COUPLING BETWEEN FLUXES IN ONE-PARTICLE PORES WITH FLUCTUATING ENERGY PROFILES

A Theoretical Study

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ABSTRACT We show theoretically that extending pore models to allow for fluctuations between configurations with different energy profiles results in the prediction of coupling between fluxes and forces of different species diffusing through singly occupied pores. Considering the case of a one-site, two-barrier pore capable of existing in two states, and using Eyring rate theory to describe the translocation of two permeant species, the flux of each is found to be linked to the driving force of the other via cross coefficients that are given as explicit functions of concentrations and potential, and that obey Onsager's relations when the system is near equilibrium. Conditions for the existence of coupling are that both states of the channel be permeable to both diffusing species and that the peaks of the two energy barrier shift by different amounts during the state transition of the pore. Some implications of this model on phenomena of biological interest are discussed briefly.

INTRODUCTION

Models for ion permeation based on Eyring rate theory have been generalized to the case of channels fluctuating between states with different energy profiles (1, 2). An interesting result of this extension is that one-ion pore models acquire characteristics that are usually regarded as indicative of multi-ion occupancy. For example, the conductance can have a maximum as a function of ion concentration, a feature well known for multi-ion channels (3), but inconsistent with one-ion pore models with fixed energy barriers (4). This paper shows that, for a one-particle pore in the presence of two permeant species, the interaction between the kinetics of permeation and that of fluctuation can induce a coupling between the fluxes similar to that postulated in nonequilibrium thermodynamics. Because the two particles can be either both ionic, or both neutral, or one ionic and one neutral, the equations we obtain are susceptible to various applications of interest in membrane transport. For example, when one species is ionic and the other neutral, they describe a simple mechanism whereby the flux of a nonelectrolyte can be driven by the electro-chemical potential gradient of an ion, as is generally postulated to occur in cotransport phenomena.

DESCRIPTION OF THE MODEL AND RESULTS

For simplicity, the analysis is restricted to a one-site, two-barrier pore, as shown in Fig. 1. The fluctuations of the energy profiles are described by single kinetic steps between two classes of states, referred to as "normal" and "polarized," according to a nomenclature used previously (1, 2). Because two permeant species are considered, A and B, and the internal site can be empty, occupied by A, or occupied by B, the channel can exist in six different states: three "normal" and three "polarized" (see Fig. 2).

For simplicity we shall make the following assumptions: (*a*) the peaks of the energy barriers are half-way between the external solutions and the internal site. (*b*) The locations of the peaks and of the internal site (measured in fractions of the applied voltage) are the same for the two particles and are not altered by the state transitions of the pore. (*c*) The rate constants for such transitions are not functions of voltage, but depend on whether the pore is empty, occupied by A or occupied by B.

The meaning of the symbols used for the rate constants should be clear from Figs. 1 and 2 and their legends. Because the rate constants for entrance, ν', and ν'', depend on the external concentrations, they can be written in the form

$$\nu' = \rho' C' \qquad \nu'' = \rho'' C''. \tag{1}$$

In Eyring theory, the rate constants are proportional to the exponential of the free energy difference per unit mole (in units of RT, the molar gas constant and absolute temperature) between the site in which the particle is located and the peak of the barrier it crosses. Writing explicitly the

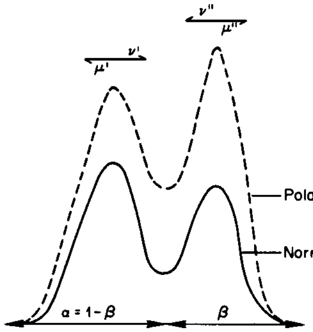

FIGURE 1 Energy profiles of the channel in its normal and polarized states. In the equations, the rate constants for permeation bear one-letter subscripts, A or B (to identify the species), in the case of normal channels, and two-letter subscripts, PA or PB, in the case of polarized channels.

electric component of the energy due to the applied voltage, ν' and ν'' become

$$\nu' = \bar{\rho}' C' e^{\alpha z u/2} \qquad \nu'' = \bar{\rho}'' C'' e^{-\beta z u/2} \tag{1a}$$

where z is the valency, α and β are the fractional electric distances shown in Fig. 1, u $(=u' - u'')$ is potential in units of RT/F, C' and C'' are external concentrations, and the bars over $\bar{\rho}'$ and $\bar{\rho}''$ designate the values of these rate constants when $u = 0$. (A bar over a symbol will be used consistently to indicate its value at zero potential.) The steady state fluxes are calculated by a standard procedure outlined in the Appendix, the most interesting result being that, in general, the flow of each species is a function of the "driving forces" conjugated with both. More precisely, the expressions for the fluxes, ϕ_A and ϕ_B, can be written in the form

$$\phi_i = L_i \left[2 \sinh \frac{z_i(u - u_i)}{2} \right] + L_{ij} \left[2 \sinh \frac{z_j(u - u_j)}{2} \right], \qquad \begin{matrix} \text{i and j} = \text{A or B} \\ \text{i} \neq \text{j} \end{matrix} \tag{2}$$

where

$$u_{i(j)} = \frac{1}{z_{i(j)}} \ln \frac{C''_{i(j)}}{C'_{i(j)}}, \qquad \text{i and j} = \text{A or B} \tag{3}$$

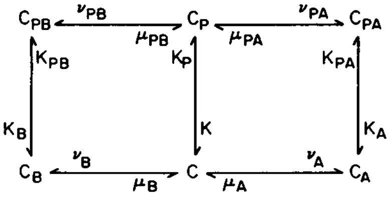

FIGURE 2 State diagram for the six possible states of the pore. The unprimed rate constants, ν and μ, stand for $\nu' + \nu''$ and $\mu' + \mu''$, respectively. The meaning of the primed rate constants is illustrated in Fig. 1. The other constants, denoted by the letter K, describe the kinetics of channel fluctuation.

and the coefficients, L, are functions of the rate constants, of the external concentrations and of the potential. Because coupling is the interesting feature of these equations, we shall concentrate on the coefficients, L_{AB} and L_{BA}, while the equations for L_A and L_B, which are rather lengthy, are given for completeness in the Appendix. L_{AB} can be expressed in the form

$$L_{AB} = -\frac{\bar{S}}{\Delta} \overline{W}_A \overline{W}_B \sqrt{C'_A C''_A C'_B C''_B} \cosh \frac{z_A(u - u_A)}{2}, \tag{4}$$

where

$$\bar{S} = \bar{\rho}'_A \bar{\rho}'_{PB} \bar{\mu}'_B \bar{\mu}'_{PA} K_B K_{PA};$$

$$\overline{W}_i = \left(\frac{\bar{\rho}''_i}{\bar{\rho}'_i} - \frac{\bar{\rho}''_{Pi}}{\bar{\rho}'_{Pi}} \right), \qquad (\text{i} = \text{A or B}). \tag{5}$$

Δ, given in Eq. A13, is a polynomial of second degree in the four variables, C'_A, C''_A, C'_B, C''_B. The reciprocal coefficient, L_{BA}, is obtained from Eq. 4 by merely interchanging the subscripts A and B. Because $\bar{S}$ and Δ remain unaltered, as is shown in the Appendix, L_{AB} will differ from L_{BA} only for the argument of the hyperbolic cosine. However, when the system is sufficiently close to equilibrium, we shall have $\cosh \simeq 1$ and $L_{AB} = L_{BA}$, in agreement with Onsager's relations. In this limit, $C'_A \simeq C''_A \equiv C_A$, $C'_B \simeq C''_B \equiv C_B$, $z_A(u - u_A) \simeq 0$, and Eq. 4 reduces to

$$L_{AB} = -\bar{S} \overline{W}_A \overline{W}_B \cdot \frac{C_A C_B}{\gamma_o + \gamma_A C_A + \gamma_B C_B + \gamma_{AA} C_A^2 + 2\gamma_{AB} C_A C_B + \gamma_{BB} C_B^2}, \tag{6}$$

where the coefficients, γ, are constants deducible from the general expression for Δ, Eq. A13. Due to the quadratic terms, C_A^2 and C_B^2, in the denominator, Eq. 6 shows that L_{AB}, viewed as a function of either C_A or C_B, increases from zero to a maximum and vanishes at high concentrations. From Eq. 4 and the corresponding equation for L_{BA}, it is also seen that, when the two solutions have the same composition and when $z_A = z_B$, the validity of the relation, $L_{AB} = L_{BA}$, is not restricted to situations close to equilibrium, since it holds true for any potential.

DISCUSSION

We have shown that in a one-particle channel the interaction between the kinetics of transport and that of channel fluctuation can give rise to cross interactions between the fluxes and forces of different permeant species. Eq. 4 shows, however, that coupling requires that the three parameters, $\bar{S}$, $\overline{W}_A$, and $\overline{W}_B$ all be different from zero. As can be seen from Eqs. 5 and A2, $\bar{S}$ will differ from zero only when both species, A and B, permeate normal as well as polarized channels. Moreover, the requirement that $\overline{W}_A$ and $\overline{W}_B$ be nonvanishing quantities is equivalent to the condition that the two energy peaks of the pore shift unequally in the transition between the two states. The justification for this statement follows from the definition of $\overline{W}_i$ in Eq. 5 and the recollection that, according to the formalism of Eyring's rate theory, the ratio, $\bar{\rho}''/\bar{\rho}'$, is equal to the exponential of the difference between the two energy peaks (in units of RT).

Note that, if $L_{AB} \not\equiv 0$, coupling can be either positive ($L_{AB} > 0$) or negative ($L_{AB} < 0$), as in principle there is no correlation between the signs of $\overline{W}_A$ and $\overline{W}_B$ when A and B are different molecules. Only when they are chemically similar (as in the case isotopes), $\overline{W}_A$ and $\overline{W}_B$ must be practically equal and L_{AB} will be negative. One interesting consequence of the fact that L_{AB} may not vanish when A and B are different isotopes of the same chemical species is that the present model will generally predict deviations from Ussing flux ratio equation, which in this case are not related either to active transport or to multi-particle occupancy.

A case of physiological interest to which this model may be relevant is that in which the flow of a nonelectrolyte is linked to the driving force of an ion (cotransport). For example, if A is neutral and B is an ion, and if we also assume for simplicity that $C'_A = C''_A$, Eq. 2 predicts that the flow of A will be driven by the electro-chemical potential gradient of B according to the equation

$$\phi_A = 2\, L_{AB} \sinh \frac{z_B(u - u_B)}{2}. \qquad (7)$$

The detailed behavior of the flux will clearly depend on the values assigned to the parameters of the model. However, Eq. 7 is compatible with the common finding that the kinetics of cotransport differ according to whether the driving force is varied by changing the concentration or the potential gradient (5), since the dependence of L_{AB} on concentrations in Eq. 4 is manifestly different from its dependence on potential.

If A and B are both ions, Eq. 2 allows one to derive an expression for the zero-current potential that can be written in the same form as the Goldman-Hodgkin-Katz equation, the permeability ratio being given by a fraction of two polynomials, linear in the variables C'_A, C''_A, C'_B, C''_B. Concentration-dependent permeability ratios have been measured both in artificial (6, 7) and biological channels (8, 9), and have been generally interpreted in terms of multi-ion pores (10, 3), or channels with modulatory binding sites (11). This model suggests the possibility of still another explanation.

APPENDIX

Proof that the Parameter $\overline{S}$ in Eqs. 4 and 5 is Invariant with Respect to the Interchange of the Subscripts A and B

Fig. 2 shows that the pore can exist in six states. From the requirement that they be in mutual equilibrium when the concentrations and the potential are the same in the two solutions, one deduces the six following relations:

$$\begin{gathered} \bar{\rho}'_i \bar{\mu}''_i = \bar{\rho}''_i \bar{\mu}'_i, \quad \bar{\rho}'_{Pi} \bar{\mu}''_{Pi} = \bar{\rho}''_{Pi} \bar{\mu}'_{Pi}, \\ \bar{\rho}'_i \bar{\mu}'_{Pi} K_{Pi} K = \bar{\rho}'_{Pi} \bar{\mu}'_i K_i K_P. \end{gathered} \qquad (i = A \text{ or } B) \qquad (A1)$$

Solving the last two separately for K_P/K and equating the results, one finds

$$\bar{\rho}'_A \bar{\mu}'_{PA} \bar{\rho}'_{PB} \bar{\mu}'_B K_B K_{PA} = \bar{\rho}'_B \bar{\mu}'_{PB} \bar{\rho}'_{PA} \bar{\mu}'_A K_A K_{PB}. \qquad (A2)$$

Because the left-hand side coincides with $\overline{S}$, defined in Eq. 5, Eq. A2 proves that this quantity is symmetrical with respect to A and B, and, consequently, that $L_{AB} = L_{BA}$ near equilibrium.

Outline of the Calculation of the Fluxes and Explicit Expressions for the Coefficients of Eq. 2

Using the subscript i to represent either A or B, the number of pores in each state per unit area will be denoted by N (normal, empty), N_P (polarized, empty), N_i (normal, occupied by i), N_{Pi} (polarized, occupied by i). Because the sum of all the Ns is assumed to be constant, we will have

$$N^T = N + N_P + N_A + N_{PA} + N_B + N_{PB}, \qquad (A3)$$

which implies that only five states are independent. If N is chosen to be the dependent variable, and if we define for brevity

$$\nu = \nu' + \nu'', \qquad \mu = \mu' + \mu'', \qquad (A4)$$

the steady-state values of the five independent Ns are described by the equations

$$\frac{dN_P}{dt} = K_P N - K N_P + [\mu_{PA} N_{PA} + \mu_{PB} N_{PB}] - [\nu_{PA} + \nu_{PB}] N_P = 0 \qquad (A5)$$

$$\frac{dN_i}{dt} = \nu_i N - \mu_i N_i + K_i N_{Pi} - K_{Pi} N_i = 0, \qquad (i = A \text{ or } B) \quad (A6)$$

$$\frac{dN_{Pi}}{dt} = \nu_{Pi} N_P - \mu_{Pi} N_{Pi} + K_{Pi} N_i - K_i N_{Pi} = 0, \qquad (i = A \text{ or } B). \quad (A7)$$

Once the Ns are evaluated, the steady-state fluxes of A and B can be calculated from

$$\phi_i = \nu'_i - \mu'_i N_i + \nu'_{Pi} N_P - \mu'_{Pi} N_{Pi} = 0, \qquad (i = A \text{ or } B). \quad (A8)$$

With algebraic rearrangements Eq. A8 can be written in the form of Eq. 2, where the coefficients are lengthy functions. Here we shall give the expressions for L_A and shall complete (by defining Δ) that for L_{AB}, it being understood that L_B and L_{BA} are obtained from the other two by simply interchanging the subscripts A and B. It is useful, in order to shorten the equations, to define the following quantities

$$\tilde{K} = K + K_P, \quad \tilde{K}_i = K_i + K_{Pi} \qquad (i = A \text{ or } B) \qquad (A9)$$

$$\Omega_i = \mu_i(K_i + \mu_{Pi}) + K_{Pi}\mu_{Pi} \qquad (i = A \text{ or } B), \qquad (A10)$$

the unprimed μ's being defined in Eq. A4. L_A is finally given by

$$L_A = \frac{T_A + T_{PA}}{\Delta} \qquad (A11)$$

where

$$\begin{aligned} T_{\mathrm{A}} = \sqrt{C'_{\mathrm{A}} C''_{\mathrm{A}}}\Big\{ & \Omega_{\mathrm{B}} \bar{\rho}'_{\mathrm{A}} \, [K\bar{\mu}''_{\mathrm{A}}(K_{\mathrm{A}} + \mu_{\mathrm{PA}}) \\ & + KK_{\mathrm{PA}}\bar{\mu}''_{\mathrm{PA}} + K_{\mathrm{A}}\bar{\mu}''_{\mathrm{A}}\nu_{\mathrm{PA}}] + \frac{\bar{S}}{\bar{\rho}'_{\mathrm{B}}\bar{\rho}'_{\mathrm{PB}}} \\ & \cdot \Big[\bar{\rho}'_{\mathrm{B}}\bar{\rho}'_{\mathrm{PB}}C'_{\mathrm{B}} + \bar{\rho}''_{\mathrm{B}}\bar{\rho}''_{\mathrm{PB}}C''_{\mathrm{B}} + \sqrt{C'_{\mathrm{B}} C''_{\mathrm{B}}}\,(\bar{\rho}'_{\mathrm{PB}}\bar{\rho}''_{\mathrm{B}} + \bar{\rho}'_{\mathrm{B}}\bar{\rho}''_{\mathrm{PB}}) \\ & \cdot \cosh\frac{z_{\mathrm{B}}(u - u_{\mathrm{B}})}{2}\Big]\frac{\bar{\rho}''_{\mathrm{PA}}}{\bar{\rho}'_{\mathrm{PA}}}\Big\} \qquad (\mathrm{A12}) \end{aligned}$$

and T_{PA} is obtained from T_{A} by interchanging normal with polarized states; that is, by removing in T_{A} the subscript P from the rate constants that bear it and adding it to those that don't. The quantity Δ, that appears in the definitions of L_{A} and L_{AB}, is found to be given by

$$\Delta = \tilde{K}\Omega_{\mathrm{A}}\Omega_{\mathrm{B}} + \Delta_{\mathrm{A}} + \Delta_{\mathrm{B}} \qquad (\mathrm{A13})$$

where

$$\begin{aligned} \Delta_{\mathrm{A}} = \Omega_{\mathrm{B}}\,\{ & [K(\tilde{K}_{\mathrm{A}} + \mu_{\mathrm{PA}}) + K_{\mathrm{PA}}\mu_{\mathrm{PA}}]\nu_{\mathrm{A}} \\ & + [K_{\mathrm{P}}(\tilde{K}_{\mathrm{A}} + \mu_{\mathrm{A}}) + K_{\mathrm{A}}\mu_{\mathrm{A}}]\nu_{\mathrm{PA}} + \tilde{K}_{\mathrm{A}}\nu_{\mathrm{A}}\nu_{\mathrm{PA}}\} \\ & + [\mu_{\mathrm{PA}}K_{\mathrm{PA}}(\tilde{K}_{\mathrm{B}} + \mu_{\mathrm{B}}) + \mu_{\mathrm{B}}K_{\mathrm{B}}(\tilde{K}_{\mathrm{A}} + \mu_{\mathrm{PA}})]\nu_{\mathrm{A}}\nu_{\mathrm{PB}} \qquad (\mathrm{A14}) \end{aligned}$$

and Δ_{B} is obtained from Δ_{A} by interchanging the subscripts A and B.

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