Biochimica et Biophysica Acta, 1111 (1992) 81–92

BBAMEM 75772

A channel model with fluctuating barrier structures

J. Nieto-Frausto [1] and B. Kleutsch

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

(Received 30 June 1992)

Key words: Ionic channel; Conformational state; Barrier structure; Dipolar group; Rate-theory analysis; Conductance; Selectivity

Following the theory 'Fluctuations of barrier structure in ionic channels' (Läuger, P., Stephan, W. and Frehland, E. (1980) Biochim. Biophys. Acta 602, 167–180), we constructed a model of a channels with several conformational states. The origin of these conformational states and the source for the transitions from one to the other are given explicitly for the presented model. In this work the effect of multiple conformational states on the ion transport process is analyzed. We considered a channel protein with two main barriers and one binding site. The site is surrounded by dipolar groups. The dipole moment of these groups can be reoriented by thermal activity and also by electrical interaction with the transported ions. Differently polarized states generate different activation energy barriers for the ions. The set of conformational states of the channel is constituted by all the possible polarized states of the binding site. Using the rate-theory analysis of ion transport (Glässtone, S., Laider, K.J. and Eyring, H. (1941) The theory of rate processes, McGraw-Hill, New York), the possible coupling between ion flux and the channel conformational transitions has been incorporated into the model by considering the dependence of the rate constants on the heights of the energy barriers. The resulting multistate kinetic equations have been solved numerically. It was shown that the simple saturation characteristic of the flux-concentration curve was obtained. For certain values of the model parameters, the channel shows a strongly different conductance for anions compared to cations. In fact, the model contains an interesting mechanism that exhibits selectivity with respect to the charge of the ions.

Introduction

The theory of fluctuating barriers [1] is based on the experimental evidence that proteins can exist in a large number of conformational states and may undergo transitions from one state to the other in a broad spectrum of time scales [2,3]. Therefore one should expect that ions passing through a channel protein have to overcome fluctuating energy barriers. These fluctuations could have significant interactions with the flux of ions crossing the channel. This is especially true when some particular mechanisms exist by which the transported ions induce conformational transitions on the protein. Such a mechanism is proposed in this model.

A general treatment of systems with coupling between transport and conformational transitions is a complex problem. Therefore we restrict the analysis to a channel having two barriers, one binding site, and several conformational states.

If the interconversions of channel states is much faster than ion transfer over the barriers, 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. But this is no longer true when the rates of both processes become comparable [1]. In this case a jump of an ion is followed by a slow transition to a more polarized state of the site, and the conductance of the channel could exhibit a depedence on ion concentration which is different from a simple saturation behavior [1]. If there is a preferred orientation of the dipolar groups, due to internal steric restrictions, the result would be a different conductance for anions compared to cations.

Description of the model

Fig. 1 is a schematic representation of the model. The transversal section of the channel-forming membrane-embedded protein is shown in Fig. 1a. In the left aqueous phase (interior or cytoplasmic side) a concentration c' of ions exist, and the potential has the value ψ'. In the right aqueous phase (exterior side) the concentration and the potential are c'' and ψ'', respectively. The protein has two wide entrance vestibules at

---

Correspondence to: J. Nieto-Frausto, Departamento de Física, CIEA IPN, Apdo Postal 14-740, México 07300 D.F., México.

[1] Permanent address: Departamento de Física, Centro de Investigación y de Estudios Avanzados del IPN, Apdo Postal 14-740, México 07300 DF., México.

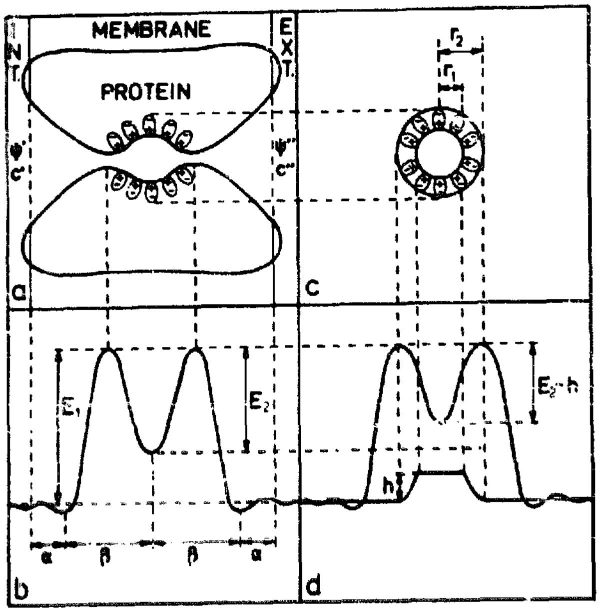


Fig. 1. (a) Transversal section of the channel-forming membrane-embedded protein. It has two wide entrance vestibules, one on each side of the membrane. The narrow central part is the binding site formed by a moiety surrounded by dipolar groups. The interior (or cytoplasmic) aqueous phase has an ion concentration c' and a potential ψ'. For the exterior aqueous phase the corresponding quantities are c'' and ψ''. (b) Potential energy profile offered by the channel to the ions. It does not contain the contribution of the polarized state of the binding site. E_1 and E_2 are the barrier heights which have to be overcome by the ion in order to leave or to enter into the site, respectively. α and β are the fractions of the membrane spanned by the indicated regions of the energy profile. (c) Approximated form of the ion binding site. In order to calculate its contribution to the total energy profile, the moiety is considered to be formed by two concentric spheres of radius r_1 and r_2, containing the dipoles within them. (d) The profile of height h (lower part) is the energy contribution of the polarized binding site. The upper profile is obtained by adding up this contribution and that in (b). This is the total energy profile. Note that the only effect due to the polarization is to change the height of the minimum in (b). This height is increased or decreased depending on the sign of h, which depends on the particular polarized states of the binding site (see text).

both ends of the channel and a narrow middle part which contains the binding site. The binding site is formed by a moiety surrounded by dipolar groups which can be reoriented.

The mechanism for the interaction between the ions and the dipolar groups may be described in the following way. When an ion jumps into the moiety creates a strong coulombic field on the neighborhood, this field tends to reorient the dipolar groups of the protein. The reorientation shifts the energy level of the binding site and the height of adjacent barriers. If the rate for conformational change induced by the ion is comparable to or smaller than the jump rate, the ion may leave the site before the protein structure has relaxed the polarized state [5]. Thus, when the ion has left the site, a certain time is required for the channel to return to the original conformation, and the next entering ion may find the structure still in a partly polarized state.

Fig. 1b represents the energy profile encountered by the ions on their pathway through the channel (to simplify this figure and the following explanation, we have taken the case $\psi' = \psi''$). The origin of these energy barriers may be easily understood from the geometry of the channel: If an ion, having a diameter slightly greater or similar than the narrow parts of the channel, intent to cross it from left to right, the ion has to remove part of its hydration shell, pass the first narrow entrance (first maximum), be trapped into the moiety (minimum), overcome the narrow exit (second maximum), and finally be released and rehydrated in the opposite side. This energy profile does not contain the contribution from the polarized state of the site, but includes all the others interactions of the ion with water, membrane, and protein. This profile is assumed symmetrical, with α and β defined as the fractions of the membrane thickness spanned by the indicated parts of the energy profile ($2(\alpha + \beta) = 1$). The contribution from an external potential $U_m = \psi' - \psi''$ (case $\psi' \neq \psi''$) will be considered below (here we use the constant field approximation).

In order to include in the energy profile the contribution from the dipolar groups, it is assumed that the binding site can be approximated by the dipolar array shown in Fig. 1c. This array is formed by two concentratic spheres with radius r_1 and r_2, respectively, containing n dipoles uniformly distributed within them. We suppose that each dipole has only two possible orientations: 'up', when their dipole moment points to the center of the moiety, and 'down', when pointing opposite to this center. In the polarized state represented in Fig. 1c, all the dipoles are 'up'.

An arbitrary polarized state of the site will be characterized by a number u of dipoles 'up' and $n-u$ dipoles 'down'. Here, to make the model tractable, we assume that it is unimportant which particular dipoles are 'up' and which are 'down', only being important how many of them are in each of this two states. Therefore, if the site contains n dipoles, there are only $n + 1$ different polarized states. The set of these $n + 1$ polarized states constitutes all the conformational states of the channel protein.

In the polarized state shown in Fig. 1c ('all up'), the energy profile offered by the binding site to a positive ion traversing the channel, is calculated as follows (see details below): Assume that the positive charges of the dipoles 'up' are distributed uniformly on the internal sphere, and their negative charges on the external one. Let h be the difference of electric potential between these two uniformly charged spheres. The resulting profile is shown in the lower part of Fig. 1d. If the polarized state is arbitrary (u dipoles 'up' and $n-u$ 'down'), the procedure to calculate h is similar: just

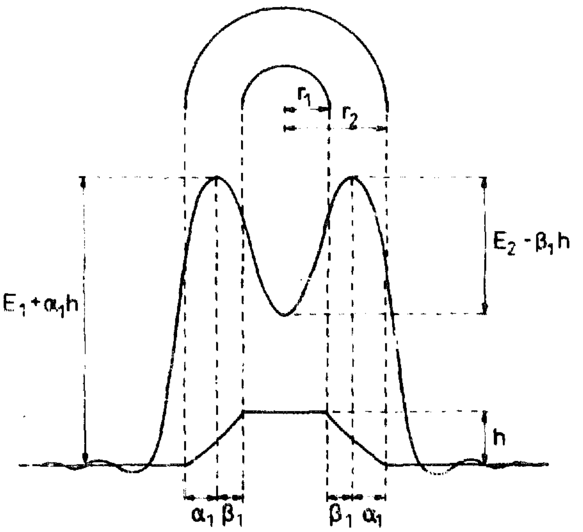


Fig. 2. A more detailed geometry concerning the positions of the dipolar groups with respect to the positions of the maxima and minimum of the energy profile. α_1 and β_1 are the fractions of the distance r_1-r_2 spanned by the indicated parts of the energy profile ($\alpha_1+\beta_1=1$). h is the energy contribution generated by the polarized binding site. The effect produced by the polarization is to shift both, the height of the minimum (E_2 becomes $E_2-\beta_1h$) and the heights of the maxima (E_1 becomes $E_1-\alpha_1h$). Implicit is the assumption that the electric potential between the spheres forming the moiety is approximately linear. The geometry shown in Fig. 1d corresponds to the case $\alpha_1=0$ ($\beta_1=1$).

distribute uniformly on the two spheres the charges of the 'heads' and 'tails' of each of the corresponding dipoles. In this case the value of h could be positive or negative, depending on the numbers u and n. Note that h is zero if n is even and $n/2$ dipoles are 'up'.

Adding up the energy profile of the polarized state (lower part of Fig. 1d) together with the profile of Fig. 1b, we get the upper part of Fig. 1d. This is the total energy profile encountered by the ions traversing the channel. Note that the only effect produced by the polarization is to shift the height of the minimum between the barriers (E_2 becomes E_2-h), increasing or decreasing it depending on the sign of h. The height E_1 of the maxima remain unchanged. This fact is a direct consequence of the geometry assumed in Fig. 1, that is, the positions of the dipolar groups with respect to the positions of the maxima and minimum of the energy profile.

A more general geometry concerning the positions of the dipolar groups with respect to the positions of the maxima and minimum of the energy profile is shown in Fig. 2. Here α_1 and β_1 are defined as the fractions of the distance r_1-r_2 spanned by the indicated parts of the energy profile ($\alpha_1+\beta_1=1$). In this situation, the effect produced by the polarization is to shift both, the height of the minimum (E_2 becomes $E_2-\beta_1h$) and the heights of the maxima (E_1 becomes $E_1-\alpha_1h$). Implicit in this new values of the heights is the assumption that the electric potential between the spheres forming the moiety is approximately linear. The geometry shown in Fig. 1 corresponds to the case $\alpha_1=0$ ($\beta_1=1$).

The geometry shown in Fig. 2 physically corresponds to the situation in which the narrow parts of the channel are located in some position between the charges of the nearest dipoles (see Fig. 1a). That is, in Fig. 1a the narrow parts of the channel are very close to the negative charge of the nearest dipoles, therefore $\alpha_1=0$. For a situation in which $\alpha_1=1$, the narrow parts of the channel would be located very close to the positive charge of the same dipoles. We will see below that the value of α_1 plays a critical role on the charge selectivity of the channel.

The contribution from an external potential $U_m=\psi'-\psi''$ will be considered as linear (assuming the constant field approximation). Thus, the fractions α and β of the membrane thickness (Fig. 1b) provide the fractions of U_m which contribute to the change in the heights of the energy profile. This fact will be used when considering the explicit expressions for the rate constants below.

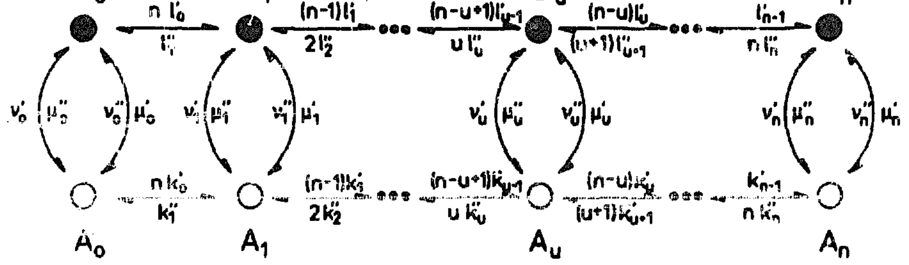


Fig. 3. Kinetic scheme for the transport reactions in the channel. n is the total number of dipoles in the binding site. There are $2(n+1)$ different states of the channel, A_u and B_u, with u being the number of dipoles 'up' ($u=0, 1, \dots, n$). A_u are the states of the channel when the moiety is empty, and B_u when it is occupied. The quantities ν_u and μ_u are the rate constants for an ion to jump into the moiety and out of the moiety, respectively. k_u and l_u are the rate constants for turning a dipole when the moiety is empty and occupied, respectively. (Explicit expressions for the rate constants are given in the text).

States of the channel and rate constants

The channel protein with n dipoles in the binding site may exist in $2(n+1)$ distinct states. These states depend on the orientation of the dipoles and on the occupancy of the site:

A_u (binding site empty, and u dipoles 'up')

B_u (binding site occupied, and u dipoles 'up')

for $u = 0, 1, 2, \ldots, n$.

We assume that only one dipole can turn its orientation at a time, in a single step, and, that this interconvertion is much faster than the ion translocation. This means that an ion crossing one barrier finds the barrier in a well defined state. Under these conditions the kinetic scheme of the system is depicted in Fig. 3. The rate constants for transitions between the states are also indicated in the figure, and are defined as follows (when u dipoles are 'up').

ν'_u, ν''_u: rate constants for an ion to jump from the left, and from the right, respectively, into the moiety.

μ'_u, μ''_u: rate constants for an ion to jump to the right, and to the left, respectively, out of the moiety.

l'_u, l''_u: rate constants for turning one dipole "down" to "up", and "up" to "down", respectively, in the occupied state.

k'_u, k''_u: rate constants for turning one dipole "down" to "up", and "up" to "down", respectively, in the empty state.

Schematically:

$$\text{ion in left phase} \underset{\mu''_u}{\overset{\nu'_u}{\rightleftharpoons}} \text{ion in moiety} \underset{\nu''_u}{\overset{\mu'_u}{\rightleftharpoons}} \text{ion in right phase}$$

$$\text{dipole 'down'} \underset{l''_u}{\overset{l'_u}{\rightleftharpoons}} \text{dipole 'up' (occupied state)}$$

$$\text{dipole 'down'} \underset{k''_u}{\overset{k'_u}{\rightleftharpoons}} \text{dipole 'up' (empty state)}$$

In Fig. 3, the line connecting B_u with B_{u+1} represents the transitions between the dipolar states when the site is occupied. The one connecting A_u with A_{u+1} represents the same transitions when the site is empty. The factors multiplying the rate constants l'_u, l''_u, k'_u, and k''_u, account for the fact that, in the indicated transition, anyone of the corresponding dipoles could be turned. The two curved lines connecting B_u with A_u, represent the interchange of an ion between the site and the aqueous phase of the left side (left curved line), or of the right one (right curved line). Note that the channel protein can be found in just one state at a time, and no more than one ion can occupy the moiety simultaneously.

The ions crosses the channel in both directions, jumping from one phase to the moiety and then to the other phase. Also, they can go back to the aqueous phase from which they jumped in the previous step. At a given time, the net flow $\phi(t)$ (from left to right in Fig. 1a) may be obtained from the net number of jumps over a given barrier.

The jump rates ν'_u and ν''_u of ions into the empty site depend on the aqueous ion concentrations c' and c'', and may be written in the form: $\nu'_u = c'\rho'_u$ and $\nu''_u = c''\rho''_u$, where ρ'_u and ρ''_u are concentration-independent quantities [7]. The jump rates of ions out of the site, μ'_u and μ''_u, may be assumed to be true monomolecular rate constants. Expressions for ρ'_u, ρ''_u, μ'_u, μ''_u, and the other rate constants are given below.

Finally, we are going to assume that the only quantities which are dependent on the external potential $U_m = \psi' - \psi''$, are those which involve the movement of the ion, that is, ρ'_u, ρ''_u and μ''_u. Then, it is considered that the necessary energy for turning a dipole is not affected by the presence of an external electric field. This is true for those dipoles which move in a plane perpendicular to this field, but not for those which move out of this plane (although some cancelation effects could be expected because of the symmetry of the moiety). In any case, our results will be valid for small values of U_m (see Eqn. 13). The main reason for doing this approximation is that, otherwise, it could result in a large complication of the energy levels and the kinetic scheme. This would be subject of a further improvement of the model.

Kinetic equations and ion transport

The mere use of rate constants gives to the model a stochastic character. This comes from the definition of a rate constant as the conditional probability for a transition to occur in the system, or, equivalently, as the average frequency of jumps over a barrier in one direction given that an ion was in the previous minimum. Then, the transport of ions through the channel is not a deterministic process, and it is necessary to use time-dependent probabilities in the description.

The dynamics of the channel is described in terms of random transitions between the conformational states. At time t, the relevant quantities are then the probabilities, $A_u(t)$ and $B_u(t)$, to find the channel in the states A_u and B_u, respectively. We use the same symbol to denote the state and its probability. The time evolution of these probabilities is given by the following set of linear kinetic equations:

$$\frac{\mathrm{d}A_0}{\mathrm{d}t} = -(nk'_0 + \nu_0)A_0 + k''_1 A_1 + \mu_0 B_0 \qquad (1)$$

$$\frac{\mathrm{d}A_1}{\mathrm{d}t} = nk'_0A_0 - (k''_1 + (n-1)k'_1 + \nu_1)A_1 + 2k''_2A_2 + \mu_1B_1 \tag{2}$$

$$\vdots$$

$$\frac{\mathrm{d}A_u}{\mathrm{d}t} = (n-u+1)k'_{u-1}A_{u-1} - (uk''_u + (n-u)k'_u + \nu_u)A_u$$

$$+ (u+1)k''_{u+1}A_{u+1} + \mu_uB_u \tag{3}$$

$$\vdots$$

$$\frac{\mathrm{d}A_{n-1}}{\mathrm{d}t} = 2k'_{n-2}A_{n-2} - ((n-1)k''_{n-1} + k'_{n-1} + \nu_{n-1})A_{n-1}$$

$$+ nk''_nA_n + \mu_{n-1}B_{n-1} \tag{4}$$

$$\frac{\mathrm{d}A_n}{\mathrm{d}t} = k'_{n-1}A_{n-1} - (nk''_n + \nu_n)A_n + \mu_nB_n \tag{5}$$

and the corresponding equations for $B(t)$:

$$\frac{\mathrm{d}B_0}{\mathrm{d}t} = -(nl'_0 + \mu_0)B_0 + l''_1B_1 + \nu_0A_0 \tag{6}$$

$$\frac{\mathrm{d}B_1}{\mathrm{d}t} = nl'_0B_0 - (l''_1 + (n-1)l'_1 + \mu_1)B_1 + 2l''_2B_2 + \nu_1A_1 \tag{7}$$

$$\vdots$$

$$\frac{\mathrm{d}B_u}{\mathrm{d}t} = (n-u+1)l'_{u-1}B_{u-1} - (ul''_u + (n-u)l'_u + \mu_u)B_u$$

$$+ (u+1)l''_{u+1}B_{u+1} + \nu_uA_u \tag{8}$$

$$\vdots$$

$$\frac{\mathrm{d}B_{n-1}}{\mathrm{d}t} = 2l'_{n-2}B_{n-2} - ((n-1)l''_{n-1} + l'_{n-1} + \mu_{n-1})B_{n-1}$$

$$+ nl''_nB_n + \nu_{n-1}A_{n-1} \tag{9}$$

$$\frac{\mathrm{d}B_n}{\mathrm{d}t} = l'_{n-1}B_{n-1} - (nl''_n + \mu_n)B_n + \nu_nA_n \tag{10}$$

where, $\mu_u \equiv \mu'_u + \mu''_u$ and $\nu_u \equiv \nu'_u + \nu''_u$.

The system of linear equations has to be solved together with the probability conservation condition:

$$\sum_{u=0}^{n} [A_u(t) + B_u(t)] = 1 \tag{11}$$

and the given initial probabilities $A_u(0)$, $B_u(0)$ for $u = 0, 1, \ldots, n$.

Solving the system of equations, one finds the quantities $A_u(t)$ and $B_u(t)$ which are expressions containing the time t and the set of rate constants. The rate constants are functions involving the ion concentrations, the external potential U_m, the temperature, and the parameters of the channel and the ion. Explicit expressions for the rate constants will be given in the next section.

The net flow of ions through the channel, $\phi(t)$, may be obtained by summing over all transitions in which an ion jumps over a given barrier. For the left-hand barrier this gives:

$$\phi(t) = \sum_{u=0}^{n} [\nu'_uA_u(t) - \mu''_uB_u(t)] \tag{12}$$

An analogous equivalent expression for $\phi(t)$ is obtained from the right-hand barrier.

If we have an ensemble of N identical channels embedded in the membrane, and they are prepared according to a given initial state, the total flow through the membrane is simply $\Phi(t) = N\phi(t)$.

By solving the system of equations under steady-state conditions ($\mathrm{d}A_u/\mathrm{d}t = 0$, $\mathrm{d}B_u/\mathrm{d}t = 0$), one finds the stationary flux $\Phi = \Phi(\infty)$. From this flux, the ohmic single-channel conductance, Λ, may be calculated. This quantity is defined by:

$$\Lambda = \frac{1}{N}\lim_{U_m \to 0}\left[\frac{Ze_0\Phi}{U_m}\right]_{c'=c''=c} \tag{13}$$

Note 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 resulting from conformational transitions may be directly observed. Such a limiting case is given by a channel with open-closed kinetics.

Explicit expressions for the rate constants

In order to write their explicit expressions for the rate constants, we are going to calculate the height h of the energy profile offered by the binding site to an ion crossing the channel. This will be done with the procedure described above, that is, using a uniform distribution of charge on the spheres forming the moiety.

Let the positive and negative charges on one dipole be se_0 and $-se_0$, respectively, where e_0 is the elementary charge, and s is a positive quantity (for the numerical calculations, a value of $s = 0.25$ was chosen, this correspond to the dipolar groups in the gramicidin A channel [6]). When u dipoles are 'up' and $n-u$ 'down', the charges on the spheres forming the moiety would be $q_1 = (2u-n)se_0$ and $q_2 = -(2u-n)se_0$, respectively. From elementary electrostatic calculations, the value of h, which is the difference of energy between the two spheres times the charge of the ion, is found to be

$$h = \frac{(Ze_0)\tau}{4\pi\epsilon\epsilon_0(r_1 r_2)}(2u - n) \tag{14}$$

where Z is the valency of the ion, $\tau \equiv (se_0)(r_1 - r_2)$ is the dipole moment, ϵ_0 the permittivity of the free space, and ϵ the dielectric constant of the medium between the ion and the dipolar groups.

In the expressions for the rate constants, the energy h will be written in the form $h/k_BT = (2u - n)f$, where k_s is the Boltzmann's constant, T is the absolute temperature, and f is a dimensionless quantity defined by

$$f = \frac{(Ze_0)\tau}{4\pi\epsilon\epsilon_0(r_1 r_2)} \frac{1}{k_B T} \tag{15}$$

It is also necessary to know the energy to turn a dipole when u dipoles are 'up' and $n - u$ 'down'. This energy is closely related to h, because h/Ze_0 is the energy required to interchange a unity of charge between the two spheres forming the moiety. The process for turning a dipole is equivalent to the process for interchanging the charges se_0 and $-se_0$ between the two spheres. The corresponding required energy is simply given by $2se_0(h/Ze_0)$. We define a dimensionless quantity g:

$$g \equiv 2sf/Z \tag{16}$$

which will be used in the energy dependence of the corresponding rate constants.

Now, using the rate theory analysis of ion transport, and according to the assumptions and approximations we have made above, the explicit expressions for the jump rates of ions, when there exists an external potential $U_m = \psi' - \psi''$, are given by:

$$\nu'_u = c'\rho'_u, \quad \nu''_u = c''\rho''_u \tag{17}$$

$$\rho'_u = \bar{\rho}'_u \exp((\alpha + \beta/2)u_m), \quad \text{with } \bar{\rho}'_u = \bar{\rho}'_{n/2} \exp(-\alpha_1(2u - n)f) \tag{18}$$

$$\rho''_u = \bar{\rho}''_u \exp(-(\alpha + \beta/2)u_m), \quad \text{with } \bar{\rho}''_u = \bar{\rho}''_{n/2} \exp(-\alpha_1(2u - n)f) \tag{19}$$

$$\mu'_u = \bar{\mu}'_u \exp((\beta/2)u_m), \quad \text{with } \bar{\mu}'_u = \bar{\mu}'_{n/2} \exp(\beta_1(2u - n)f) \tag{20}$$

$$\mu''_u = \bar{\mu}''_u \exp(-(\beta/2)u_m), \quad \text{with } \bar{\mu}''_u = \bar{\mu}''_{n/2} \exp(\beta_1(2u - n)f) \tag{21}$$

where u_m is the dimensionless external potential defined by

$$u_m = Ze_0U_m/(k_BT) \tag{22}$$

and, $\bar{\rho}'_{n/2}$, $\bar{\rho}''_{n/2}$, $\bar{\mu}'_{n/2}$, and $\bar{\mu}''_{n/2}$ are constants independent of u and u_m, they only depend on the undisturbed internal potential profile when there is no external potential U_m and there is no polarization in the site ($u = n/2$). Note that because of the assumed symmetry of the potential energy profile, the equalities: $\bar{\rho}'_{n/2} = \bar{\rho}''_{n/2}$ and $\bar{\mu}'_{n/2} = \bar{\mu}''_{n/2}$ are fulfilled. The factors containing α, β, α_1 and β_1 in the exponentials, accounts for the fractions of u_m and f that contribute to the change in the height of the energy barriers (see Figs. 1b and 2).

Similarly, the rate constants for turning the dipoles are given by:

$$k'_u = \bar{k}'_{n/2} \exp(-(2u - n)g/2), \quad k''_u = \bar{k}''_{n/2} \exp((2u - n)g/2) \tag{23}$$

$$l'_u = \bar{l}'_{n/2} \exp(-(2u - n)g/2), \quad l''_u = \bar{l}''_{n/2} \exp((2u - n)g/2) \tag{24}$$

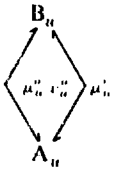
where $\bar{k}'_{n/2}$ and $\bar{k}''_{n/2}$ are constants independent of u, and they only depend on the internal energy barrier of the binding site (not shown) when there is no polarization ($u = n/2$). The dimensionless energy g is defined in Eqn. 16. The factor $1/2$ in the exponentials takes the implicit assumption that the internal energy barrier of the site is symmetrical. It also assumes that the difference of potential between the spheres is approximately linear (see the lower part of Figs. 1d and 2). On the other hand, the quantities $\bar{l}'_{n/2}$ and $\bar{l}''_{n/2}$ are related with $\bar{k}''_{n/2}$ and $k''_{n/2}$ because the former are obtained from the later when considering their modification due to the presence of an ion into the moiety. If this ion only contributes with its electric field to modify the internal barrier of the site, the energy required to turn a dipole is increased by the quantity $2(f \cdot k_BT)/2$ as may be seen from elementary electrostatic claculations (the factor 2 in the numerator accounts for the two changes of a dipole, and that in the denominator accounts for the symmetrical internal barrier of the site). Then, the quantities $\bar{l}'_n/2$ and $l''_{n/2}$ are given by the following expressions:

$$\bar{l}'_{n/2} = \bar{k}'_{n/2} \exp(-f), \quad \text{and } \bar{l}''_{n/2} = \bar{k}''_{n/2} \exp(f) \tag{25}$$

Detailed balance

If the channel model constructed here has to be consistent with the microscopic reversibility, then, in the equilibrium state the set of rate constants have to satisfy the requirement of the detailed balance. That is, in any closed circuit taken in Fig. 3, the forward product (counterclockwise) of the involved rate constants should be equal to the backward product. Let us check this requirement for some closed circuits of the reaction scheme.

Take the circuit:



The detailed balance requires: $(\nu''_u\mu''_u)/(\nu'_u\mu'_u) = 1$. Substituting the expression for the rate constants given in Eqns. 17–22, we get:

$$\frac{\nu''_u\mu''_u}{\nu'_u\ \mu'_u} = \frac{c''\rho''_u\mu''_u}{c'\rho'_u\mu'_u} = \{c''\bar{\rho}''_{n/2}\exp(-(\alpha+\beta/2)u_{\mathrm{m}})$$

$$\times\exp(-\alpha_1(2u-n)f)\bar{\mu}''_{n/2}\exp(\beta_1(2u-n)f)$$

$$\times\exp(-(\beta/2)u_{\mathrm{m}})\}$$

$$\times\{c'\bar{\rho}'_{n/2}\exp((\alpha+\beta/2)u_{\mathrm{m}})$$

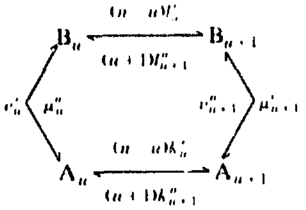
$$\times\exp(-\alpha_1(2u-n)f)\bar{\mu}'_{n/2}$$

$$\times\exp(\beta_1(2u-n)f)\exp((\beta/2)u_{\mathrm{m}})\}^{-1}$$

$$= \frac{c''\bar{\rho}''_{n/2}\bar{\mu}''_{n/2}}{c'\bar{\rho}'_{n/2}\bar{\mu}'_{n/2}}\exp(-u_{\mathrm{m}}) = \exp(u_0 - u_{\mathrm{m}}) = 1$$

where we have used the relations $\bar{\rho}'_{n/2} = \bar{\rho}''_{n/2}$, $\bar{\mu}'_{n/2} = \bar{\mu}''_{n/2}$, $2(\alpha+\beta) = 1$, and $c'' = c'\exp(u_0)$, with u_0 being the Nernst potential. The Nernst potential u_0 is equal to u_{m} in equilibrium.

Take now the closed circuit:



the requirement of the detailed balance is

$$\frac{\nu''_{u+1}(u+1)l''_{u+1}\mu''_u(n-u)k'_u}{\nu'_u(n-u)l'_u\mu'_{u+1}(u+1)k''_{u+1}} = 1$$

substituting the expressions of Eqns. 17–25 for the rate constants involved here, it is found again that the left hand term, reduces to $\exp(u_0 - u_{\mathrm{m}})$ which is unity at equilibrium ($u_0 = u_{\mathrm{m}}$).

Similarly, it can be shown that the requirement of the detailed balance is fulfilled in every closed circuit chosen from Fig. 3. Therefore, the model is consistent with the microscopic reversibility. Note that, in a non-equilibrium state, the quantity $\exp(u_0 - u_{\mathrm{m}})$ is the driving force for the cycles in the system.

Solution of the system of kinetic equations

We are interested in the behavior of the ohmic single-channel conductance, Λ, for different values of the model parameters. Therefore, we have to determine the stationary solution of the system of linear kinetic equations. The method used to find this solution for arbitrary value of n, was a numerical one assigning explicit values to the parameters of the model. In addition, the solutions can be bound analytically for $n = 0$ and $n = 1$, and because they are not too complicated their general characteristics may be analyzed in detail In case $n = 0$, the model correspond to a rigid pore which has been well studied before [7]. In case $n = 1$, the model represents a channel with two conformational states, and it has been treated in general in Ref. 1. As mentioned before, the general treatment considers the conformational states as abstract states of a channel protein, and it is not assumed any particular mechanism for the conformational transitions induced by the ions. In the following, we derive some of those results adapting them to the present model.

For $n = 1$, only one dipolar group exists in the site. It is clear that, in this case, the method of uniformly charged spheres that we used to calculate the energy profile of the site in the polarized state, is very rude, and it has to be considered only as a gross approximation. Nevertheless, as will be shown later, the main features of the model for $n > 1$, are found also in this case.

For one dipole in the site, the channel protein may exist in four distint states: A$_0$, A$_1$, B$_0$ and B$_1$ (see Fig. 3, with $n = 1$). The system of kinetic equations (Eqns. 1–11) consists of four equations with four unknowns. As explained before, in steady state, the net flow of ions through a membrane having embedded N identical channels, is given by $\Phi = N\phi$, being ϕ the net flow per channel (see Eqn. 12):

$$\phi = [\nu'_0 A_0 - \mu''_0 B_0] + [\nu'_1 A_1 - \mu''_1 B_1] \tag{26}$$

where A_0, A_1, B_0, and B_1 are here the stationary probabilities for the corresponding states. These probabilities are found to be:

$$A_0 = [\mu_0 l''_1(\nu_1 + k''_1) + \nu_1 k''_1(\mu_0 + l''_0)]/\sigma \tag{27}$$

$$A_1 = [\mu_1 l'_0(\nu_0 + k'_0) + \mu_0 k'_0(\mu_1 + l''_1)]/\sigma \tag{28}$$

$$B_0 = [\nu_1 l''_1(\nu_0 + k'_0) + \nu_1 k''_1(\mu_1 + l''_1)]/\sigma \tag{29}$$

$$B_1 = [\nu_0 l'_0(\nu_1 + k''_1) + \nu_1 k'_0(\mu_0 + l'_0)]/\sigma \tag{30}$$

where $\mu_0 \equiv \mu'_0 + \mu''_0$, $\mu_1 \equiv \mu'_1 + \mu''_1$, $\nu_0 \equiv \nu'_0 + \nu''_0$, $\nu_1 \equiv$

$\nu'_1 + \nu''_1$, and, the denominator σ is defined by:

$$\begin{aligned}\sigma = {} & (k''_1 + k'_0)(\mu_0\mu_1 + \mu_0 l''_1 + \mu_1 l'_0) \\ & + (l''_1 + l'_0)(\nu_0\nu_1 + \nu_0 k''_1 + \nu_1 k'_0) \\ & + \nu_0\mu_1(k''_1 + l'_0) \\ & + \nu_1\mu_0(l''_1 + k'_0) \qquad (31)\end{aligned}$$

Inserting Eqns. 17, 27–31 into Eqn. 26, we get:

$$\phi = \frac{\gamma}{\sigma}[1 - \exp(u_0 - u_m)] \qquad (32)$$

with,

$$\begin{aligned}\gamma = {} & \nu'_0\mu'_0(\nu_1 l''_1 + k''_1 l''_1 + \mu_1 k''_1) + \nu'_1\mu'_1(\nu_0 l'_0 + k'_0 l'_0 + \mu_0 k'_0) \\ & + \nu'_0\mu'_1 k''_1 l'_0 + \nu'_1\mu'_0 l''_1 k'_0 \qquad (33)\end{aligned}$$

From this flux, the signle-channel conductance can be calculated using Eqn. 13 (with $\Phi = N\phi$):

$$\Lambda = \lim_{U_m \to 0}\left[\frac{Ze_0\phi}{U_m}\right]_{c' = c'' = c} \qquad (34)$$

this limit yields Λ as a function of the ion concentration c:

$$\Lambda(c) = \frac{(Ze_0)^2}{k_B T}\cdot\frac{c(D + Ec)}{F + Gc + Hc^2} \qquad (35)$$

where,

$$D = \rho'_0\mu'_0 k''_1(\mu_1 + l''_1) + \rho'_1\mu'_1 k'_0(\mu_0 + l'_0) + \rho'_0\mu'_1 k''_1 l'_0 + \rho'_1\mu'_0 l''_1 k'_0 \qquad (36)$$

$$E = \rho'_0\rho_1\mu'_0 l''_1 + \rho'_1\rho_0\mu'_1 l'_0 \qquad (37)$$

$$F = (k''_1 + k'_0)(\mu_0\mu_1 + \mu_0 l''_1 + \mu_1 l'_0) \qquad (38)$$

$$G = (l''_1 + l'_0)(\rho_0 k''_1 + \rho_1 k'_0) + \rho_0\mu_1(k''_1 + l'_0) + \rho_1\mu_0(l''_1 + k'_0) \qquad (39)$$

$$H = \rho_0\rho_1(l''_1 + l'_0) \qquad (40)$$

with $\rho_0 \equiv \rho'_0 + \rho''_0$, $\rho_1 \equiv \rho'_1 + \rho''_1$, $\mu_0 \equiv \mu'_0 + \mu''_0$, and $\mu_1 \equiv \mu'_1 + \mu''_1$. Note that according to the definition of Λ in Eqn. 34 the rate constants appearing in Eqns. 36–40 refer to a voltage $U_m = 0$, (see Eqns. 18–22).

It is seen from Eqn. 35 that $\Lambda(c)$ is a nonlinear function of 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 ($n = 0$) which always exhibits a simple Michaelis-Menten saturation characteristic [7]:

$$\Lambda(c) = \frac{(Ze_0)^2}{k_B T}\cdot\frac{\rho'_0\mu'_0 c}{\mu_0 + \rho_0 c} \qquad (41)$$

As expected, Eqn. 35 reduces to Eqn. 41 in the limit $k'_0 = l'_0 = 0$.

Results

Saturation behavior

(a) Case $n = 1$

In the general treatment given in ref. 1 for a channel with one site and two conformational states, it is pointed out that the difference between the behavior of $\Lambda(c)$ from Eqn. 35 and from Eqn. 41 is clearly manifested using some combinations of rate constants. The conductance in Eqn. 35 goes through a maximum with increasing ion concentration c, whenever the relationship $DH > EG$ is fulfilled. This relationship, which is directly obtained from Eqn. 35, is equivalent to the condition:

$$\begin{aligned}(l''_1 + l'_0)\left[\frac{\mu'_0}{\mu_0} - \frac{\mu'_1}{\mu_1}\right]^2 & \\ > \left[\frac{1}{k''_1} + \frac{1}{k'_0}\right]\left[\frac{\mu'_0\mu''_0}{\mu_0} l''_1 + \frac{\mu'_1\mu''_1}{\mu_1} l'_0\right] & \qquad (42)\end{aligned}$$

Eqn. 42 is a general relation for a channel with one site and two conformational states. That is, every channel model of this type, having rate constants satisfying this relation, should show a maximum in its conductance.

Let us check if the particular channel model treated here satisfy Eqn. 42. Using the expressions for the rate constants given by Eqns. 20–22 (with $U_m = 0$), it is found that,

$$\begin{aligned}\left[\frac{\mu'_0}{\mu_0} - \frac{\mu'_1}{\mu_1}\right] = \Bigg[& \frac{\bar{\mu}'_{1/2}\exp(-f/2)}{\bar{\mu}'_{1/2}\exp(-f/2) + \bar{\mu}''_{1/2}\exp(-f/2)} \\ & - \frac{\bar{\mu}'_{1/2}\exp(f/2)}{\bar{\mu}'_{1/2}\exp(f/2) + \mu''_{1/2}\exp(f/2)}\Bigg] = 0 \qquad (43)\end{aligned}$$

but, the right-hand term in Eqn. 42 is a positive quantity (rate constants are positive quantities). This means that the condition for the existence of a maximum in the conductance $\Lambda(c)$ (Eqn. 35) is not fulfilled by the rate constants of our model with $n = 1$. In other words, a channel with one dipolar group, has a conductance $\Lambda(c)$ which always behaves like a saturation curve. In fact, no matter what the particular values of the rate constants were used (as long as they were consistent with Eqns. 17–25), $\Lambda(c)$ from Eqn. 35 was always well fitted by Michaelis-Menten curves $\Lambda_{max}c/(k_m + c)$. These results are shown in Fig. 4, where the conductance $\Lambda(c)$ (in arbitrary units) is plotted for several sets of parameters of the model. The fitting curves can not be distinguished from the $\Lambda(c)$ curves.

An intuitive explanation for the inverse relation between the conductance and the parameter g, as shown in Fig. 4, can be given with the help of Eqns. 16, 23–25. This equations show that g determines the values of the rate constants l_0' and l_1'' in such a way that l_0' increases and l_1'' decreases as g increases (see the values of the remaining parameters in Fig. (4). This situation determines, partially, a greater probability for the 'all down' (B_0) compared to the 'all up' (B_1) states (see Fig. 3, with $n = 1$). In the B_0 state the corresponding minimum is deeper and, therefore, the average time an ion spends in the site is greater compared with that in B_1. This fact decreases the chance for the ions to enter into the moiety and, consequently, the net flux is decreased. This point will be extended in the section dealing with the selectivity of the channel.

(b) Case n > 1

For a channel with one site and $n + 1$ abstract conformational states, $\Lambda(c)$ can be shown to have the general form [1]:

$$\Lambda(c) = c\frac{P_n(c)}{Q_{n+1}(c)} \tag{44}$$

where $P_n(c)$, and $Q_{n+1}(c)$ are polynomials of n-th and $(n + 1)$-th order, respectively. The concentration dependence of the conductance, as described by this equation, may become rather complicated.

Nevertheless, for the particular model of channel we are treating here, it is found that the conductance always presents a simple saturation behavior. As mentioned before, to find the solution of the steady state for $n > 1$, we solve numerically the system of kinetic equations (Eqns. 1–12). Using this solution, we obtain the conductance $\Lambda(c)$ as given by Eqns. 34 and 12. Even when the numerically determined conductance has to have the general form of Eqn. 44, no complicated dependence on c was observed. For all the values of $n > 1$ studied, and for all the sets of parameters chosen, the conductances obtained by Eqn. 34, were always well fitted by Michaelis-Menten curves.

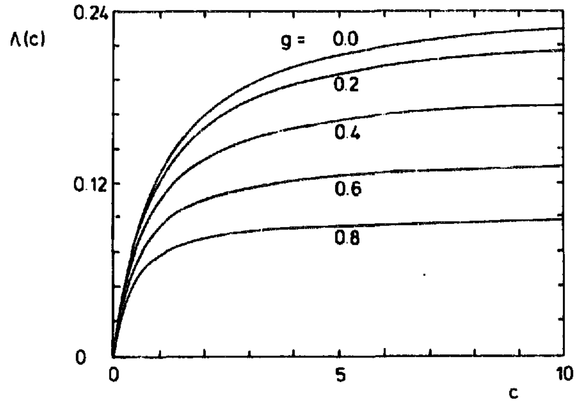


Fig. 4. Conductance $\Lambda(c)$ as a function of the concentration c (arbitrary units). There is only one dipole in the binding site ($n = 1$). The curves are presented for several values of the energy g. The rest of the parameters used in these calculations are: $\alpha = 0.3$, $\alpha_1 = 0$, $s = 0.25$, and $Z = 1$. The values of the rate constants (in s^{-1}) are: $\bar{k}'_{n/2} = 1.0$, $\bar{k}''_{n/2} = 1.0$, $\bar{\rho}'_{n/2} = 1.0$, and $\bar{\mu}'_{n/2} = 1.0$. All the conductances are well fitted by Michaalis-Menten curves $\Lambda_{max}c/(k_m + c)$. The values of the parameters (Λ_{max}, k_m) corresponding to $g = 0.0$, 0.2, 0.4, 0.6, and 0.8 are found to be: (0.25, 1.0), (0.23, 0.92), (0.19, 0.75), (0.14, 0.55) and (0.97, 0.39), respectively.

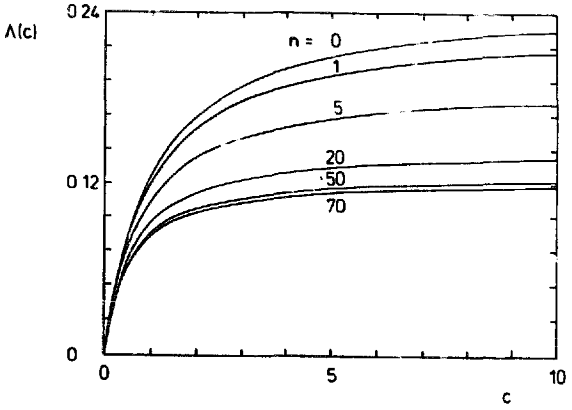


Fig. 5. Conductance $\Lambda(c)$ as a function of the concentration c (arbitrary units). The curves are presented for several numbers n of dipoles in the binding site. The parameters used in these calculations are those in Fig. 4 with $g = 0.2$. All the conductances found with these and many other combinations of parameters are well fitted by Michaelis-Menten curves $\Lambda_{max}c/(k_m + c)$. The parameters (Λ_{max}, k_m) corresponding to $n = 0$, 1, 5, 20, 50, and 70 are found to be: (0.249, 0.99), (0.231, 0.925), (0.189, 0.76), (0.145, 0.581), (0.128, 0.506), and (0.124, 0.5), respectively.

This interesting result can be explained considering the way in which the rate constants in Eqns. 17–25 combine to form the coefficients of the polynomials in Eqn. 44. The general form of these coefficients is rather complicated and it is not easy to find a general condition for the existence of a maximum in $\Lambda(c)$, as it was the case for $n = 1$ (see Eqn. 42). In Fig. 5, the conductance of channels with different number n of dipoles is shown. The inverse relation between the conductance and n is explained below. In these calculations the quantities $\bar{\rho}_{n/2}$, $\bar{\mu}'_{n/2}$, $\bar{k}'_{n/2}$ and $\bar{k}''_{n/2}$ were supposed independent of n. All curves were well fitted by Michaelis-Menten curves. Analogous curves were found using several sets of model-parameters.

Let us now turn to the second interesting behavior encountered in the model, that is, the different conductance that the channel exhibits depending on the sign of the ionic charge.

Selectivity

For all curves of $\Lambda(c)$ presented above, the sign of the ionic charge was chosen positive. This was done when electing the value of the valency Z. The dependence on this quantity is introduced in the model by the relations in Eqns. 15 and 22. Also, in all those curves, the values of α_1 was chosen equal to zero, representing the geomtry shown in Fig. 1d. In the following we investigate the consequences on the conductance $\Lambda(c)$ for anions and cations when α_1 is varied.

(a) Case $\alpha_l = 0$

If $\alpha_1 = 0$, the conductance for cations ($Z = 1$) and for anions ($Z = -1$) is depicted in Fig. 6a, the values of the remaining parameters used are indicated there. For anions the conductance is calculated using Eqn. 34 with $U_m < 0$. As can be seen in this figure, the conductance strongly decrease when the transported ions are cations. The magnitude of the effect depends primarily on the ratio of the rate constants $\bar{k}'_{n/2}$ and $\bar{k}''_{n/2}$. For this plot it was chosen $\bar{k}''_{n/2}/\bar{k}'_{n/2} = 5$. The effect also depends on the number n of dipoles in the site, for anions the conductivity increases with this number, and for cations it decreases. As expected, for $n = 0$ (rigid pore), the conductance is the same for both types of ions.

This selectivity is explained because the ratio of the rates $\bar{k}''_{n/2}$ and $\bar{k}'_{n/2}$ determines the preferential equilibrium position of the dipoles. This fact can be clearly understood considering the extreme case $\bar{k}''_{n/2} \gg \bar{k}'_{n/2}$ where the site is preferentially found by the ions in the 'all down' polarized state. In this state the site presents a total energy profile where the minimum in Fig. 1d is deeper for cations compared to anions. In this situation the anions escape more easily from the moiety, and rapidly give chance to other anions to occupy the site (remember that only one ion can occupy the site at a time). The cations spend more time in the moiety before they acquire enough energy to escape and, correspondingly, more time passes before other cations can enter into the site. Because of the value $\alpha_1 = 0$, the height of the barriers does not depend on the polarized state of the site nor on the charge of the ions, therefore the rate to enter is the same for both types of ions. The resulting effect is a greater anion over cation conductance.

Similarly, if the relation $\bar{k}''_{n/2} < \bar{k}'_{n/2}$ holds, the result is a greater conductance for cations compared to anions. And, as expected, in the case $\bar{k}'_{n/2} = \bar{k}''_{n/2}$, the conductance is the same for both types of ions.

The dependence of the conductance on the number n of dipoles in the site can be explained because the average height of the minimum in the total energy profile depends directly on this number (apart from $\bar{k}'_{n/2}$ and $\bar{k}''_{n/2}$). This effect is opposite for anions compared to cations. In Fig. 6a the conductances for $n = 10$ and $n = 20$ are shown.

(b) Case $\alpha_l = 1$

In this case, the conductance for cations and for anions is depicted in Fig. 6b. The role of both types of

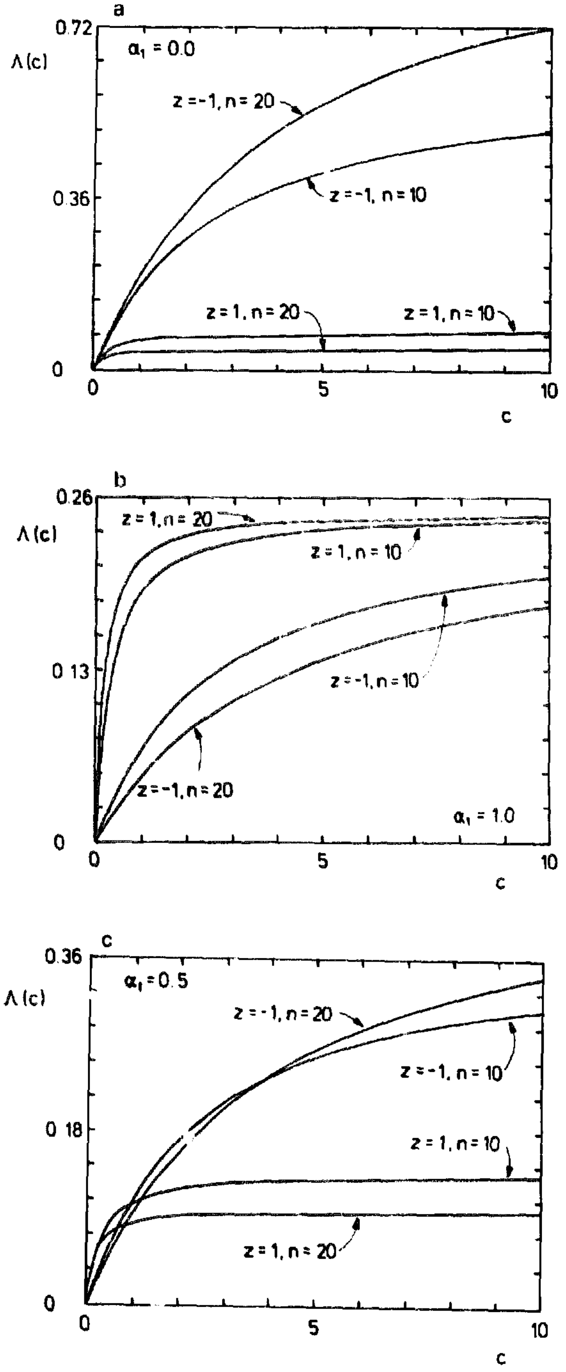


Fig. 6. Conductance $\Lambda(c)$ as a function of the concentration c (arbitrary units), in the cases: (a) $\alpha_1 = 0$, (b) $\alpha_1 = 1$, and (c) $\alpha_1 = 0.5$. α_1 is defined in Fig. 2. Z is the valency of the transported ions, n is the number of dipoles in the binding site, and the rest of the parameters used in these calculations are: $g = 0.1$, $a = 0.3$, $s = 0.25$, $\bar{\rho}'_{n/2} = 1.0$, $\bar{\mu}'_{n/2} = 1.0$, $\bar{k}'_{n/2} = 1.0$ and $\bar{k}''_{n/2} = 5.0$. The values of the rate constants $\bar{k}'_{n/2}$ and $\bar{k}''_{n/2}$ determine the preferred orientation of the dipoles in the binding site, this fact facilitates the transport of ions of one type and hinders the other. Which type is hindered depends critically on the value of the parameter α_1. All conductances are well fitted by Michaelis-Menten curves $\Lambda_{max}c/(k_m + c)$. In case (a), the parameters (Λ_{max}, k_m) corresponding to $Z = -1$ and $Z = 1$, respectively, are found to be: (1.01, 4.04) and (0.043, 0.16) for $n = 20$, and, (0.62, 2.49) and (0.08, 0.33) for $n = 10$. In case (b): (0.25, 4.05) and (0.25, 0.17) for $n = 20$, and, (0.25, 0.32) and (0.25, 2.49) for $n = 10$. In case (c): (0.48, 4.04) and (0.098, 0.17) for $n = 20$, and, (0.38, 2.49) and (0.14, 0.32) for $n = 10$.

ions is now inverted as compared to the case (a). That is, the conductance is now greater for cations. The magnitude of this effect depends on the ratio of the rate constants $\bar{k}'_{n/2}$ and $\bar{k}''_{n/2}$, and also on the number n of dipoles in the site. For $n = 0$ (rigid pole), the conductance is the same for both types of ions.

An explanation for this inversion in the charge selectivity may be given easily using Fig. 2. For $\alpha_1 = 1$ ($\beta_1 = 0$), the only contribution of the polarization is to change the heights of the maxima in the energy profile without changing the relative position of the minimum. Therefore, the entrance barriers encountered by an anion are higher if the polarization is near the 'all down' state. This fact decreases its probability to enter into the binding site. The opposite occurs if the transported ions are positive.

The explanation for the dependence of the conductance on the number of dipoles in the site is similar as in case (a).

The inversion in the cation/anion selectivity is not symmetric, as may be seen comparing Figs. 6a and 6b. In case $\alpha_1 = 1$, all the four conductances tend to the same limit as c increases. This common limit is explained because the constant heights of the exit barriers are the same in the four cases (in contrast to the case $\alpha_1 = 0$), and, the rates for entrance depend on the concentration c. In case $\alpha_1 = 0$, the four different average heights of the exit barriers determine the limiting rates for the transport processes, therefore each one of the four conductances has its own limiting value.

(c) Case $\alpha_l = 0.5$

In this case the contribution from the polarization results in changing both, maxima and minimum, of the energy profile (see Fig. 2). This fact is manifested on the charge selectivity, which behaves intermediate between the cases (a) and (b), and it also depends on the concentration c. Fig. 6c shows these findings. For low values of the concentration c, the selectivity behaves like in case (b), but for high values of c behaves like in (a). As α_1 varies from 0 to 1, the crossing point for cation and anion conductances moves to the right. The dependence of $\Lambda(c)$ on the number of dipoles is more complicated than in the cases (a) and (b). For $n = 0$, the conductance is the same for both types of ions.

All curves in Fig. 6 were well fitted by Michaelis-Menten type curves: $\Lambda_{\max} c/(k_m + c)$. The corresponding parameters ($\Lambda_{\max}$, k_m) for each curve are indicated there.

Discussion

We have analyzed a model of channels with fluctuations in their internal barrier structure. Fluctuations in the energy profile of a channel may be expected to arise from transitions between the different conformational states of the channel protein. The general problem is complex, therefore, a simple but more realistic model in comparison to a rigid channel structure have been considered. The channel has two (main) barriers and a single (main) binding site. The rates of conformational transitions are comparable with the jump rates of the ions. The nature of the conformational states is proposed to arise from the movement of dipolar groups in the site. A jump of one ion into the energy minimum is followed by transitions into more polarized states. Under these conditions coupling between transport and conformational transitions occurs and the channel conductance Λ exhibits a peculiar dependence on the concentration c. According to the general theory of fluctuations in barrier structure in channels [1], if the rate constants satisfy certain conditions, Λ could show a non-simple saturation behavior. Nevertheless, for the presented model, it was found that the rate constants contributed in such a way that simple saturation curves were obtained. This was true for all the sets of parameter used in the calculations. In the case of only one dipole in the site ($n = 1$), it was shown that the corresponding rate constants do not satisfy the requirements for the existence of a maximum in the conductance $\Lambda(c)$ and, in fact all the $\Lambda(c)$ curves were well fitted by Michaelis-Menten curves.

The second interesting property found in this model is the selectivity for the sign of the ions. This was clearly manifested by the very different conductances presented by anions and cations for certain values of the model parameters. If the dipoles of the binding site have a preferred orientation (close to the 'all up', or to the 'all down' states), ions with different sign in charge 'see' different energy barriers when they try to get out or to get in the site. The consequence may be a strongly different cation over anion transport rates. This cation/anion selectivity critically depends on the value of the parameter α_1, which determine the positions of the dipoles with respect to the maxima of the energy profile. As shown in Fig. 6, the extreme opposite situations correspond to the cases where only the heights of the minimum ($\alpha_1 = 0$) or of the maxima ($\alpha_1 = 1$) are changed due to the polarization of the site. For $\alpha_1 = 0.5$, both tendencies compete, and the resulting selectivity depends also on the concentration of permeant ion in the aqueous phases.

Other interesting characteristics of this model will be presented in a forthcoming publication. The consequences of considering ions with different size and net charge, and the case of a non symmetric energy profile will be analyzed. Also, the time relaxation of the ion flux will be investigated when an external parameter such as the concentration or voltage is changed suddenly [8–12].

Acknowledgements

The authors wish to express their deep gratitude to the late Prof. P. Läuger, who initiated and enabled this work. It has been financially supported by the Deutsche Forschungsgemeinschaft (LA 214/11-1).

References

1 Läuger, P., Stephan, W. and Frehland, E. (1980) Biochim. Biophys. Acta 602, 167-180.

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

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

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

5 Weber, J. (1965) In Molecular Biophysics (Pullman, B. and Weissbluth, M., eds.), pp. 369-396, Academic Press, New York.

6 Fischer, W., Brickmann, J. and Läuger, P. (1981) Biophys. Chem. 13, 105-116.

7 Läuger, P. (1973) Biochim. Biophys. Acta 311, 423-441.

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

9 Nieto-Frausto, J., Läuger, P. and Apell, H.-J. (1992) Biophys. J. 61, 83-95.

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

11 Skrabal, A. (1930) Z. Phys. Chem. 6, 382-422.

12 Frehland, E. (1982) in Stochastic Transport Processes in Discrete Biological Systems, Lecture Notes in Biomathematics, Vol. 47, (Levin, S., ed.), pp. 42-133, Springer-Verlag, Berlin, Heidelberg, New York.