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THE EFFECT OF SURFACE CHARGE ON THE WATER PERMEABILITY OF PHOSPHOLIPID BILAYERS

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SUMMARY

1. Bilayers have been made from phosphatidylcholine and phosphatidylserine.

2. By means of an osmotic technique, the water permeability coefficient P_{os} has been measured for different values of pH.

3. The value of P_{os} for phosphatidylcholine bilayers ($1.8 \cdot 10^{-5}$ m$\cdot$s^{-1}) does not vary with pH within the range of 3.0–7.4.

4. The value of P_{os} for phosphatidylserine varies from $0.86 \cdot 10^{-5}$ m$\cdot$s^{-1} at pH 3.0 to $1.25 \cdot 10^{-5}$ m$\cdot$s^{-1} at pH 9.0.

5. The increase in P_{os} with pH for phosphatidylserine bilayers has been interpreted in terms of an increase of configurational freedom of hydrocarbon chains resulting from an increase in surface area per molecule.

6. The large difference between the phosphatidylcholine value for the water permeability coefficient and the phosphatidylserine values is interpreted in terms of differences in hydrocarbon chain unsaturation.

INTRODUCTION

Lipid membrane 'models' have recently been used to investigate membrane phenomena normally inaccessible to experiment in natural systems, on account of technical reasons or simply biological complexity. Two model systems are; (1) the phospholipid vesicles, which consist of one or several phospholipid bilayers and (2) the black lipid bilayer, a single bilayer separating two aqueous phases. Each system has its advantages and disadvantages. Phospholipid vesicles of known composition can be prepared in large quantities, free of solvents; but until recently non-uniformity of size and number of bilayers have hampered interpretations. However, the vesicle systems have now been characterised[1–5] and single shelled vesicles extracted, although the difficulty of inhomogeneity of size of vesicles still remains. The black lipid bilayer is usually formed from solutions of phospholipids in solvents foreign to natural membranes and has composition which is not well understood. However, its convenient size and physical structure allows direct measurements to be made of solute and

Abbreviation: PD, potential difference.

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volume flows across it. These models have been extensively reviewed by Bangham[6] and Goldup *et al.*[7].

In studies of water permeability through lipid bilayers, the most important advances have been the demonstrations that the high permeability coefficients are in the range of values for biological membranes and that permeation does not necessarily take place through water-filled pores[8,9,10]. Though it has been shown that water permeability depends on the phospholipids used[11] and the surface zeta potential[12], it has not been possible to say to what extent observed differences in permeability are due to difference in polar group charges, or hydrocarbon chain composition or both.

The present study investigates the dependence of the water permeability of bilayers on the net surface charge. The net surface charge was varied by varying the pH of the aqueous phases. To minimise the effect of unstirred layers close to the bilayer[13], the water permeability coefficient, P_{os}, was determined by the osmotic gradient technique.

MATERIALS

The phosphatidylserine was a gift from Dr H. Hauser (Unilever Research Laboratory, Welwyn). The phosphatidylcholine was extracted from egg yolks and purified on an alumina column by a method similar to that of Singleton *et al.*[14]. Both lipids gave a single spot by thin layer chromatography (Merck silica gel F_{254}, 0.25 mm plates; solvent system used was chloroform–methanol–7 M ammonia solution (230:90:15, by vol.)). All solutes were A.R. grade. Light petroleum (Fisons fraction 60–80 °C) and *n*-decane (Koch Light) were passed through alumina before use. The water was twice distilled in an all-glass still.

METHODS

Black lipid bilayers were formed in a horizontal plane by the use of the brush technique[15]. The apparatus used was of the type described by Henson *et al.*[16].

The measurements were carried out as follows. The solution in the upper compartment was exchanged for a new one with a different osmotic pressure. The resulting volume flow caused the bilayer to become non-planar. By withdrawing from the lower compartment a volume equal to the volume flow across the bilayer, it was restored to its original planar state. This enabled direct measurements of volume flow to be made over measured intervals of time, accurate to within $\pm 0.01 \cdot 10^{-12}$ $m^3 \cdot s^{-1}$ (see Fig. 1).

The bilayer area was measured by means of an eye piece graticule, accurate to within $\pm 2 \cdot 10^{-7}$ m^2. The osmotic pressures of the upper and lower solutions were measured by the use of an Advanced Osmometer Model 31LA to within $\pm$ 2%. The sample from the upper compartment was taken after the experiment, with the bilayer still intact. Buffer solutions used were: phosphate (approx. 6 mM) for measurements at pH 7 and bicarbonate (approx. 20 mM). Measurements were made at temperatures within the range 20–22 °C.

The permeability coefficient P_{os}, was calculated according to the following relations:

$$J_v = \sigma L_p \Delta\Pi \quad (1)$$

where σ is the reflection coefficient ($= 1$ for the solutes used), $\Delta\Pi$ the osmotic pressure difference and L_p is the hydraulic conductivity

$$P_{os} = \frac{L_p RT}{\overline{V}_w} \quad (2)$$

where $\overline{V}_w$ is the partial molar volume of water.

The analysis of phosphatidylcholine and phosphatidylserine fatty acids was determined by gas-phase chromatography of their methyl esters. The apparatus used was a Perkin–Elmer F11 gas chromatograph fitted with ionisation detector. The methyl esters were formed by adding 25% tetrabutylammonium hydroxide in methanol (w/v) to the dry phospholipids followed by extraction with 60–80 °C light petroleum. The chromatogram areas used for the estimation of fatty acid composition were calculated as the product of response height and the width at half the response height.

RESULTS

Phosphatidylcholine bilayers were formed and measurements of volume flow were made at pH values 3, 7.4 and 9. The progress of a typical experiment is shown in Fig. 1. Unsuccessful attempts were made to form phosphatidylserine bilayers at pH 11.0, 10.0 and 9.5. At pH 11.0, stroking the brush across the aperture did not even leave a thick film. At pH 10.0 thick films formed but these would not drain. At pH 9.5, films drained to the coloured fringe stage; black islands were observed on those films across which a potential difference (PD) of approx. 0.1 V was maintained, but these disappeared when the PD was removed. This experience is similar to that described by Ohki[18]. At pH 9.0 bilayers were formed with difficulty but these were fragile, frequently broke during experiment, and only two values for P_{os} obtained. Phosphatidylserine bilayers formed readily at pH 3.0 and pH 7.4.

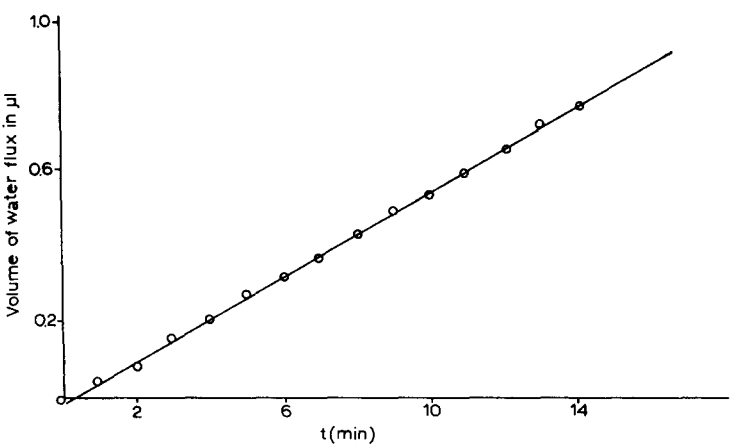

Fig. 1. Volume flow across a phosphatidylcholine bilayer. Sucrose gradient 640 mosM. Bilayer area $4.93 \cdot 10^{-6}$ m^2.

The values of P_{os} obtained for phosphatidylcholine and phosphatidylserine are summarised in Table I and it is clear that there is a significant difference between the behaviour of phosphatidylcholine and phosphatidylserine bilayers with respect to variation of pH.

TABLE I

OSMOTIC WATER PERMEABILITY COEFFICIENTS WITH STANDARD ERROR OF THE MEAN

The aqueous phase was 0.1 M NaCl.

pH	$P_{os} \times 10^5$ ($m \cdot s^{-1}$)		*No. of experiments*	
	Phosphatidyl-choline	*Phosphatidyl-serine*	*Phosphatidyl-choline*	*Phosphatidyl-serine*
3.0	1.86 ± 0.08	0.86 ± 0.02	6	5
7.4	1.82 ± 0.11	1.09 ± 0.03	9	7
9.0	—	1.25 ± 0.04	—	2

DISCUSSION

The mechanism of water movement through lipid bilayers has been considered by a number of authors[8,9,11,17]. The difference between osmotic and tracer permeability coefficients has been accounted for in lipid bilayers by the unstirred layer present adjacent to the bilayer. These partially control water movement in tracer experiments where there is no net volume flow, but are absent as a controlling effect in osmotic experiments.

Price and Thompson[8] have examined several mechanisms for water movement. They conclude that solubility–diffusion is the most probable process. The corresponding energy profiles are illustrated in Fig. 2. Following Zwolinski *et al.*[19],

$$\frac{1}{P_{os}} = \frac{2}{k_{sm}} + \frac{m}{k_m \lambda \frac{k_{sm}}{k_{ms}}} \qquad (3)$$

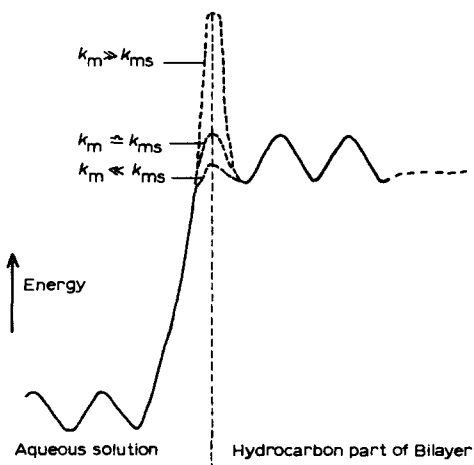

Fig. 2. Energy profile for transport of water across the bilayer interface.

where λ is the mean free path for water, m is the number of jumps required to cross the bilayer, k_{sm} and k_{ms} are velocity constants for the interface and k_m the velocity constant for the hydrocarbon part of the bilayer. The ratio k_{sm}/k_{ms} is the partition coefficient B of water between the bilayer and the aqueous solution.

Eqn 3 may be rewritten

$$P_{os} = \frac{k_m k_{sm} \lambda}{2k_m \lambda + m k_{ms}} \tag{4}$$

Assuming λ to be of the order of a few tenths of a nanometre the following limiting conditions can be derived (see Fig. 2)

(i) if $k_m \ll k_{ms}$, Eqn 4 becomes

$$P_{os} \simeq \frac{k_m}{m} \lambda \frac{k_{sm}}{k_{ms}} \tag{5}$$

(ii) if $k_m \gg k_{ms}$, Eqn 4 becomes

$$P_{os} \simeq \frac{k_{sm}}{2} \tag{6}$$

(iii) if k_m and k_{ms} are of the same order of magnitude, P_{os} would be a function of the three velocity constants.

If it can be assumed that the velocity constant depends upon the temperature according to

$$k_i = A_i \exp\left(-\frac{\Delta E_i}{RT}\right) \tag{7}$$

where A_i is a constant for each velocity constant, E_i is the activation energy, then P_{os} would be a non-linear function of $1/T$. However, it has been shown[8,19] that P_{os} is a linear function of $1/T$; therefore the condition (iii) that k_m and k_{ms} are of the same order of magnitude can be rejected.

The question which remains to be answered is: to what extent is water movement in these membranes controlled by k_m, (Condition i) and thus the hydrocarbon composition, and to what extent is it controlled by k_{sm} (Condition ii) and thus the surfaces of the bilayer?

In Table I it is shown that P_{os} for phosphatidylcholine bilayers is not dependent on pH; there is no reason why it should be since phosphatidylcholine possesses no net charge between pH 3 and pH 11[21], and there is no change in surface packing on a monolayer in the same pH range (D. E. Graham and G. T. Rich, unpublished). However, the P_{os} for phosphatidylserine bilayers increases with pH (Table 1). From electrophoretic mobility measurements of dispersions it has been shown[22] that phosphatidylserine has an increase in net negative charge between pH 1.4–4.5. In addition it has been shown (D. E. Graham, H. Hauser and M. C. Phillips, unpublished) that at pH values greater than 8.0 the phosphatidylserine molecule attains a greater net charge, due to the dissociation of the amino group. The resulting increase in coulombic repulsion between neighbouring molecules causes an increase in surface area per molecule in the bilayer[23]. An analogous increase in surface area per molecule with pH has been observed in phosphatidylserine monolayers (D. E. Graham and G. T. Rich, unpublished).

The values of P_{os} for phosphatidylserine are all lower than for phosphatidylcholine. This may be partially explained in terms of the degree of hydrocarbon chain saturation. The average saturation of hydrocarbon chains is greater for phosphatidylserine than phosphatidylcholine (see Table II). The ratio saturated:unsaturated is approx. 1 and 1.5 for phosphatidylcholine and phosphatidylserine hydrocarbon chains, respectively. The analysis also shows that phosphatidylserine contains a greater fraction of long chain fatty acids than phosphatidylcholine. Phosphatidylserine has 5.9 % of its fatty acid chains shorter than C_{18}, whereas phosphatidylcholine has 33.2 % shorter than C_{18}. Data in Table III (taken from Englin et al.[24]) shows that the solubility, X_w, of water in hydrocarbon decreases with increasing chain length and, moreover, is greater for the alkenes than the alkanes. It is probable that P_{os} would behave in a similar way, and this offers a qualitative explanation for the low values of P_{os} for phosphatidylserine. This view is supported by the observations of de Gier *et al.*[25] who have shown that the permeability of phosphatidylcholine liposomes to glycerol is similarly dependent on hydrocarbon chain length and degree of saturation. The variation of polar charges and molecular packing with pH could

TABLE II

GAS–LIQUID CHROMATOGRAPHIC ANALYSIS OF THE PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLSERINE METHYL ESTERS

Phosphatidylcholine satd:unsatd = 47.5:52.5, approx. 1; and phosphatidylserine satd:unsatd = 59.6:40.8, approx. 1.5. t means a trace was seen. Phosphatidylserine also showed a trace of 16:3.

C No.:	*12:0*	*12 unsatd*	*14:0*	*14 unsatd*	*16:0*	*16:1*	*16:2*
Phosphatidylcholine	—	—	0.4	0.4	29.0	2.4	t
Phosphatidylserine	0.8	0.6	0.5	0.4	3.3	0.4	0.2

C No.:	*18:0*	*18:1*	*18:2*	*18:3*	*20:0*	*20:1*	*20:2*	*22:0*	*22:1*	*20:3*
Phosphatidylcholine	17.1	37.0	12.3	0.4	t	t	t	—	—	1.0
Phosphatidylserine	48.3	29.4	2.4	1.4	2.7	3.1	2.1	3.0	1.1	t

TABLE III

WATER SOLUBILITIES (TAKEN FROM ENGLIN *et al.*[24]) AT 20 °C

Substance	X_w (*wt* %)
Hexadecane	0.0069
Hexane	0.0101
Heptane	0.0096
Octane	0.0095
Hexene	0.0477*
Heptene	0.0249
Octene	0.0355
Undecene	0.0195

* 30 °C.

explain the difference in the water permeability. The larger the net charge on the molecule, the greater the coulombic repulsion forces, and thus the larger the area per molecule. The hydrocarbon chains will thus tend to have greater configurational freedom and a greater number of cavities to accommodate water molecules will be created enhancing the solubility of water in the hydrocarbon interior, by reducing the energy barrier for water moving through it.

We have suggested that an increase in water flux may qualitatively be explained by an increase in water solubility within the hydrocarbon region of the bilayer. However, it is possible that the cavities may allow an increase in the mobility of water molecules. Since the water flux within the hydrocarbon chains is a function of both concentration and mobility, it is reasonable to expect these two factors to control the water permeability. The effect of cholesterol lends support to this view. Finkelstein and Cass[11] have shown that the addition of cholesterol to phosphatidylcholine bilayers reduces the water permeability coefficient P_{os}. Cholesterol has been shown[28–30] to reduce the mobility of the hydrocarbon chains and thus the thermal cavities, hence the water solubility in the bilayer interior.

This study suggests the indirect involvement of the polar group regions of phospholipids in the control of water permeability. It is possible that under certain conditions water movement across natural membranes may be similarly controlled, *e.g.* as a result of interaction between proteins, ions and lipids. Such interactions would alter the molecular packing and as a result alter the solubility of water in the membrane, and thus the water permeability.

CONCLUSIONS

The present work indicates that an increase in P_{os} seems to be related to an increase in surface area per polar group. There is no proof that the P_{os} behaviour is not due to a change in surface area per polar group but the different behaviour of phosphatidylcholine and phosphatidylserine and the fact that P_{os} is a linear function of $1/T$ lends credence to the hypothesis that water permeability is controlled in the hydrocarbon region. From the foregoing experimental results and theoretical considerations certain general conclusions can therefore be drawn concerning the control of water movement through bilayers.

1. Water movement is directly controlled by the packing of the hydrocarbon chains.

2. The longer and more saturated the hydrocarbon chains the smaller the water content and thus water permeability.

3. The greater the surface area per molecule (in the case of phosphatidylserine a consequence of the dependence of net charge on pH) the greater the configurational freedom of the hydrocarbon chains and thus the greater the permeability to water.

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