J. Membrane Biol. 52, 161–164 (1980)

The Journal of
Membrane Biology

Anomalous Permeation of a Reversibly Associating Substance: Hydraulic Conductivity and Tracer Water Diffusion

H. Schönert
Institut für Physikalische Chemie, Abteilung Biopolymere, RWTH Aachen, D-51 Aachen, Germany

Summary. The permeability coefficient determined with isotopically labeled solvent (water) and the permeability coefficient determined from the volume flow under the influence of an osmotic gradient are different, if the solvent is considered to be reversibly associated. This is shown by application of the equations of irreversible thermodynamics to systems with an associating substance. An equation is derived which relates the permeability ratio to the average cluster size of the solvent.

The determination of permeability coefficients for the solvent (water) by essentially two different methods, tracer diffusion and osmotically induced volume flow, has led to the concept of water transport through equivalent pores. Since the introduction of this model by Koefoed-Johnsen and Ussing [7] (*see also* [9]), it has been applied and refined by several authors [2, 6, 12, 14]. Critical reviews have been given [12, 13].

An alternative suggestion for the experimental finding, that the two permeabilities are different and that this can be traced back to a water/water interaction [14], has been put forward by Stein [13]: the water diffuses through the membrane in the form of clusters which are breaking and forming at random. It is this model that we want to study within the framework of irreversible thermodynamics.

Theory

The following derivation is based on the treatment of membrane transport processes as given by Kedem and Katchalsky [5]. Therefore, some of their relationships are restated here.

The phenomenological equations for the flux J_w of water and the flux J_s of the solute are:

$$\begin{aligned} J_w &= L_{ww}\Delta\mu_w + L_{ws}\Delta\mu_s \\ J_s &= L_{sw}\Delta\mu_w + L_{ss}\Delta\mu_s \end{aligned} \tag{1}$$

where $\Delta\mu_w$ and $\Delta\mu_s$ are the differences of the chemical potentials μ_w and μ_s of water and solute, respectively, across the membrane. The L_{ik} are the phenomenological coefficients.

For dilute solutions (V_i = partial molar volume of substance i)

$$c_w V_w \approx 1 \tag{2}$$

where the concentration c_s of the solute is small compared to the concentration c_w of water, the $\Delta\mu_i$ can be approximated by

$$\begin{aligned} \Delta\mu_w &= V_w \Delta P - RT\Delta c_s/c_w, \\ \Delta\mu_s &= V_s \Delta P + RT\Delta c_s/c_s. \end{aligned} \tag{3}$$

Here, P is the hydrostatic pressure, R the gas constant and T the absolute temperature. The concentrations c_w and c_s are appropriate averages.

Introducing the volume flux J_v and an exchange flux J_D by

$$\begin{aligned} J_v &= V_w J_w + V_s J_s \\ J_D &= (J_s/c_s) - (J_w/c_w) \end{aligned} \tag{4}$$

the set of Eqs. (3) and (4) constitute a transformation which leaves the entropy production unchanged.

Thus, the phenomenological equations and the phenomenological coefficients are transformed to:

$$\begin{aligned} J_v &= L_P \Delta P + L_{PD}(RT\Delta c_s); \\ J_D &= L_{DP}\Delta P + L_D(RT\Delta c_s) \end{aligned} \tag{5}$$

0022-2631/80/0052-0161 $01.00

and

$$\begin{aligned} L_P &= L_{ww} V_w V_w + 2L_{sw} V_w V_s + L_{ss} V_s V_s; \\ L_{PD} &= L_{DP} = -(L_{ww} V_w/c_w) \\ &\quad + L_{ws}(V_w/c_s - V_s/c_w) + (L_{ss} V_s/c_s), \\ L_D &= (L_{ww}/c_w c_w) - 2(L_{sw}/c_s c_w) + (L_{ss}/c_s c_s). \end{aligned} \tag{6}$$

Here, the Onsager reciprocity relation $L_{ws} = L_{sw}$ has been used. Furthermore, the mobility ω of the solute has been introduced

$$\omega = c_s(L_P L_D - L_{PD} L_{DP})/L_P \tag{7}$$

which, with the help of Eq. (6), can be transformed to

$$\begin{aligned} \omega &= (L_{ww} L_{ss} - L_{ws} L_{sw})/ \\ &\quad \cdot (L_{ww} V_w V_w + 2L_{ws} V_w V_s + L_{ss} V_s V_s) c_s c_w c_w. \end{aligned} \tag{8}$$

Now, if the solute is a tracer to the solvent, there exist several relationships between the phenomenological coefficients of the two isotopes. These can be expressed either in terms of the frictional coefficients [4, 14] or in terms of the generalized conductivity coefficients L_{ik} [10]. In this derivation it is advantageous to use the latter relations. They are:

$$\begin{aligned} L_{ww} &= \frac{c_w^1 c_w^1}{c\,c} L_{ww}^B + \frac{D_w^* c_w^1 c_w^2}{RTc}; \\ L_{ws} &= L_{sw} = \frac{c_w^1 c_w^2}{c\,c} L_{ww}^B - \frac{D_w^* c_w^1 c_w^2}{RTc}; \\ L_{ss} &= \frac{c_w^2 c_w^2}{c\,c} L_{ww}^B + \frac{D_w^* c_w^1 c_w^2}{RTc}. \end{aligned} \tag{9}$$

Here and in the following, we use the superscripts 1 and 2 to denote the two isotopes of the solvent, i.e., we identify c_w^1 with the concentration c_w of the abundant isotope

$$c_w^1 = c_w \quad \text{and} \quad V_w^1 = V_w \tag{10a}$$

and the concentration c_w^2 of the trace isotope with the concentration c_s of the solute

$$c_w^2 = c_s \quad \text{and} \quad V_w^2 = V_s \tag{10b}$$

and c with the total concentration of the constituent

$$c = c_w^1 + c_w^2. \tag{11}$$

The coefficient L_{ww}^B describes the transport of the total constituent under the influence of its own gradient and D_w^* is the tracer-diffusion coefficient measured under the condition $\Delta c = 0$.

Combination of Eqs. (6) and (9)–(11) leads to

$$\begin{aligned} L_P &= V_w V_w L_{ww}^B; \quad L_{PD} = 0; \\ L_D &= c D_w^*/RTc_w^1 c_w^2. \end{aligned} \tag{12}$$

Here, use has been made of the isotope relation $V_w^1 = V_w^2$. This in turn brings us to the consequence that the reflection coefficient $\sigma_w^2 = \sigma$ for the tracer, defined by

$$\sigma_w^2 = -L_{PD}/L_P = 0 \tag{13}$$

vanishes: the membrane has no selectivity towards a tracer of the solvent. This result has only been cited to show the consistency of the relations (9).

Furthermore, if the tracer is added in vanishingly small amounts

$$c_w^2 \ll c_w^1 \quad \text{or} \quad c_w^1 \approx c,$$

the flux of the tracer $J_w^2 = J_s$, as given by

$$\begin{aligned} J_w^2 &= c_s J_v(1-\sigma) + c_s(L_D - \sigma\sigma L_P)(RT\Delta c_s) \\ &= c_w^2 J_v(1-\sigma_w^2) + c_w^2(L_D - \sigma_w^2 \sigma_w^2 L_P)(RT\Delta c_w^2) \end{aligned}$$

reduces to

$$J_w^2 = c_w^2 J_v + D_w^2 \Delta c_w^2 \tag{14}$$

which is just the formula one would expect.

The ratio of the two permeabilities [6, 14] turns out to be

$$L_P/\omega V_w = RTV_w L_{ww}^B/D_w^*. \tag{15}$$

At this point, the assumptions of a reversibly associating substance can be introduced. One assumes that the solute can exist as monomer (subscript $i = 1 = M$), dimer (subscript $i = 2 = D$), and so on. Each of these species is assigned a diffusion coefficient D_i, which is the same for both isotopes. Also, the association-dissociation reactions have to be so fast as compared to the transport processes that local chemical equilibria prevail. The first of these model assumptions means that the transport is a purely diffusional one, and it excludes the quasi-laminar flux which can lead to the observation that the osmotic permeability is some 700 times greater than the diffusion permeability [8]. With these model assumptions one finds [10]

$$\begin{aligned} L_{ww}^B &= (\Sigma\, i\, c_i)(\Sigma\, i^2 c_i D_i)/RT(\Sigma\, i^2 c_i); \\ D_w^* &= (\Sigma\, i\, c_i D_i)/(\Sigma\, i\, c_i) \end{aligned} \tag{16}$$

where the summation extends over all species: monomers, dimers, trimers, etc.

Now we remember: L_{ww}^B describes the osmotically induced total flux of the constituent, whereas D_w^*

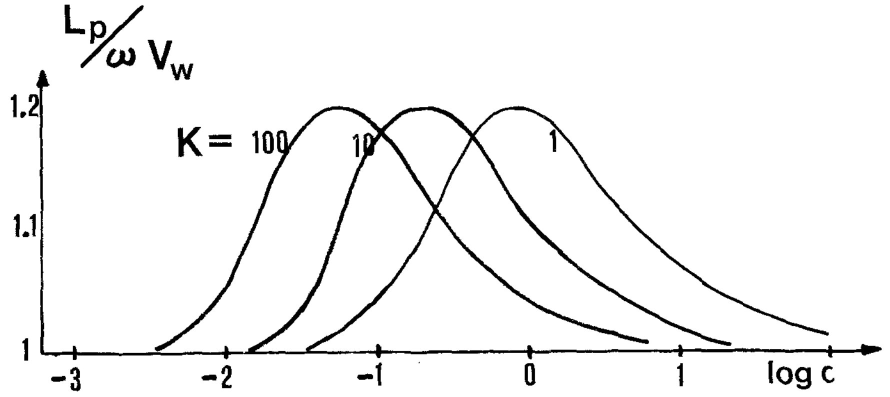

Fig. 1. The permeability ratio, Eq. (19), as a function of concentration c, for a trimerization reaction with $D_M/D_k = 0.5$ and various values of the equilibrium constant K

describes the tracer exchange flux. In osmotically induced transport, all species move all together through the membrane, whereas the tracer may be transported by exchange from cluster to cluster without a movement of the clusters. This different mode of transport is reflected in the different weights given to the species diffusion coefficients D_i in the two parts of Eqs. (16).

Combination of Eqs. (15) and (16) yields

$$L_P/\omega V_w = (\Sigma i^2 c_i D_i)(\Sigma i c_i)/(\Sigma i c_i D_i)(\Sigma i^2 c_i) \qquad (17)$$

if one observes that Eq. (2) is in terms of species

$\Sigma i c_i V_w \approx 1$.

The same result can be obtained if the derivation goes along the lines as given by Kedem *et al.* [6]: use of friction coefficients within the membrane; introduction of the appropriate isotope relations, which are equivalent to Eq. (9); integration across the membrane, and use of distribution coefficients at the membrane-solution interface. This second route, which is much more involved and therefore not reproduced here, shows that the relation (2), which applies to the bathing solutions, is not a necessary assumption within the membrane, *i.e.*, the solvent may be at any concentration within the membrane.

Discussion

If the solvent exists in the form of only one species k, we have $c_i = 0$ for $i \neq k$, and therefore from Eq. (17)

$$L_P/\omega V_w = 1; \quad \text{for } c_k \neq 0;$$
$$c_i = 0 \quad \text{for } i \neq k. \qquad (18)$$

In this case, the two permeabilities are equal as it should be for a diffusion-controlled process.

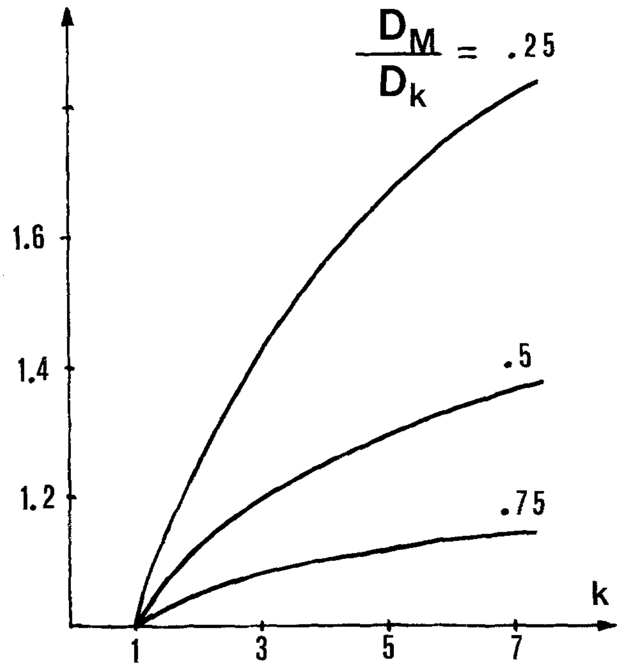

Fig. 2. The maximum permeability ratio as a function of the cluster size k

If besides monomers the main association product is a k-mer, we have from Eq. (17)

$$L_P/\omega V_w = (c_M D_M + k^2 c_k D_k)(c_M + k c_k)/(c_M D_M + k c_k D_k)(c_M + k^2 c_k). \qquad (19)$$

The departure of this expression from unity depends on the ratio D_M/D_k. At this point we take up the suggestion of Stein [13], which is based on the formulation of water transport by Kedem *et al.* [4, 6, 14]: "The drag between water molecules within such a cluster is likely to be substantial and, in particular, to be greater than that between an isolated water molecule and the lipid side chains [13]." This then means within the framework of this model

$D_M/D_k < 1$.

With this condition we can calculate with the help of Eq. (19) that there is

$L_P/\omega V_w > 1.$

The result of this calculation is shown in Fig. 1, where this ratio is plotted as a function of the total solvent concentration c, with $D_M/D_k = 0.5$, $k=3$ and various values of the trimerization constant K. One can see that at infinite dilution, where we have only monomers, and at very high concentrations, where the k-mers dominate exclusively, this ratio is 1 (*see* Eq. (18)), whereas in between it rises to a maximum, the height of which is nearly independent of the value of the association constant.

Figure 2 depicts this maximum as a function of the cluster size k for various values of the parameter D_M/D_k.

This maximum spans a range from 1 to 2 for the permeability ratio, which is the range where the equivalent pore model might not be satisfactorily applied and where the suggestion of Stein [13] could be an alternative explanation for some experimental findings [1, 14].

The support of the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 160) is gratefully acknowledged.

References

1. Cass, A., Finkelstein, A. 1967. Water permeability of thin lipid membranes. *J. Gen. Physiol.* **50**:1765
2. Dainty, J., Ginzburg, B.Z. 1963. Irreversible thermodynamics and frictional models of membrane processes, with particular reference to the cell membrane. *J. Theor. Biol.* **5**:256
3. Goldstein, D.A., Solomon, A.K. 1960. Determination of equivalent pore radius for human red cells by osmotic pressure measurements. *J. Gen. Physiol.* **44**:1
4. Kedem, O., Essig, A. 1965. Isotope flows and flux ratios in biological membranes. *J. Gen. Physiol.* **48**:1047
5. Kedem, O., Katchalsky, A. 1958. Thermodynamic analysis of the permeability of biological membranes to nonelectrolytes. *Biochim. Biophys. Acta* **27**:229
6. Kedem, O., Katchalsky, A. 1961. A physical interpretation of the phenomenological coefficients of membrane permeability. *J. Gen. Physiol.* **45**:143
7. Koefoed-Johnsen, V., Ussing, H.H. 1953. The contributions of diffusion and flow to the passage of D_2O through living membranes. *Acta Physiol. Scand.* **28**:60
8. Mauro, A. 1957. Nature of solvent transfer in osmosis. *Science* **126**:252
9. Pappenheimer, J.R., Renkin, E.M., Borrero, L.M. 1951. Filtration, diffusion and molecular sieving through peripheral capillary membranes. *Am. J. Physiol.* **167**:13
10. Schönert, H. 1978. Transport of isotopes in systems with reversibly reacting species. I. General equations. *Ber. Bunsenges. Phys. Chem.* **82**:726
11. Schönert, H. 1978. Transport of isotopes in systems with reversibly reacting species. II. Binary and ternary non-electrolyte solutions with association reactions. *Ber. Bunsenges. Phys. Chem.* **82**:731
12. Solomon, A.K. 1968. Characterization of biological membranes by equivalent pores. *J. Gen. Physiol.* **51**:335
13. Stein, W.D. 1967. The Movement of Molecules across Cell Membranes. Academic Press, New York
14. Thau, G., Bloch, R., Kedem, O. 1966. Water transport in porous and non-porous membranes. *Desalination* **1**:129

Received 13 April 1979; revised 2 October 1979