THE KINETICS OF OSMOTIC TRANSPORT THROUGH PORES OF MOLECULAR DIMENSIONS

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ABSTRACT This paper presents a theoretical analysis of the kinetics of osmotic transport across a semipermeable membrane. There is a thermodynamic connection between the rate of flow under a hydrostatic pressure difference and the rate of flow due to a difference in solute concentration on the two sides. One might therefore attempt to calculate the osmotic transport coefficient by applying Poiseuille's equation to the flow produced by a difference in hydrostatic pressure. Such a procedure is, however, inappropriate if the pores in the membrane are too small to allow molecules to "overtake." It then becomes necessary to perform a statistical calculation of the transport coefficient, and such a calculation is described in this paper. The resulting expression for the number of solvent molecules passing through a pore per second is

$$J = m \; D_1 \; \delta n_1 / l^2$$

where m is the number of solvent molecules in the pore, l is the length of the pore, D_1 is the self-diffusion coefficient of the solute, and δn_1 the difference in solvent mole fraction on the two sides of the membrane. This equation is used for estimating the number of pores per unit area of the squid axon membrane; the result is 6×10^9 pores/cm^2.

1. INTRODUCTION

Few phenomena are so well understood thermodynamically, or so ill understood kinetically, as the osmotic flow of a solvent through a semipermeable membrane. As a result, measurements of osmotic transport across biological membranes have yielded disappointingly little information about the microscopic structure of such membranes, in spite of the comparative abundance of experimental data. In this paper we discuss the transport coefficient for osmotic flow in the light of non-equilibrium thermodynamics. In principle one could calculate this coefficient either by considering flow under a hydrostatic pressure difference or under a difference in solvent activity on the two sides of the membrane, as there is a thermodynamic connection between the rates of the two processes. This connection underlies the concept of osmotic pressure, and we explain it in section 2. In section 3 we describe a hydrodynamic calculation of the osmotic transport coefficient, based on Poiseuille's equation, but suggest that such a calculation is misconceived if the pores in the membrane are so narrow that solvent molecules cannot "overtake" inside a pore. In section 4 we consider this case of exceedingly narrow pores and attempt to calculate the osmotic transport coefficient from statistical first principles. The result is an equation of the diffusion type, and in section 5 we discuss the validity of this equation and apply it to the squid axon membrane, obtaining an order of magnitude estimate of the number of pores per square centimeter of the resting membrane.

2. THERMODYNAMIC CONSIDERATIONS

In the thermodynamic theory of irreversible processes a key quantity is the rate of production of entropy (1). We therefore begin by calculating the increase in entropy which accompanies the transfer of a mole of solvent from one solution to another through the pores in a semipermeable membrane.

Suppose that we have two solutions separated by a semipermeable membrane which allows the passage of solvent but not solute. The solutions are at the same temperature, T, but their hydrostatic pressures p' and p'' may be different. Now support that dN solvent molecules pass from the first solution to the second, with accompanying volume changes dV' and dV'' on the two sides. By the first law of thermodynamics the increase in energy of the whole system is

$$dE = -p'\,dV' - p''\,dV''. \tag{2.1}$$

The second law, applied to each solution separately, gives

$$dE' = T\,dS' - p'\,dV' - \mu_1'\,dN, \tag{2.2}$$

and

$$dE'' = T\,dS'' - p''\,dV'' + \mu_1''\,dN, \quad \text{where} \tag{2.3}$$

μ_1' and μ_1'', are the solvent chemical potentials in the first and the second solution, respectively. Setting dE equal to $dE' + dE''$, we obtain the total entropy increase as

$$dS = dS' + dS'' = (\mu_1' - \mu_1'')\,dN/T. \tag{2.4}$$

Differentiating both sides with respect to time we obtain the rate of production of entropy as

$$dS/dt = JX, \tag{2.5}$$

$$\text{where} \quad J = dN/dt \quad \text{and} \quad X = (\mu_1' - \mu_1'')/T = \delta\mu_1/T. \tag{2.6}$$

Osmosis will occur, then, if the solvent has unequal chemical potentials on the two sides, and if the difference is small, J will be proportional to X:

$$J = LX = L\,\delta\mu_1/T. \tag{2.7}$$

The coefficient L is the osmotic transport coefficient.

Now a discrepancy between the values of μ_1' and μ_1'' may arise from either or both of two different causes: a difference in solute mole fraction or a difference between hydrostatic pressures p' and p''. Denoting mole fractions of solvent and solute by n_1 and n_2, and assuming the solutions to be ideal and dilute, we may write

$$\delta\mu_1 = v_1\ \delta p + kT\ \delta \log n_1 = v_1\ \delta p - kT\ \delta n_2, \tag{2.8}$$

where v_1 denotes the volume of the solvent per molecule. Therefore

$$J = L(v_1\ \delta p - kT\ \delta n_2)/T, \tag{2.9}$$

and J will vanish if and only if

$$\delta p = (kT/v_1)\delta n_2. \tag{2.10}$$

Equation (2.10) is the classical thermostatic formula for the osmotic pressure; i.e., the hydrostatic pressure difference needed to balance a given difference in solute concentration. But the important equation for our purposes is equation (2.9), which shows that L may be determined by either of two distinct methods: either by finding the ratio of J to δp when $\delta n_2 = 0$, or by finding the ratio of J to δn_2 when $\delta p = 0$.

3. HYDRODYNAMIC AND DIFFUSIONAL THEORIES

Various authors (2–6) have suggested that one might determine L by considering the case $\delta n_2 = 0$ and calculating the rate of flow under a hydrostatic pressure difference δp by classical hydrodynamics. For a cylindrical pore of radius r and length l, Poiseuille's equation gives the rate of flow in cm^3 per second as

$$Jv_1 = \pi r^4\ \delta p/8l\eta_1, \tag{3.1}$$

where η_1 is the viscosity of the solvent. Comparison with equation (2.9) then gives the osmotic transport coefficient as

$$L = \pi r^4 T/8\, l v_1^2 \eta_1. \tag{3.2}$$

But Poiseuille's equation is derived by investigating the velocity profile of a continuous fluid flowing through a cylindrical tube. If the tube is so small that molecules cannot overtake one another inside it, then one can scarcely speak of the variation of velocity across the tube. So for pores which have radii nearly as small as the molecular radius of the solvent, equation (2.12) loses all physical justification.

The unreliability of a hydrodynamic calculation for such small pores has led a number of authors (3, 5, 7, 8) to try and estimate L from a "diffusional" model of osmosis. The simplest model of this kind is perhaps the following. One assumes that there is no hydrostatic pressure difference between the two solutions, but notes that if there is a difference of solute concentration, there will necessarily be a difference in solvent activity a_1 between the two ends of the pore. In actual fact

$$\delta a_1 = -\delta n_2/v_1, \tag{3.3}$$

where v_1 is the volume per molecule of the solvent. If one now thinks of the solvent molecules diffusing along the pore under the influence of their activity gradient one might guess that the solvent current would satisfy the diffusional equation

$$J = \pi r^2 D_1 \, \delta a_1/l \tag{3.4}$$

$$= -\pi r^2 D_1 \, \delta n_2/lv_1, \tag{3.5}$$

where D_1 is a suitably defined diffusion constant, which might be taken to be the self-diffusion constant of the solvent. Comparison with equation (2.9) gives a second estimate of the osmotic transport coefficient L, namely

$$L = \pi r^2 D_1/klv_1. \tag{3.6}$$

The arguments leading to this result look even more shaky than those used for deriving equation (3.2). It is imperative, therefore, to try to obtain L from a sound statistical theory, for the case of pores which are comparable in radius to the solvent molecules themselves. We now present such a theory.

4. A STATISTICAL CALCULATION OF *L*

We treat the membrane as an inert partition, punctuated by a number of very small pores which allow the passage of solvent molecules, not more than one abreast, but not solute molecules. Our problem is to calculate the osmotic transport coefficient L defined in section 2. To do this we make use of a general relation (10) which expresses the value of any transport coefficient as a correlation integral referring to an equilibrium ensemble. For a dissipative process involving a single flux–as in our case–the coefficient L is given by

$$L = k^{-1} \int_0^\infty \langle \dot{\alpha}(0)\dot{\alpha}(t) \rangle \, dt \tag{4.1}$$

where k is Boltzmann's constant, $\dot{\alpha}$ is the instantaneous value of the flux J, and the notation $\langle \ldots \rangle$ implies an average over the members of an equilibrium ensemble. The possibility of writing L in this form depends on being able to find a function $\alpha(x_1, x_2, \ldots)$ whose time derivative is the instantaneous value of J. We must find such a function.

We require α to increase by unity for every solvent molecule transferred from the first solution to the second. A function of this kind may be defined as follows. Considering a single pore, let us measure the x coordinate of each molecule from the left-hand end of the pore, and define for each solvent molecule a number β_i, as follows: β_i is 0 if the molecule is in the first solution (which we take to be on the left), and is 1 if the molecule is in the second solution (which we take to be on the right); for a molecule in the pore β_i is assigned the value x_i/l, and this increases from 0 to 1 as the molecule moves along the pore from left to right. The function

$\alpha(x_1, x_2, \ldots)$ is then defined as the sum of all the β_i, and its time derivative is clearly the flux $J = dN/dt$, as required. We are now ready to calculate L.

We begin by nothing that only molecules which are actually inside the pore will contribute anything to α, so that L may be written

$$L = k^{-1} \int_0^\infty \langle \sum_i \dot{\beta}_i(0) \sum_j \dot{\beta}_j(t) \rangle \, dt$$

or

$$L = k^{-1} l^{-2} \int_0^\infty \langle \sum_i \dot{x}_i(0) \sum_j \dot{x}_j(t) \rangle \, dt, \tag{4.2}$$

where each sum is restricted to the molecules in the pore. The integrand in equation (4.2) is awkward as it stands, but it can be simplified very greatly by the following argument. Each molecule in the pore suffers two kinds of collision–with the walls and with other molecules proceeding along the pore. If two solvent molecules collide with one another, the sum of their velocities is unaffected by the collision, so that in calculating the integrand in equation (3.2) it is legitimate to ignore such collisions and regard the molecules as proceeding independently along the pore, though of course each collides continually with the lining of the pore. Assuming, then, that

$$\langle \dot{x}_i(0) \dot{x}_j(t) \rangle = 0, \qquad i \neq j, \tag{4.3}$$

we may reduce L to the simpler expression

$$\begin{aligned} L &= k^{-1} l^{-2} \int_0^\infty \langle \sum_i \dot{x}_i(0) \dot{x}_i(t) \rangle \, dt \\ &= k^{-1} l^{-2} m \int_0^\infty \langle \dot{x}_1(0) \dot{x}_1(t) \rangle \, dt, \end{aligned} \tag{4.4}$$

where $\dot{x}_1$ is the velocity of a typical solvent molecule in the pore and m is the total number of molecules in the pore.

At this point we pause in the derivation and remark that the integral on the right-hand side of the equation (4.4) is identical with that which occurs in the statistical expression for the diffusion coefficient of a solute in dilute solution, namely (11)

$$D = \int_0^\infty \langle \dot{x}(0) \dot{x}(t) \rangle \, dt, \tag{4.5}$$

where $\dot{x}$ is the x component of the instantaneous velocity of a typical solute molecule relative to the hydrodynamic velocity of the surrounding medium. It is therefore appropriate to write

$$L = m D_1 / k l^2, \tag{4.6}$$

where

$$D_1 = \int_0^\infty \langle \dot{x}_1(0)\dot{x}_1(t)\rangle \, dt \tag{4.7}$$

is the "diffusion coefficient" of a single solvent molecule along the pore.

Returning to the calculation of J, we obtain the flux through the pore in molecules per second as

$$J = (mD_1/kl^2)(v_1\delta p - kT\delta n_2)/T. \tag{4.8}$$

which becomes, when there is no hydrostatic pressure difference between the two solutions.

$$J = -mD_1\delta n_2/l^2. \tag{4.9}$$

5. GENERAL REMARKS

There is a close resemblance between our statistically derived equation (4.9) and the equation (3.5) which emerged from a plausibility argument based upon a diffusion model of osmosis. The two equations are (4.9)

$$J = -mD_1\delta n_2/l^2 \tag{5.1}$$

and (3.5)

$$J = -\pi r^2 D_1\delta n_2/lv. \tag{5.2}$$

In the former equation m denotes the number of molecules in the pore. In the latter, which may be written

$$J = -(\pi r^2 l/v_1)D_1\delta n_2/l^2,$$

the term $\pi r^2 l/v_1$ is the volume of a pore divided by the molecular volume of the solvent. Equation (3.5) therefore follows from equation (4.9) if one supposes that the pore is "full of solvent at its usual density." For very small pores, then, equation (3.5) has a better basis than we realized in section 3.

It is important to notice that one cannot apply both the hydrodynamic equation (3.1) and the diffusional equation (3.5) to the same membrane. The reason is that according to equation (3.1)

$$L = \pi r^4 T/8lv_1^2\eta_1, \tag{5.3}$$

whereas equation (3.5) implies that

$$L = \pi r^2 D_1/klv_1. \tag{5.4}$$

For these two equations to agree, r must have the value

$$r_1 = (8D_1v_1\eta_1/kT)^{1/2}; \tag{5.5}$$

in other words, unless the pores happen to have this particular radius the two approaches will give thermodynamically inconsistent results. This in turn raises the question what value to take for D_1. We suggest that D_1 is likely to be of the same order of magnitude as the self-diffusion coefficient of the solvent. A water molecule diffusing through a pore in a plasma membrane will probably be subject to forces and impulses similar to those which it experiences in liquid water. If this were not so, the pore would either not admit water, or would bind it so strongly that diffusion could not occur. We therefore put D_1 equal to the self-diffusion coefficient of the solvent.

For water at 25°C D_1 has the value 2.5×10^{-5} cm^2/sec. Inserting this value into equation (5.5) we obtain $r_1 = 4.5$ A, which is more than twice the radius of a water molecule. Pores with a smaller radius than this will transport water by an essentially diffusional mechanism; pores of greater radius by a hydrodynamic mechanism. It is interesting, if mildly inconvenient, that 4.5 A is rather close to published estimates of the pore radii of a number of biological membranes. It is also interesting that for collodion membranes the diffusional type of theory has been found wanting (9); this indicates that at least some of the holes in a collodion membrane have a radius larger than 4.5 A.

6. AN APPLICATION

Perhaps the best use that can be made of our equation (4.9), or the related equation (5.4), is for calculating the number of pores per unit area of a plasma membrane, in cases where the pore length l and capacity m can be determined by other means, and the pores are known to be narrow enough for the diffusional approach to be the right one. A good example is the squid axon membrane, in its resting state. The membrane thickness l is approximately 80 A (13), and the pore radius, estimated from the sizes of those solutes which will penetrate the membrane, is about 4 A (14). The permeability coefficient for water, defined as the number of cm^3 crossing 1 cm^2 per sec at a solute concentration difference δc_2 of 1 osmol per cm^3, is (13) 2.0×10^{-2}. Defining Z as the number of pores per cm^2 of membrane, we may write

$$Z J v_1 = -P_w \delta c_2 = -P_w \delta n_2 / N v_1, \tag{6.1}$$

where Nv_1 is the molar volume of water. Comparing this with equation (5.3) we deduce that

$$Z = P_w l / \pi r^2 N v_1 D_1; \tag{6.2}$$

setting $P_w = 0.02$, $l = 8 \times 10^{-7}$, $r = 4 \times 10^{-8}$, $D_1 = 2.5 \times 10^{-5}$, we obtain

$$Z = 6 \times 10^9 \text{ cm}^{-2}, \qquad Z\pi r^2 = 3 \times 10^{-5},$$

the latter figure representing the fraction of the membrane surface that is covered by pores. It is interesting to compare this number with the measured ratio of the specific resistances of sea water and the resting membrane, namely (12) 4×10^{-8}; the apparent discrepancy may be accounted for by supposing that the liquid in the pores (if it can properly be described as a liquid) has a specific conductivity only 1/750 that of sea water. Perhaps it would be less misleading just to say that the ratio of ions to water molecules seems to be very much smaller in the pores than in the ambient medium, when the membrane is at rest.

Received for publication 5 January 1965.

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