Frehland, E. (1979) Biophys. Struct. Mech. 5, 91—106

19 Frehland, E. and Läuger, P. (1974) J. Theor. Biol. 47, 189—207

20 Skrabal, A. (1930) Z. Physik. Chem. 6, 382—422

21 Weber, J. (1965) in Molecular Biophysics (Pullman, B. and Weissbluth, M., eds.), pp. 369—396, Academic Press, New York

22 Urry, D.W. (1971) Proc. Natl. Acad. Sci. U.S.A. 68, 672—676

23 Hladky, S.B. and Haydon, D.A. (1972) Biochim. Biophys. Acta 274, 294—312

24 Bamberg, E. and Läuger, P. (1973) J. Membrane Biol. 11, 177—194

25 Neher, E., Sandblom, J. and Eisenman, G. (1978) J. Membrane Biol. 40, 97—116

26 Andersen, O.S. (1980) Acta Physiol. Scand. Suppl. 481, 27—35

27 Urry, D.W., Venkatachalam, C.M., Spisni, A., Läuger, P. and Khaled, M.A. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 2028—2032

28 Schagina, L.V., Grinfeldt, A.E. and Lev, A.A. (1978) Nature 273, 243—244

29 Andersen, O.S. (1975) 5th Int. Biophys. Congress, Copenhagen, p. 369

30 Hille, B. (1975) J. Gen. Physiol. 66, 535—560

31 Cahalan, M. and Begenisich, T. (1976) J. Gen. Physiol. 68, 111—125

32 French, R.J. and Wells, J.B. (1977) J. Gen. Physiol. 70, 707—724

33 Adelman, W.J., Jr. and French, R.J. (1978) J. Physiol. 276, 13—25

34 Hille, B. and Schwarz, W. (1978) J. Gen. Physiol. 72, 409—442

35 Kolb, H.-A. and Bamberg, E. (1977) Biochim. Biophys. Acta 464, 127—141

36 Stühmer, W. and Conti, F. (1979) in Annual Meeting of the Deutsche Gesellschaft für Biophysik, Konstanz 1979 (Adam, G. and Stark, G., eds.), p. 84, Springer-Verlag, Berlin

37 Stark, G., Ketterer, B., Benz, R. and Läuger, P. (1971) Biophys. J. 11, 981—994

38 Frehland, E. and Stephan, W. (1979) Biochim. Biophys. Acta 553, 326—341