A Method for the Determination of the Filtration Coefficient for Spherical Bilayers Prepared from Bulk Erythrocyte Membrane Lipids

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Abstract. Water transport through spherical lipid bilayers consisting of bovine red cell lipids has been studied. A new experimental method for the determination of volume flow induced by concentration gradient is described. Changes of the filtration coefficient indicate phase transition in membrane lipid taking place at 33—36 °C.

Key words: Water transport — Spherical bilayer membrane — Erythrocyte lipids

Introduction

Changes in the permeability to water of lipid bilayers may provide information about membrane structure (Sha'afi 1973; Carruthers and Melchior 1983). One simple model which predicts water permeability of a lipid bilayer is the liquid hydrocarbon model (Vreeman 1966; Finkelstein and Cass 1967). According to this model the membrane is viewed as a thin slice of hydrocarbon liquid. Water passes through such a membrane by dissolving in the nonpolar interior and diffusing across it, just as it would through hydrocarbon liquid. Several studies on lipid bilayers have indicated that the water permeability coefficient is in reasonable agreement with the predictions of the liquid hydrocarbon model (Fettiplace and Haydon 1980). Recent studies by Petersen (Petersen 1980, 1983) have shown that the water permeability of a monooleine/n-hexadecane planar bilayer membrane differed significantly from the values predicted by the model, and also from the measured water permeability through hydrocarbon liquid. The inferred source of this discrepancy was the greater degree of organization of hydrocarbon chains in a lipid bilayer than in hydrocarbon liquid.

In recent years the water permeability of lipid bilayers has been widely studied by several methods: radioactive tracer movement (Sha'afi 1973; Macey 1979;

Fettiplace and Haydon 1980; Anderson 1973), osmotic volume changes in liposomes (Carruthers and Melchior 1983), and volume flow measurements through planar lipid bilayers (Fettiplace and Haydon 1980; Petersen 1983).

The present method of estimation of volume flow through a spherical lipid bilayer is related to the technique presented by Waldbilling and Szabo (1979), based on osmotically induced surface changes of a planar membrane. Because of the small surface area of such membranes (2—5 mm^2), the error of the estimation of the surface area, and of volume changes may be large. Moreover, the surface area of a planar membrane is restricted in view of the possibility of bursting. The method presented in this paper eliminates those difficulties and allows the determination of volume flow through spherical lipid bilayers with a large surface area (of about 1 cm^2).

Materials and Methods

Lipids

Fresh heparinised bovine blood was used. Plasma and leukocytes were removed. Erythrocytes were washed four times with an isotonic phosphate buffer-NaCl solution, pH = 7.4. The lipids were extracted from erythrocytes with *n*-butyl alcohol at 0 °C, according to Dodge et al. (1963).

Formation of spherical black membranes

Spherical black membranes were formed at room temperature in an aqueous KCl solution of lipids dispersed in *n*-decane and *n*-butanol, at a ratio 1 : 1 (v : v), to obtain a concentration of 10—30 mg/ml. The membranes were prepared according to the technique of Schagina et al. (1976) on a teflon capillary tube in a KCl concentration gradient $C_2/C_0 = 10$ where C_0 and C_2 are the KCl concentrations inside and outside the membrane, respectively; $C_0 = 0.01$ N KCl (Fig. 1) which induced membrane shrinkage. The membranes were stable enough to permit an up to five-fold decrease of the volume limited by the membrane (during 10—14 hours).

Determination of water filtration coefficient

The filtration coefficient was determined from volume flow through the membrane induced by different KCl concentrations on either side of the membranes. Changes of volume limited by the membrane were determined from geometrical changes of the membrane dimensions measured by a microscope equipped with a metric eyepiece. The system considered here consisted of three compartments (Fig. 1): a space under the teflon capillary with the KCl concentration $C_0(1)$, a space limited by the spherical membrane with a KCl concentration of $C(2)$, an aqueous medium with a KCl concentration of $C_2(3)$. We assume that volumes 1 and 3 were much greater than volume 2. In the first approximation we considered all compartments as homogenous. According to the definition of mass transport we have:

$$\frac{dn}{dt} = \frac{dn_{32}}{dt} - \frac{dn_{21}}{dt} \tag{1}$$

$$-\frac{dn_{21}}{dt} = S_0 P_1 (C - C_0) \tag{2}$$

$$\frac{dn_{32}}{dt} = SP(C_2 - C) \tag{3}$$

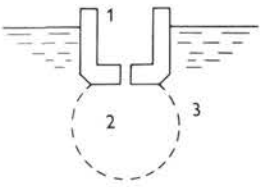

Fig. 1. A schematic outline of the system used to study volume flow in spherical membranes. 1,2 and 3 are solution compartments separated by the capillary and the membrane. We assume that compartments 1 and 3 are large and stable as compared to compartment 2.

were dn/dt — total mass flow, dn_{21}/dt and dn_{32}/dt — mass flow from compartment 2 to 1, and from compartment 3 to 2, S — membrane surface area, S_0 — crossection of the teflon capillary connecting compartments 1 and 2 (0.5 mm^2), $P_1 = D/l$ — permeability coefficient for KCl through the capillary, l — length of the capillary (3.2 mm), D — diffusion coefficient for KCl in water, $D = 2 \times 10^{-5}$ cm^2/s (Koryta et al. 1975). Therefore, $P_1 = 6.25 \times 10^{-5}$ cm/s, P — permeability coefficient for KCl through the membrane (4×10^{-10} cm/s, Pagano and Thompson 1968). Under the conditions of our experiments $S_0P_1 > SP$; we could therefore neglect KCl flow through the membrane, hence:

$$\frac{dn}{dt} = -\frac{dn_{21}}{dt} = S_0P_1(C - C_0) \tag{4}$$

since $n = CV$:

$$\frac{dC}{dt} + \left(\frac{dV}{Vdt} - \frac{P_1S_0}{V}\right) \cdot C + \frac{C_0S_0P_1}{V} = 0 \tag{5}$$

where V — volume of the space limited by the membrane, t — time. This differential equation has time-dependent coefficients since $V = V(t)$. With a good approximation (correlation coefficient $r > 0.99$) we have assumed that the volume of compartment 2 is a linear function of time:

$$V = at + b \tag{6}$$

Coefficients a and b were determined by the least squares method from the experimental data. On introducing equation (6) into equation (5) and with the boundary condition $C(t = 0) = C_0$, we obtained the solution of equation (5) in the form:

$$C(t) = C_0 \cdot \left(1 + \frac{S_0P_1}{b} \cdot t \exp(W)\right) \exp\left(-W \ln \frac{at + b}{b}\right) \tag{7}$$

were $W = 1 - S_0P_1/a$.

$C(t)$ represents the time-dependence of the KCl concentration in space 2.
A linear equation of nonequilibrium thermodynamics (Mason and Viehland 1978; Mikulecky 1969; Rothschild et al. 1980) was used to estimate the filtration coefficient. According to the Kedem-Katchalsky equation (Katchalsky and Curran 1967) we may write:

$$J_v = L_p(\Delta P - \sigma \Delta \pi) \tag{8}$$

where J_v — volume flow, L_p — filtration coefficient,

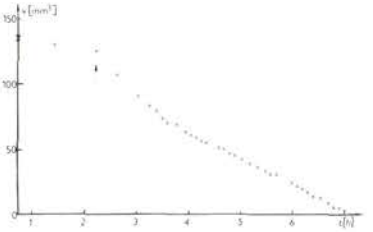

Fig. 2. The volume of the compartment limited by the membrane (2) plotted against time. The arrow indicates the moment when the membrane bilayer formation starts. Results were obtained in a single experiment.

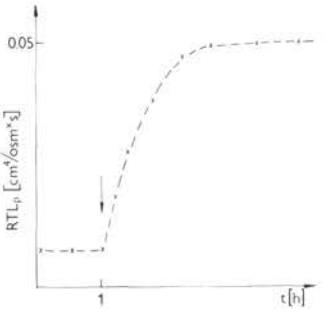

Fig. 3. The RTL_p plotted against time for a single membrane. The arrow indicates the moment when the membrane bilayer formation starts.

ΔP — pressure associated with membrane curvature, $\Delta\pi$ — osmotic pressure, σ — reflection coefficient. It was determined experimentally that ΔP was much smaller than the osmotic pressure, and assuming that the solvent flow through the membrane was negligible, then $\sigma = 1$. Under these conditions equation (8) can be written as:

$$J_v = \frac{dV}{dt} \cdot \frac{1}{S} = -L_p \Delta\pi = -L_p RT(C_2 - C(t)) \tag{9}$$

where R — gas constant, T — absolute temperature, dV/dt — experimentally measured volume flow, and $C(t)$ was calculated from equation (7). Equation (9) finally yields the filtration coefficient.

Results

The data presented in this paper were obtained on a single representative membrane, but the results obtained on other membranes were qualitatively identical.

Figure 2 illustrates how volume, limited by the membrane, changed over time. From this figure it is evident that when the "blacking" of the membrane starts (an

Table 1. Water filtration coefficient (L_p) for erythrocytes and artificial membranes. R — gas constant, T — absolute temperature. The RTL_p is expressed in $cm^4 osm^{-1} s^{-1}$.

Membrane	RTL_p	References
Human erythrocyte	0.41	Farmer and Macey (1970)
Bovine erythrocyte	0.45	Farmer and Macey (1970)
Chicken erythrocyte	0.015	Farmer and Macey (1970)
Lipids from bovine erythrocytes	0.05 ± 0.015	Farmer and Macey (1970) present paper
Egg phosphatidylcholine to cholesterol molar ratio:		
1:2	0.23—0.29	Finkelstein and Cass (1967)
1:8	0.11—0.34	Finkelstein and Cass (1967)

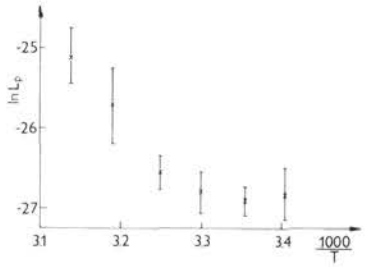

Fig. 4. The logarithm of the filtration coefficient plotted against $1/T$. The filtration coefficient is expreessed in $cm^3 N^{-1} s^{-1} \times 10^{-5}$. The values are means $\pm$ S.D. from at least five experiments.

arrow in the figure indicates this moment) volume flow rapidly increases, thus indicating an increase of the filtration coefficient. This parameter reached its maximal value and became stable when bilayer formation was complete. Changes in the filtration coefficient during the formation of the black membrane are shown in Figure 3. Initially the value of the filtration coefficient was less than 1.0×10^{-8} cm^3 N^{-1} s^{-1}. During bilayer formation it increased to a value of $(2.05 \pm 0.39) \times 10^{-7}$ cm^3 N^{-1} s^{-1}. It was not possible to measure volume flow accurately when the membrane was thick, because the membrane was not stable in this state, and the associated time interval was too short to allow accurate measurements of volume flow. Consequently, this value varied from membrane to membrane. But the process of bilayer formation was in all cases similar. The obtained value of RTL_p (given as mean $\pm$ S.D. for eight membranes) at room temperature is displayed in Table 1 in relation to results obtained elsewhere.

Figure 4 shows the logarithm of the filtration coefficient plotted against $1/T$. Each point in this figure is a mean value ($\pm$ S.D.) for 5—6 measurements made on