Kinetic Analysis of Water Transport through a Single-File Pore

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ABSTRACT We apply the diagrammatic method developed by Hill (1977. Free Energy Transduction in Biology. Academic Press, New York) to analyze single-file water transport. We use this formalism to derive explicit expressions for the osmotic and diffusive permeabilities P_f and P_d of a pore. We first consider a vacancy mechanism of transport analogous to the one-vacancy pore model previously used by Kohler and Heckmann (1979. *J. Theor. Biol.* 79:381–401). (*a*) For the general one-vacancy case, we find that the permeability ratio can be expressed by $P_f/P_d = (P_f/P_d)_{eq} f(w_A, w_B)$, where the second factor is a function of the water activities in the two adjoining compartments A and B. As a consequence, the permeability ratio in general can effectively differ from its value at equilibrium. We also find that $n - 1 \leq (P_f/P_d)_{eq} \leq n$, a result already proposed by Kohler and Heckmann (1979. *J. Theor. Biol.* 79:381–401). (*b*) When vacancy states are transient intermediates, the model can be reduced to a diagram consisting of only fully occupied states. Such a diagram resembles the one describing a no-vacancy mechanism of transport (*c*), but in spite of the similarity the expressions obtained for the permeability coefficients still retain the basic relationships of the original (*a*) nonreduced one-vacancy model. (*c*) We then propose a kinetic description of a no-vacancy mechanism of single-file water transport. In this case, the expressions derived for P_f and P_d are formally equivalent to those obtained by Finkelstein and Rosenberg (1979. Membrane Transport Processes. Vol. 3. C.F. Stevens and R.W. Tsien, editors. Raven Press, New York. 73–88.) A main difference with the vacancy mechanism is that here the permeability coefficients are independent of the water activities.

INTRODUCTION

Descriptions of the rate of water movement across biological membranes have usually involved the determination of permeability coefficients for water. The diffusive permeability of water, P_d, is often obtained as the ratio between a net water tracer flux and the tracer concentration difference under the condition of water equilibrium. The osmotic permeability coefficient, P_f, is defined as RTL_p/V_w, where R is the universal gas constant, T the temperature in kelvin, V_w the partial molar volume of

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water, and L_p the hydraulic permeability coefficient per unit area of the membrane. In this context, L_p is obtained as the ratio between a water volume flow and the osmotic (or hydrostatic) pressure difference. The significance of these permeability coefficients in terms of molecular mechanisms of water permeation has become a matter of controversy. Thus, the interpretation of data on the P_f/P_d ratio in human erythrocytes using macroscopic hydrodynamic theories leads to conclusions about the water pore dimensions that have been questioned (Galey and Brahm, 1985). This is because hydrodynamic models (Solomon, 1968) assume that water is a smooth, continuous fluid and that water transport through pores can be represented by Poiseuille-type flow. This could be an accurate description for pores with radii several times larger than that of water. For pores with smaller radii, such models may not adequately represent the possibly discrete nature of the mechanism of transport of water molecules, and therefore could not constitute proper descriptions to interpret macroscopic determinations, such as diffusive and osmotic permeabilities.

For the limiting case of single-file pores, continuum models have been used (Levitt, 1974; Finkelstein and Rosenberg, 1979) to derive expressions for P_d and P_f. The basic assumption underlying these types of dynamical descriptions is that the water pores are constantly completely filled with water molecules. In this "no-vacancy" mechanism, molecules are packed together inside the pore. As a consequence, during the transport process the pore contents are displaced as a whole. Hence, individual molecules are not free to move back and forth to vacant positions, but are forced to move by the pushing of the neighboring molecules. These models derive expressions in the near-equilibrium region, as emerges from the fact that derivations depend on the exact balancing of the forces involved in volume displacement in the diffusive and osmotic processes. However, as we discuss below, since the "independence principle" holds, the permeability coefficients are not affected by the water activities in the compartments. Therefore, the permeability ratio remains constant arbitrarily away from equilibrium. These models provide explicit expressions for P_d and P_f that could result in accurate interpretation of the transport properties of some pores, like the gramicidin A pore, provided that the transport process only takes place by this no-vacancy, or hydrodynamic, mechanism. The formalism of linear irreversible thermodynamics has also been used to derive expressions for the water permeabilities (Levitt, 1984), which have basically confirmed the results obtained with the dynamical continuum theories.

On the other hand, if the single-file water pore consists of a nonuniform structure and the transport process takes place by a vacancy mechanism, a discrete description will be more appropriate than a continuous model. In such a type of mechanism, the movements of the molecules can be envisaged as "jumps" over energy barriers between adjacent positions, from an occupied position to a vacant one. A description of this type of diffusion in nonuniform media is provided by the kinetic formalism. The basic general aspects of a kinetic model of a tracer–main isotope transport process through a single-file pore mediated by vacancies have been analyzed by Kohler and Heckmann (1979, 1980). Although expressions for the permeabilities of the two species are not explicitly derived, these authors examine the restrictions that govern the unidirectional flux ratio, directly related near equilibrium to the permeability ratio (Hille and Schwartz, 1978). Our purpose in this paper is to show how the kinetic formalism can provide an alternative way to interpret experimental data about water permeability which is more general than the classical continuous descriptions.

In the first part, we derive explicit steady-state kinetic expressions for P_d and P_f from the analysis of a single-file model of water transport formally analogous to the one-vacancy model studied by Kohler and Heckmann (1979). We show there, in particular, that the relation $(P_f/P_d) \leq n$, where n is the number of positions inside the pore, is strictly valid for this kind of pore only in the near-equilibrium region, and that far from equilibrium the permeability ratio can be larger than n. We comment on this result because of the previous idea that this ratio would not exceed n (Finkelstein, 1987). We also obtain here expressions for the permeability coefficients in the particular case of the uniform pore.

In the second part of the paper we analyze the model under the assumption that the vacancy pore states are transient intermediates. Under this condition, the kinetic model can be "reduced" to a diagram of only fully occupied pore states, where the new kinetic constants are complicated functions of the primitive constants. Although this reduced model resembles the description of the permanently fully occupied pore, the rate constants are overall entities with complex internal structure. Analyzed in their explicit expressions, the permeability coefficients obtained in this case retain the basic relationships of the original nonreduced model. This is a consequence of the fact that although vacancies are not made explicit in the reduced diagram, their existence remains as the basic mechanism involved in this type of transport process.

In the third part we describe the water tracer transport process through a permanently completely filled, single-file pore. We obtain the expressions for the permeability coefficients of this type of pore, which turn out to be formally analogous to the ones derived by Finkelstein and Rosenberg (1979). We also discuss the fact that in this no-vacancy mechanism the permeability coefficients are independent of the water activities in the compartments, and therefore that the ratio P_f/P_d remains constant arbitrarily away from equilibrium. Finally, we compare the expressions obtained with those of the one-vacancy uniform pore.

In general, the basic assumption implicit in the kinetic description of water transport through pores is that this process can be adequately represented by the movement of molecules between well-defined sequential positions inside the pore. The definition of positions permits us to establish a correspondence between a particular position of the water tracer molecule and/or the available vacancy, and a particular state in the kinetic diagram. Accordingly, the rate constants of transition between states can be related to the rate constants of displacement between positions. Throughout the paper, the kinetic formalism developed by Hill (1977) provides the basic technique for the derivation of the steady-state expressions.

THE ONE-VACANCY SINGLE-FILE WATER PORE: GENERAL KINETIC MODEL

A membrane separating two aqueous compartments, A and B, is traversed by a total number $\tilde{N}$ of identical pores that contain, when saturated, a single file of water molecules located at n fixed positions inside each pore. In the time scale of the model, at most only one vacancy can exist per pore. Since vacancies are formed at the positions in contact with the two compartments, this assumption is justified by the very large activity of water in most aqueous solutions (Kohler and Heckmann, 1980). Each compartment contains a very small concentration of a tracer (labeled water molecules) that has diffusive properties identical to those of common (unlabeled) water molecules. Since the tracer concentrations are negligible, there is at most only one tracer per pore. To pass from one compartment to the other, a tracer molecule must sequentially "bind" to the n sites along the pore. From these considerations, the kinetic diagram shown in Fig. 1 describes the process of diffusion of tracer and common water molecules across the $\bar{N}$-pore ensemble. This model is formally identical to the one analyzed by Kohler and Heckmann (1979).

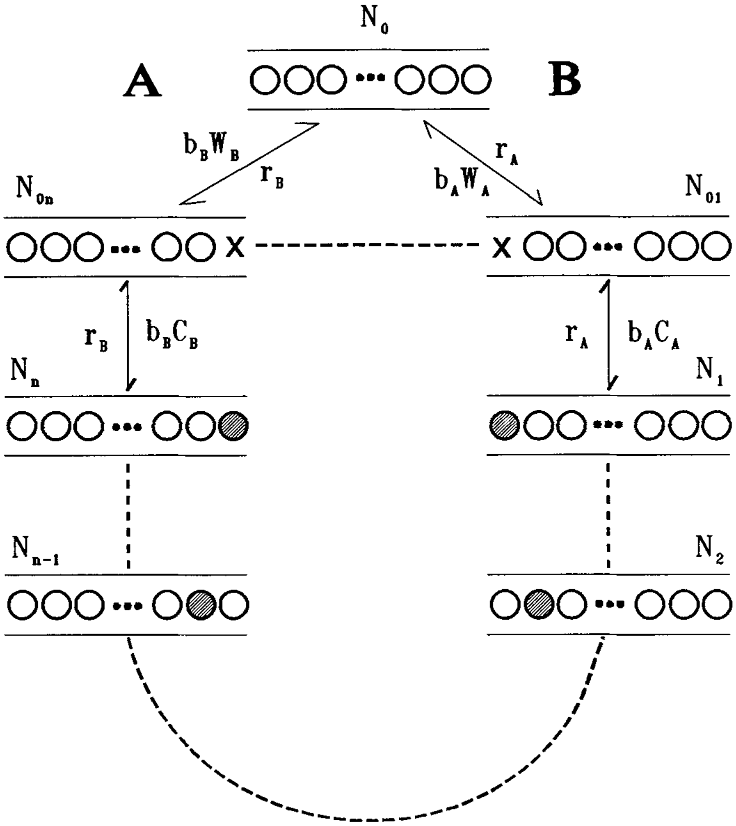


FIGURE 1. The one-vacancy kinetic model of labeled and unlabeled water diffusion through a single-file pore. x, vacancies; open circles, positions occupied by unlabeled water molecules; filled circles, positions occupied by labeled water molecules. Compartment A is assumed to be on the left side, compartment B on the right side. Solid lines are actual transitions; dashed lines summarize many intermediate pore states. Positions (sites) within the pore are numbered 1, 2, . . . , n from A to B. See text for further details.

In the context of the analysis performed here, the symbols represent the following:

b_A, b_B	rate constants of binding from compartments A and B
r_A, r_B	rate constants of release to compartments A and B
$\tilde{N}_i$	total number of saturated pores in state N_i, with the tracer located in the *i*th site ($i = 1, 2, \ldots, n$)
$\tilde{N}_0$	total number of saturated pores in state N_0, without a tracer molecule
$\tilde{N}_{ij}$	total number of pores in state N_{ij}, having the tracer at the *i*th site and the vacancy at the *j*th site ($j = 1, 2, \ldots, n$)
$\tilde{N}_{0j}$	total number of pores in state N_{0j}, without a tracer having the vacancy at the *j*th site
w_A, w_B	water activities in compartments A and B
c_A, c_B	tracer activities in compartments A and B

To avoid overloading Fig. 1, the diagram has been drawn simplifying transitions N_{0n} ----- N_{01} and N_i ----- N_{i+1}, which consist of intermediate successive steps. Every transition N_i ----- N_{i+1}, for $i = 1, 2, \ldots, n - 1$, actually consists of the following $n + 1$ steps:

$$N_i \underset{k_{(n+1)n}}{\overset{k_{n\,(n+1)}}{\longleftrightarrow}} X_n \underset{k_{n(n-1)}}{\overset{k_{n\,(n-1)n}}{\longleftrightarrow}} X_{n-1} \cdots X_2 \underset{k_{21}}{\overset{k_{12}}{\leftrightarrow}} X_1 \underset{k_{10}}{\overset{k_{01}}{\leftrightarrow}} N_{i+1}$$

where X_j represents a state with the vacancy at the *j*th site. Under the assumptions of the model, $k_{i(i+1)}$ and $k_{(i+1)i}$ (for $i = 1, 2, \ldots n - 1$) can be considered as the rate constants of displacement (of either unlabeled or labeled water molecules) between positions i and $i + 1$ in the A to B and B to A directions, respectively. We have also defined:

$$\begin{aligned} k_{01} &= b_A w_A, \\ k_{10} &= r_A, \\ k_{n\,(n+1)} &= r_B, \text{ and} \\ k_{(n+1)n} &= b_B w_B \end{aligned} \tag{1}$$

In turn, transition N_{0n} ----- N_{01} actually consists of

$$N_{0\mathrm{n}} \underset{k_{n\,(n-1)}}{\overset{k_{(n-1)n}}{\longleftrightarrow}} N_{0(n-1)} \cdots N_{02} \underset{k_{21}}{\overset{k_{12}}{\longleftrightarrow}} N_{01}$$

The kinetic scheme of Fig. 1 involves three diagram cycles, named a, b, and c, which are shown in Fig. 2. The tracer and (common) water fluxes can be expressed in terms of the corresponding cycle fluxes. In Appendix I, we show the main steady-state expressions obtained from the analysis of the model by using the algorithmic method developed by Hill (1977) and by imposing the necessary thermodynamic restrictions. From the results shown there, we now derive explicit expressions for the tracer and water permeabilities.

From Eqs. A2–A5, the water permeability for the condition $c_A = c_B = 0$ is given by

$$P_w = \tilde{N}\pi/\Omega_b \tag{2}$$

When $w_A = w_B = w$, we obtain the tracer permeability for water equilibrium from

Eqs. A6–A11 as

$$P_{tr} = \tilde{N}(\pi w)^{n-1}[Lr_A r_B b_A b_B w + \pi(r_A + r_B)]/(\Sigma)_w \tag{3}$$

Considering the assumptions for the derivation of Eqs. 2 and 3, we may establish the following relations:

$$P_f = P_w \quad \text{and} \quad P_d = P_{tr} \tag{4}$$

From Eqs. 2–4, and using the relations obtained in Appendix I, we can express the permeability ratio by

$$P_f/P_d = \frac{(\Omega_b)_w[(n-1)r_A r_B b_A b_B wL + n(r_A + r_B)\pi]}{\Omega_b[r_A r_B b_A b_B wL + (r_A + r_B)\pi]} \tag{5}$$

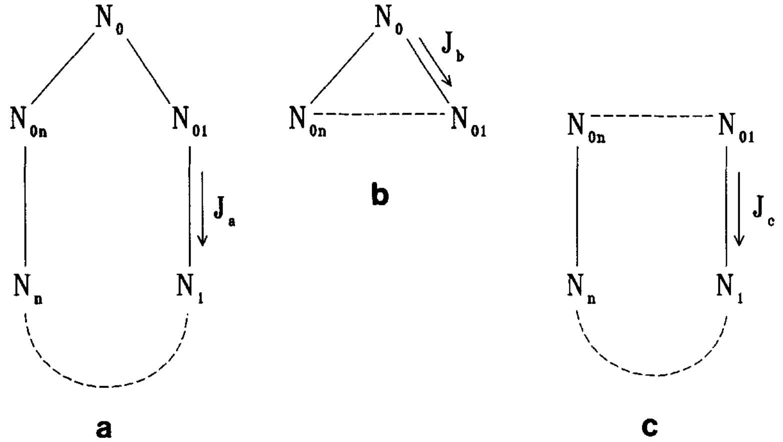


FIGURE 2. The three cycles arising from the kinetic diagram of Fig. 1. J_a, J_b, and J_c are the cycle fluxes, taken positive in the clockwise direction.

When $w_A \approx w_B = w$, $\Omega_b \approx (\Omega_b)_w$, and we obtain the near-equilibrium permeability ratio:

$$(P_f/P_d)_{eq} = \frac{[(n-1)r_A r_B b_A b_B wL + n(r_A + r_B)\pi]}{[r_A r_B b_A b_B wL + (r_A + r_B)\pi]} \tag{6}$$

From Eq. 6 we deduce that

$$n - 1 \leq (P_f/P_d)_{eq} \leq n \tag{7}$$

a result already encountered from considerations about the restrictions imposed on the unidirectional flux ratio of the permeating species in the particular case of single-file transport (Hille and Schwartz, 1978; Kohler and Heckmann, 1979).

From Eqs. 5 and 6, we can express the permeability ratio by

$$P_f/P_d = (P_f/P_d)_{eq}[(\Omega_b)_w/\Omega_b] \tag{8}$$

The term $(\Omega_b)_w/\Omega_b$, a function of w_A and w_B, determines the effective difference between the actual permeability ratio P_f/P_d and the limiting values defined by Eq. 7. From Eqs. A4 and A5 we see that, expressed in terms of common factors containing the water activities, Ω_b is of the form $R_1w_Aw_B + R_2w_A + R_3w_B + R_4$, where $R_1, \ldots, R_4$ are functions of the rate constants. Hence, $(\Omega_b)_w$ is given by $R_1w^2 + (R_2 + R_3)w + R_4$. Therefore, if $w_A = w$ and $w_B < w$, then $(\Omega_b)_w/\Omega_b > 1$, and P_f/P_d can be larger than n. This result is a direct consequence of the fact that, in this discontinuous diffusion process, the permeability coefficients depend on the water activities. We comment on this property below, when comparing this model with the no-vacancy pore model.

We define the uniform pore as the one where

$$\begin{aligned} &k_{i(i+1)} = k_{(i+1)i} = k \text{ (for all } i = 1, 2, \ldots, n-1), \\ &r_A = r_B = k, \text{ and} \\ &b_A = b_B = k/w \end{aligned} \tag{9}$$

where w is the activity of pure water.

Substitution of these definitions (Eq. 9) into Eq. 2 allows us to obtain

$$P_f = \frac{\tilde{N}(k/w)}{\{(n-1)w_Aw_B/w^2 + [(n^2 - n + 2)/2](w_A + w_B)/w + 2n\}} \tag{10}$$

Analogously we obtain, from Eq. 3,

$$P_d = \frac{\tilde{N}(k/w)}{(n^2 + 1)(n + 1)} \tag{11}$$

Therefore, the near-equilibrium osmotic permeability is given, for the uniform one-vacancy pore, by

$$(P_f)_{eq} = \frac{\tilde{N}(k/w)}{(n + 1)^2} \tag{12}$$

From Eq. 6, and also from Eqs. 11 and 12, the near-equilibrium permeability ratio becomes, in this case,

$$(P_f/P_d)_{eq} = (n^2 + 1)/(n + 1) \tag{13}$$

Hence, for a sufficiently large n, the one-vacancy uniform pore model exhibits a near-equilibrium permeability ratio that approximately equals n.

THE ONE-VACANCY SINGLE-FILE PORE: VACANCY STATES ARE TRANSIENT INTERMEDIATES

When the pore states containing the vacancy are transient intermediates, the model in Fig. 1 can be reduced to a diagram comprising only fully occupied states. In Appendix II we perform the model reduction using the methods described in Hill (1977). Fig. 3 shows the resulting reduced diagram. In this scheme, every transition

$N_i \leftrightarrow N_{i+1}$ ($i = 1, 2, \ldots, n - 1$) is of the form

$$N_i \xleftrightarrow{w_A \quad w_B} N_{i+1}$$

The rate constants α, β, and σ have a complex internal structure, given by Eqs. A15, A19, and A20.

The reduced model strictly maintains the basic kinetic relationships of the extended diagram. Vacancies occur with mean lives significantly smaller than the time scale of the model, and although the transport process microscopically takes place by a vacancy mechanism, the pore kinetics can be completely described by considering the apparent constants α, β, and σ.

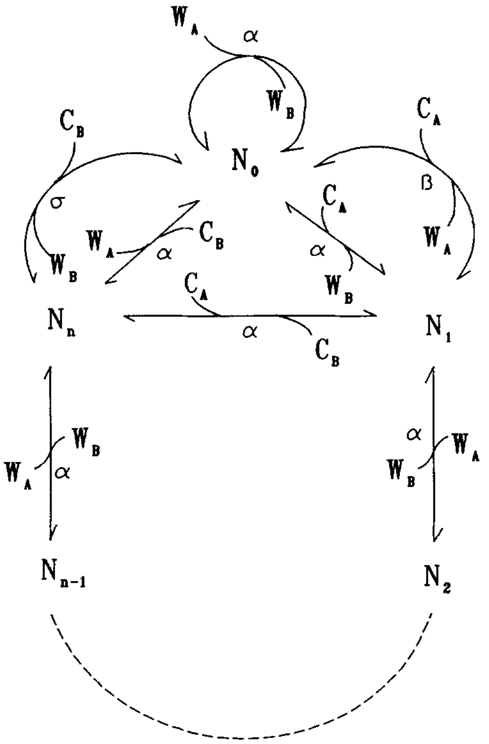


FIGURE 3. The "reduced" kinetic diagram. The rate constants α, β, and σ are defined by Eqs. A15, A19, and A20 (see Appendix II).

From considerations similar to those of the previous section, we obtain for this model the water permeability under the condition that $c_A = c_B = 0$ as

$$P_f = \tilde{N}\alpha \tag{14}$$

Analogously, when $w_A = w_B = w$, we can obtain the equilibrium diffusive permeability:

$$P_d = \frac{[\tilde{N} h_w w^n (\beta_w + \alpha_w)(\sigma_w + \alpha_w)]}{[h_w w^n (\beta_w + \sigma_w + 2\alpha_w) + w^2 (\beta_w + \alpha_w)(\sigma_w + \alpha_w)]} \tag{15}$$

As in the previous case, we have neglected terms containing c_A and/or c_B. In Eq. 15, α_w, β_w, σ_w, and h_w are given by Eqs. A15 and A19–A21 under the condition $w_A = w_B = w$.

From Eqs. 14 and 15 we obtain the permeability ratio

$$P_f/P_d = (P_f/P_d)_{eq}(D_w/D) \tag{16}$$

where $D_w = D$ for $w_A = w_B = w$ (Eq. A11), and where

$$(P_f/P_d)_{eq} = \frac{[\alpha_w(\beta_w + \sigma_w + 2\alpha_w) + (n-1)(\beta_w + \alpha_w)(\sigma_w + \alpha_w)]}{[(\beta_w + \alpha_w)(\sigma_w + \alpha_w)]} \tag{17}$$

Since it can be demonstrated from Eqs. A15, A19, and A20 that α cannot be simultaneously larger than β and σ, Eq. 17 conforms to the restriction contained in Eq. 7. This is a consequence of the fact that although the reduced model only represents no-vacancy states, the basic mechanism of transport remains the same as in the general model of Fig. 1, that is, the generation of vacancies at the pore-compartment interfaces.

It can be demonstrated that the necessary conditions (Eqs. A12 and A16) for model reduction (Hill, 1977, pp. 196–197) are fulfilled if

$$b_A w_A,\ b_B w_B \gg r_A,\ r_B \tag{18}$$

Substitution of the definitions of Appendix II into Eqs. 14 and 15 allows one to obtain the permeability coefficients and permeability ratio of the reduced model in terms of the basic rate constants. Thus, applying Eq. 18, the osmotic and diffusive permeability given by Eqs. 14 and 15 are equivalently expressed by

$$P_f = \tilde{N}\pi/D \tag{19}$$

and

$$P_d = \tilde{N}\pi/[(n-1)D_w] \tag{20}$$

From Eqs. 19 and 20 we see that, for the reduced model,

$$(P_f/P_d)_{eq} = n - 1 \tag{21}$$

Eq. 21 can be obtained by the above mentioned substitution, or by the following direct reasoning. It can be demonstrated that Eq. 18 is also a necessary condition for obtaining the limiting value $(n-1)$ for the permeability ratio in Eq. 6 (see also Kohler and Heckmann, 1979). Therefore we can directly obtain Eq. 21 as the particular value of this expression in the case of the reduced model. In relation to this, it can be demonstrated that Eq. 18 makes the rate constants β_w and σ_w significantly larger than α_w.

The reduced diagram shown in Fig. 3 represents a particular type of behavior of the general one-vacancy mechanism. When Eq. 18 applies, a complete kinetic description of the process can be done by considering only the no-vacancy states. As shown below, this description resembles the one given for the no-vacancy mechanism of transport by the single-file water pore. The significance of the overall rate constants involved is, however, quite different. In the present case of the reduced model, these constants are complicated expressions of the basic rate constants, which is a consequence of the discontinuous nature of the basic process of transport involved in the vacancy mechanism.

KINETIC DESCRIPTION OF THE NO-VACANCY PORE

In the no-vacancy mechanism, molecules present at the mouths of the pore are pushed inside the pore by the pressure effect of the surrounding molecules in each compartment. In turn, since there are no empty spaces inside the pore, the pore contents are compactly displaced as a whole. Therefore, in a simplified scheme of this type of process, one water molecule enters the pore across one mouth and one simultaneously leaves the pore across the other. The situation is depicted in Fig. 4, including the case of a tracer molecule. This transport process constitutes a mechanism analogous to the one considered in such dynamical approaches as those by Levitt (1974) or Finkelstein and Rosenberg (1979). Notice that since no empty spaces exist near the mouths of the pore, entering molecules do not exchange with molecules present at the pore inner extreme positions. This no-vacancy process is also equivalent to the "knock-on" mechanical description first introduced by

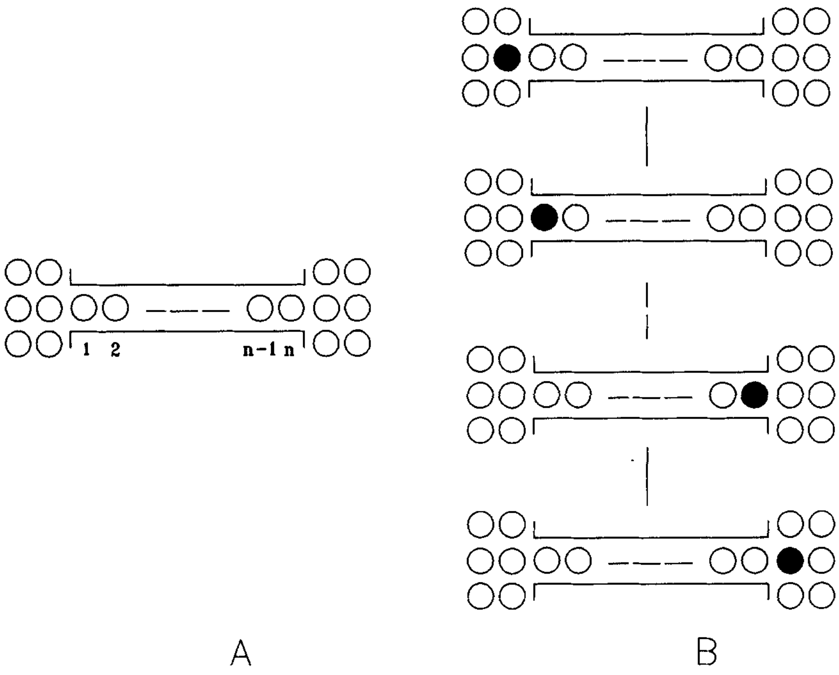


FIGURE 4. The no-vacancy single-file water pore. (*A*) The water molecules occupy positions 1, 2, . . . , *n* inside the pore, analogous to the vacancy pore model of Fig. 1. In this case, no vacancies are generated at the pore-compartment interfaces. (*B*) The process of displacement of a tracer molecule through the pore in the no-vacancy mechanism. As a result of the pressure effect of the surrounding molecules, the tracer is introduced inside the pore at one extreme while a water molecule simultaneously leaves the pore at the other.

Hodgkin and Keynes (1955) to interpret permeability properties of potassium channels.

The kinetic description of this kind of no-vacancy water and tracer transport model is shown in Fig. 5. Since every transition $N_i \leftrightarrow N_{i+1}$ involves the same type of molecular events, the same rate constant is used for all the transitions. We symbolize that constant by α^* to recall the analogy with the reduced one-vacancy model. As in the vacancy models, in order to traverse the pore completely a water or tracer molecule must experience $n + 1$ transitions. In the analysis performed by Finkelstein and Rosenberg (1979), it is assumed that the tracer molecule starts from position 1

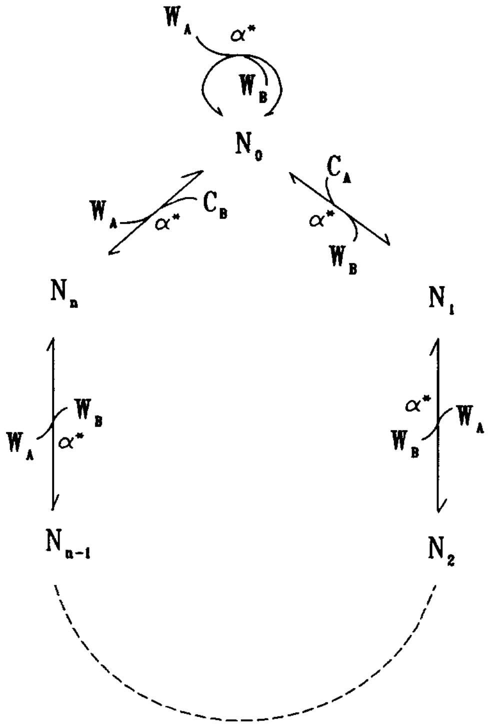


FIGURE 5. Kinetic model of the no-vacancy mechanism of transport of water through a single-file pore.

(or from n), therefore lowering the number of transitions to n. As mentioned above, the kinetic model of Fig. 5 contains pore states analogous to those of the one-vacancy reduced model.

This no-vacancy transport process is therefore characterized by the continuity of the water phase at the molecular level, even inside the pore. The use of a discrete state diagram to represent it deserves a word of caution. Whenever this kind of discrete description is used, some assumptions are implicit (for a discussion on this general problem see Hill, 1977, pp. 57–59). That continuity is what dictates that α^* is independent of the water activities in the compartments (independence principle).

This constitutes a basic difference from the vacancy mechanism (see Eqs. A11 and A15), where the presence of vacancies inside the pore allows for a discontinuity of the diffusion process. As is well recognized (Schultz, 1980), permeability coefficients depend on species activities in this type of transport processes. In contrast, in the no-vacancy mechanism the overall constant α^* obeys the independence principle.

Due to the simplicity of the scheme of Fig. 5, the steady-state diagrammatic analysis can proceed in a straightforward manner. We now show the expressions obtained.

For this model, the osmotic permeability is given by

$$P_f = \tilde{N}\alpha^* \tag{22}$$

The tracer flux is given by the cycle flux of the single cycle involved in the tracer transport in this no-vacancy case. Under the condition $w_A = w_B = w$, the diffusive permeability becomes

$$P_d = \tilde{N}\alpha^*/(n + 1) \tag{23}$$

From Eqs. 22 and 23, the permeability ratio is

$$P_f/P_d = n + 1 \tag{24}$$

From the above considerations, this ratio is independent of the water activities, and therefore remains constant arbitrarily away from equilibrium. As can be seen, this is a consequence of the independence principle (Schultz, 1980, p. 33) that holds for the water permeability coefficients in this no-vacancy mechanism of transport.

Every time an α^* transition takes place, $n + 1$ molecule displacements occur. Hence, we define

$$k^* = (n + 1)\alpha^* \tag{25}$$

where k^* is the number of individual displacements between sequential positions per transition of the state diagram.

From Eqs. 22, 23, and 25 we express the permeability coefficients by

$$P_f = \tilde{N}k^*/(n + 1) \tag{26}$$

and

$$P_d = \tilde{N}k^*/(n + 1)^2 \tag{27}$$

Comparison of Eqs. 10–12 and 26–27 shows the basic difference between the two types of mechanisms. In terms of the dependence upon n, we see that the vacancy mechanism represents a slower process. This is intuitively acceptable, since vacancies cause a larger rate of "distractions" to the molecules moving inside the pore.

Expressions similar to Eqs. 26 and 27 have been previously derived for the water permeability (Finkelstein and Rosenberg, 1979), although in the denominator n appears instead of $n + 1$. As noted above, the difference relates to the fact that n transitions of the water molecules are considered by those authors, while $n + 1$ transitions are shown here to take place in the process. From comparison with the expressions obtained by those authors, the rate constant k^* can be interpreted in terms of the frictional coefficient per water molecule.

DISCUSSION

Membrane proteins have been proposed to act as water pores across biological membranes (Macey, 1984; Parisi and Bourguet, 1985). Some of the possible candidates for that role, like the anion (Solomon, Chasan, Dix, Lukacovic, Toon, and Verkman, 1983) or glucose (Fischbarg, Kuang, Vera, Arant, Silverstein, Loike, and Rosen, 1990) transporters, are complex structures for which a description of the pore properties using a continuum pore model could not represent a good approximation. As shown in this article, the kinetic formalism can be used to represent the water transport process in diverse situations, thus providing a more versatile tool for the interpretation of the water permeability properties of biological membranes. A main advantage of the kinetic formalism is that it permits us to extend derivations arbitrarily far from the near-equilibrium region, fundamentally allowing us to test the validity of the equilibrium approximation in particular situations.

Two different mechanisms of water transport through single-file pores are distinguished here: a one-vacancy and a no-vacancy mechanism. For both of them, the analysis of the kinetic descriptions leads to expressions for the diffusive and osmotic permeability coefficients. One of the main differences is that, in the no-vacancy mechanism, the permeability coefficients obey the independence principle, while in the vacancy mechanism the coefficients are functions of the water activities in the compartments. As a consequence, the permeability ratio also exhibits differences. The far-from-equilibrium analysis of the kinetic expressions derived for the water permeabilities in the one-vacancy model shows that, contrary to what has been suggested (Finkelstein, 1987, p. 54), single-file water transport may exhibit permeability ratios P_f/P_d larger than n. However, in all probability, significant deviations from the rule $P_f/P_d \leq n$ could be achieved given only relatively large differences in water activity. If that is the case, the conclusion could therefore be that the near-equilibrium hypothesis is satisfactory, and that n (or $n + 1$, if permeation is by a no-vacancy mechanism) sets an upper limit to the value of the permeability ratio in the vast majority of situations involving single-file transport, including perhaps physiological ones. It is worth noting that the upper limits of the near-equilibrium permeability ratio obtained here (n, $n + 1$) are the same as those previously derived by Heckmann and Vollmerhaus (1970) from the general analysis of (*a*) one-vacancy and (*b*) no-vacancy (or knock-on) mechanisms of single-file transport, respectively.

The kinetic diagram proposed here to describe the no-vacancy mechanism of transport represents a kinetic analogue of the knock-on mechanical model of Hodgkin and Keynes (1955) and of dynamical models of the type of those by Levitt (1974) and Finkelstein and Rosenberg (1979). In the course of their analysis, the latter authors derive expressions assuming near-equilibrium conditions. As mentioned above, however, since the independence principle holds, the expressions obtained for the permeability coefficients in a no-vacancy model remain valid arbitrarily away from equilibrium. In agreement with this, we show here that it is not possible to derive the kinetic description of the no-vacancy mechanism as a limiting case of the one-vacancy model. The reduction of the one-vacancy diagram in the case where vacancy states are transient intermediates shows that, although the kinetic description only retains the fully occupied states, the apparent rate constants (α, β, and σ) are complicated expressions of the primitive ones. This is not the case in the no-vacancy model, where the overall rate constant (α^*) can be expressed in a simple form in terms of only the total number of molecule displacements between sequential positions per transition.

To the question, "Is water transport through biological pores carried on by a vacancy mechanism?" a possible answer may emerge from applying the above mentioned conclusions, which provide a basis for an experimental distinction between vacancy and no-vacancy mechanisms. What remain to be understood, however, are the basic theoretical aspects of the mechanisms involved in the generation of vacancies. Among other reasons, such an analysis would be crucial for the following possibility: water transport across biological pores could take place by the two basic regimes discussed here, the "hydrodynamic" continuum no-vacancy mechanism and the "diffusive" discontinuous vacancy mechanism. If an ensemble of pores has mixed properties, the permeability coefficients determined macroscopically would then represent a function of all the permeability coefficients typical of the different basic mechanisms of water transport involved. In another case, for example, a pore through which discontinuous water transport mediated by vacancies can take place could also be the site for continuous no-vacancy transport. A unifying formalism is needed that will be able to properly describe the transition between both regimes.

A plausible theoretical model of the kinetic constants of displacement of the water molecules between sequential positions inside the pore, especially one describing their relation with diffusional parameters, is a prerequisite for facing this problem properly. We now sketch speculative guidelines on this subject.

A possible formal dependency of the kinetic constants is of the Arrhenius type:

$$k_\mathrm{d} = k_\mathrm{d}^0 \exp\left(-E_\mathrm{a}/k_\mathrm{B}T\right)$$

where E_a is the activation energy, k_B is Boltzmann's constant, and the preexponential factor k_d^0 could be proportional to an activation energy–independent diffusion coefficient (for a discussion on this topic see Cooper, Gates, and Eisenberg, 1988; see also Zwanzig, 1988). The mean life of the vacancies might depend on the height of the energy barriers between positions. If the activation energy is large in comparison with the thermal energy, the rate constant will exhibit an important dependency on the exponential factor, and the mean life of the vacancy can be relatively long. In this case, discontinuous diffusion takes place. On the other extreme, a high hydrostatic pressure could diminish activation energies significantly. Under this condition, the corresponding rate constant could be of the form of a diffusion coefficient, since it would be affected very little by the exponential term. The overcoming of the energy barriers would then take place as a consequence of the high pressure condition, and the no-vacancy mechanism would dominate. An adequate physical theory of the rate constants, perhaps based on some of these ideas, therefore seems to be necessary in order to account for the basic phenomena involved in the process of water transport.

APPENDIX I

Steady-State Solution of the One-Vacancy Single-File Kinetic Model

We show here the main steady-state expressions obtained from the analysis of the model shown in Fig. 1, using the method developed by Hill (1977). For the application of this method the conservation condition (Hill, 1977, pp. 1–16) is necessarily assumed for the total number $\tilde{N}$ of pore states.

The water flux j_w (A to B positive) is determined by the cycle fluxes J_a, J_b, and J_c (Fig. 2):

$$j_w = (n-2)J_a + (n-1)J_c - J_b, \tag{A1}$$

where the cycle fluxes have been taken positive in the clockwise direction. In particular, when

$$c_A = c_B = 0, J_a = J_c = 0, \text{ and } j_w = -J_b$$

it follows that:

$$j_w = (\tilde{N}\pi/\Omega_b)(w_A - w_B) \tag{A2}$$

where, as mentioned, $\tilde{N}$ is the total number of pores and where, from the detailed balance restriction,

$$\pi = r_A b_B \prod_{i=1}^{n-1} k_{(i+1)i} = r_B b_A \prod_{i=1}^{n-1} k_{i(i+1)}. \tag{A3}$$

The denominator Ω_b is the sum of all the directional diagrams of all the states of the single cycle b. Expressed in terms of factors containing the rate constants of binding and release, Ω_b is given by

$$\begin{aligned}\Omega_b = {} & (b_A w_A r_B + b_B w_B r_A + b_A w_A b_B w_B)L + (r_A + r_B)A \\ & + (b_A w_A + r_A + r_B)\prod_{i=1}^{n-1} k_{i(i+1)} + (b_B w_B + r_A + r_B)\prod_{i=1}^{n-1} k_{(i+1)i} \\ & + b_A w_A r_B\left(\prod_{i=2}^{n-1} k_{i(i+1)} + B\right) + b_B w_B r_A\left(\prod_{i=1}^{n-2} k_{(i+1)i} + C\right)\end{aligned} \tag{A4}$$

where

$$L = \sum_{j=1}^{n-3}\left(\prod_{i=1}^{j} k_{i(i+1)} \prod_{i=1}^{n-j-2} k_{(n-i+1)(n-i)}\right) + \prod_{i=1}^{n-2} k_{i(i+1)} + \prod_{i=2}^{n-1} k_{(i+1)i}$$

$$A = \sum_{j=1}^{n-2}\left(\prod_{i=1}^{j} k_{(i+1)i} \prod_{i=j}^{n-2} k_{(i+1)(i+2)}\right)$$

$$B = \sum_{j=2}^{n-2}\left\{\prod_{i=j+1}^{n-1} k_{i(i+1)}\left[\prod_{i=1}^{j-1} k_{i(i+1)} + \prod_{i=1}^{j-1} k_{(i+2)(i+1)} + \sum_{m=1}^{j-2}\left(\prod_{i=1}^{m} k_{i(i+1)} \prod_{i=m+2}^{j} k_{(i+1)i}\right)\right]\right\}$$

and

$$C = \sum_{j=2}^{n-2}\left\{\prod_{i=1}^{n-j-1} k_{(i+1)i}\left[\prod_{i=n-j+2}^{n} k_{i(i-1)} + \prod_{i=n-j+2}^{n} k_{(i-2)(i-1)} + \sum_{m=1}^{j-2}\left(\prod_{i=n-j}^{n-m-2} k_{i(i+1)} \prod_{i=n-m}^{n-1} k_{(i+1)i}\right)\right]\right\} \tag{A5}$$

The tracer flux is given by

$$j_{tr} = J_a + J_c \tag{A6}$$

When $w_A = w_B = w$

$$J_a = [\tilde{N}Lr_A r_B b_A b_B(\pi w)^{n-1} w(c_A - c_B)]/(\Sigma)_w \tag{A7}$$

and

$$J_c = [\tilde{N}\pi(\pi w)^{n-1}(r_A + r_B)(c_A - c_B)]/(\Sigma)_w \tag{A8}$$

where the thermodynamic restriction of detailed balance has been imposed.

The denominator $(\Sigma)_w$ is the sum of all the directional diagrams of all the states arising from the model of Fig. 1, for the particular case where $w_A = w_B = w$. If we neglect terms containing c_A and/or c_B,

$$(\Sigma)_w = (\Sigma_b)_w(\Omega_b)_w \tag{A9}$$

where $(\Omega_b)_w = (\Omega_b)$ for $w_A = w_B = w$, and where $(\Sigma_b)_w$ is the sum of all the parts of the flux diagrams that feed into cycle b, also for $w_A = w_B = w$. Therefore,

$$(\Sigma_b)_w = (n - 1)r_A r_B(\pi w)^{n-2}D_w + (r_A + r_B)(\pi w)^{n-1} \tag{A10}$$

where $D_w = D$ for $w_A = w_B = w$, with

$$D = b_A w_A b_B w_B L + b_A w_A \prod_{i=1}^{n-1} k_{i(i+1)} + b_B w_B \prod_{i=1}^{n-1} k_{(i+1)i} \tag{A11}$$

APPENDIX II

Reduction of the Diagram for the Case That Vacancy States Are Transient Intermediates

If the vacancy states are transient intermediates, the diagram in Fig. 1 can be reduced to a diagram comprising only the fully occupied states. The methodology used here follows that discussed in Hill (1977), pp. 193–200.

1. Reduction of transition N_i ----- N_{i+1}. The necessary condition for reduction is that, in any situation,

$$P(N_{ij}) \ll P(N_i), P(N_{i+1}) \tag{A12}$$

with $i = 1, 2, \ldots, n - 1$; $j = 1, 2, \ldots, n$, and where $P(\mathrm{X})$ is the probability (frequency) of state X in the total pore ensemble.

From Eq. A12, we may assume that

$$\mathrm{d}\tilde{N}_{ij}/\mathrm{d}t \approx 0 \tag{A13}$$

applies to every state N_{ij}.

From Eq. A13 we can proceed by successive substitutions to obtain $\mathrm{d}\tilde{N}_i/\mathrm{d}t$ and $\mathrm{d}\tilde{N}_{i+1}/\mathrm{d}t$ as explicit functions of $\tilde{N}_i$, $\tilde{N}_{i+1}$, and the intermediate rate constants. From these expressions, the transition flux $J_{i(i+1)}$ can be obtained as

$$J_{i(i+1)} = \alpha(w_A\tilde{N}_i - w_B\tilde{N}_{i+1}) \tag{A14}$$

for $i = 1, 2, \ldots, n - 1$, and where, from detailed balance,

$$\alpha = \pi/D \tag{A15}$$

2. Reduction of transitions N_n ----- N_1, N_n ----- N_0 *and* N_0 ----- N_1. We proceed in a way analogous to that in the previous case. The condition for reduction is

$$P(N_{0j}) \ll P(N_0), P(N_1), P(N_n) \tag{A16}$$

with $j = 1, 2, \ldots, n$.

From Eq. A16 we assume that, for every state N_{0j},

$$\mathrm{d}\tilde{N}_{0j}/\mathrm{d}t \approx 0 \tag{A17}$$

From Eq. A17 we can proceed by successive substitutions to obtain $\mathrm{d}\tilde{N}_0/\mathrm{d}t$, $\mathrm{d}\tilde{N}_1/\mathrm{d}t$, and $\mathrm{d}\tilde{N}_n/\mathrm{d}t$ as explicit functions of $\tilde{N}_0$, $\tilde{N}_1$, $\tilde{N}_n$, and the intermediate rate constants. We show the expressions obtained:

$$\begin{aligned} d\bar{N}_0/dt &= (\beta w_A + \alpha w_B)\bar{N}_1 + (\sigma w_B + \alpha w_A)\bar{N}_n + (\alpha w_A - \alpha w_B)\bar{N}_0 \\ &\quad + (\alpha w_B - \alpha w_A)\bar{N}_0 - [(\alpha + \beta)c_A + (\alpha + \sigma)c_B]\bar{N}_0 \\ d\bar{N}_1/dt &= (\alpha c_A + \mathrm{h}w_B^{n-1})\bar{N}_n + (\alpha + \beta)c_A\bar{N}_0 - [\alpha(c_B + w_B) + \beta w_A + hw_A^{n-1}]\bar{N}_1 \end{aligned}$$

and

$$d\bar{N}_n/dt = (\alpha c_B + hw_A^{n-1})\bar{N}_1 + (\alpha + \sigma)c_B\bar{\mathrm{N}}_0 - [\alpha(c_A + w_A) + \sigma w_B + hw_B^{n-1}]\bar{N}_n \tag{A18}$$

In these expressions, α is given by Eq. A15, and

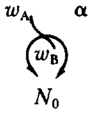
$$\beta = \left[r_A b_A \left(b_B w_B L + \prod_{i=1}^{n-1} k_{i(i+1)}\right) k_{i(i+1)}\right] \Big/ D \tag{A19}$$

$$\sigma = \left[r_B b_B \left(b_A w_A L + \prod_{i=1}^{n-1} k_{(i+1)i}\right)\right] \Big/ D \tag{A20}$$

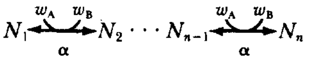
and

$$h = \pi \Big/ \left(D \sum_{i=0}^{n-2} w_A^i w_B^{n-i-2}\right) \tag{A21}$$

In Eq. A18 the expression for $d\bar{N}_0/dt$ explicitly shows the result, obtained in the derivation, that determines the cyclic transition



For the derivation of Eqs. A18–A21, transition



has been assumed to take place in steady state. It has also been assumed that $c_A \ll w_A$ and $c_B \ll w_B$.

Eqs. A15 and A18 allow us to establish the kinetic relationships between the fully occupied pore states in the form of the diagram of Fig. 3.

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