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THE EFFECT OF CHAIN LENGTH AND LIPID PHASE TRANSITIONS ON THE SELECTIVE PERMEABILITY PROPERTIES OF LIPOSOMES

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SUMMARY

This paper describes experiments showing the importance of the fatty acid chain length on the barrier properties of liposomal bilayers, prepared from saturated lecithins, under conditions of lateral phase separation.

1. Above the gel to liquid crystalline phase transition temperature, liposomes prepared from saturated lecithins with 14 or more carbon atoms per acyl chain exist as stable bilayers, which are practically impermeable to ions.

2. At temperatures well above the transition temperature dilauroyl phosphatidylcholine liposomes exhibited osmotic shrinkage, which was dependent on the ionic size of the solute used to bring about the osmotic gradient, indicating that the permeation through these less stable bilayers takes place mainly via individual diffusion of the permeating ions.

3. An enhanced release of trapped potassium from liposomes was demonstrated in the vicinity of the transition temperature. The extent of the increase, however, depended strongly on the length of the paraffin chain.

4. From measurements of the shrinkage behaviour of liposomes in the vicinity of the transition temperature it is concluded that the increased permeability decreases with increasing diameter of the permeating ion. This finding implies that the increased permeability at the transition temperature cannot be ascribed to "macroscopic" rupture of the liposomal membrane.

The maximum permeability in the vicinity of the T_c is discussed in terms of probability and size distribution of statistical pore formation at the boundaries of liquid and solid domains.

INTRODUCTION

Recently considerable attention has been focused on gel to liquid crystalline lipid phase transitions in artificial and biological membranes, and a variety of physical techniques have been used to analyse the structural and dynamical consequences of these transitions (for reviews see refs 1–4). In a number of studies discontinuities in Arrhenius plots of membrane-bound enzymes and transport systems have been

correlated with lipid phase transitions [3, 5–7], indicating the possible physiological significance of this phenomenon.

Data concerning the effect of lipid phase transitions on the barrier properties of pure lipid bilayers, which can be studied in model membrane systems, are still rather limited. It has been shown that release of ions and non-electrolytes is maximal at temperatures where the bilayers undergo a gel to liquid crystalline phase transition [8–11]. With vesicles of dipalmitoyl phosphatidylglycerol it was found that the rate of self-diffusion of ^{22}Na at the transition temperature was elevated much more than that of [^{14}C]-sucrose [9], suggesting that the increase of the permeability depends on the properties of the permeating compound. The data from the literature [11] furthermore suggest that the enhancement of the permeability of ions at the T_c depends on the length of the paraffin chain, being much less elevated for dipalmitoyl phosphatidylcholine liposomes in comparison with dimyristoyl phosphatidylcholine liposomes.

The present study was done to verify whether there is a solute dependent increase of the permeability at the phase transition temperature, and secondly to study the dependence of this phenomenon on the length of the paraffin chain.

MATERIALS AND METHODS

All phospholipids used in this study were prepared in our laboratory. Egg phosphatidic acid was prepared from egg lecithin by phospholipase D degradation [12]. Lecithins with defined fatty acid composition were synthesized according to the method described by van Deenen and de Haas [13]. The lecithins were pure as shown by thin-layer chromatography. Fatty acid impurity was less than 1 % as shown by gas chromatography, except the distearoyl phosphatidylcholine which contained 5 % of palmitate. All reagents used were commercial and of analytical reagent grade.

Multilayered liposomes were prepared at temperatures well above the T_c of the lecithins, in 150 mM KCl or 100 mM K_2SO_4, both buffered at pH 7.5 with 10 mM Tris · HCl and Tris · H_2SO_4, respectively, as previously described [14]. In experiments in which the release of K^+ had to be measured, the liposomes were subsequently cooled down in the same medium in which they were prepared to 0–4 °C, followed by dialysis at this low temperature against 150 mM $MgSO_4$ or 150 mM choline chloride, also buffered at pH 7.5 with 10 mM Tris · H_2SO_4 orTris · HCl. In experiments where the osmotic shrinkage of the liposomes was studied, the liposomes were prepared in 20 mM glucose, 10 mM Tris · HCl at pH 7.5. To measure the release of K^+ from the liposomes small samples of the dialysed liposome suspension (1.25–2.5 μmol of lipid) were transferred into 5.0 ml of isotonic medium, identical to the dialysis medium and equilibrated at the desired temperature. Changes in K^+ activities were monitored by a Schott and Gen K^+-glass electrode (Jena Glaswerk, Mainz, Germany) connected to a pH meter (Radiometer, type PHM 26) and a recorder. The amount of potassium trapped in the liposomes was determined by lysing them with Triton X-100. Calibration of the electrode was made at each temperature by adding, in a separate sample under identical experimental conditions, a series of fixed amounts of K^+.

For the osmotic shrinkage measurements the liposomes were first diluted in the medium in which they were prepared and transferred into a thermostated cuvet, which was vigorously stirred. After temperature equilibration an osmotic shock was given by rapidly injecting a small volume of a concentrated solute, preincubated at the

same temperature. Changes in the absorbance were recorded at 662 nm with a spectrophotometer (Vitatron, type MPS) connected with a recorder.

RESULTS

Fig. 1 shows the release of K^+ from liposomes of dimyristoyl phosphatidylcholine. The liposomes very rapidly lost all their K^+ when incubated at temperatures in the vicinity of the T_c, whereas they still contained large amounts of potassium after an incubation period of 5 min when incubated several degrees above or below the transition temperature.

The very rapid release of K^+ from dimyristoyl phosphatidylcholine liposomes in the vicinity of the T_c may be caused by a much increased instability of the membranes at these temperatures, resulting in a spontaneous breaking and resealing of the lipid bilayers. On the other hand it may be the result of a strongly enhanced permeability of the membrane for the enclosed ions. In the latter case the increase of the permeability at the transition temperature will be largely determined by the size of the permeating ion. This is not expected to be so if the release of K^+ has to be ascribed to breaking and resealing of the membrane. To discriminate between these two possibilities the osmotic shrinkage of liposomes was studied by giving an osmotic shock with various solutes, e.g. a series of alkali chlorides, where it is known that the diameter of the hydrated cation decreases with increasing atomic number. Since lipid bilayers normally are hardly permeable to ions, whereas the water permeability is very high [15, 16], the shrinkage of liposomes is expected to be independent of the alkali chloride used. Furthermore, if the refractive index of the various solutes is identical, the

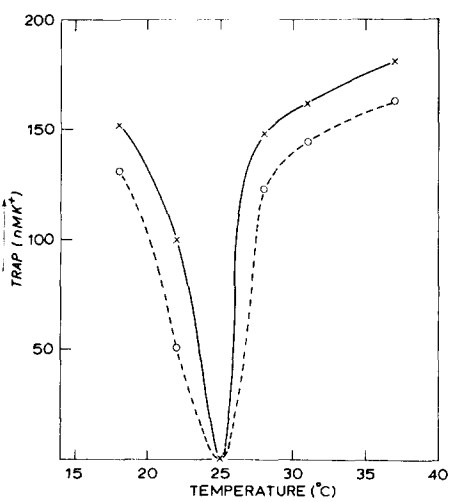

Fig. 1. Determination of the amount of potassium trapped by liposomes, prepared from dimyristoyl phosphatidylcholine with 2 mol % egg phosphatidic acid, after incubation for various time periods at different temperatures. Liposomes were prepared in 150 mM KCl, 10 mM Tris · HCl, pH 7.5; the external medium was 150 mM $MgSO_4$, 10 mM Tris · H_2SO_4, pH 7.5. (×) and (○), the potassium content of the liposomes 30 s and 5 min, respectively, after injection at the temperature indicated. For experimental details see Materials and Methods.

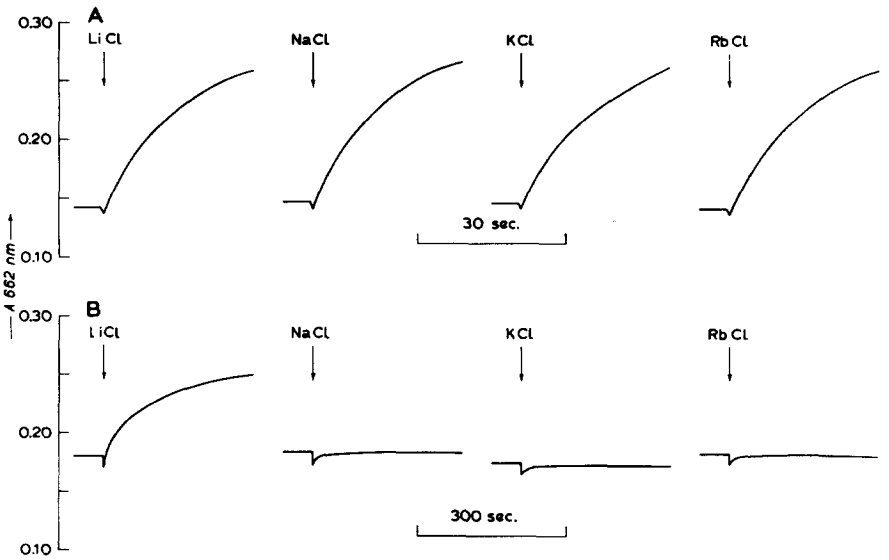

Fig. 2. Osmotic shrinkage of liposomes, prepared from dimyristoyl phosphatidylcholine with 4 mol % of phosphatidic acid, following an osmotic shock with various alkali chlorides. After temperature equilibration 0.4 ml 1.0 M alkali chloride, 10 mM Tris · HCl, pH 7.5, was rapidly injected into 9.6 ml liposome suspension (0.4 μmol of lipid per ml in 20 mM glucose, 10 mM Tris · HCl, pH 7.5) at the moment indicated by the arrow. Upper part of the figure (A): 34.5 °C; lower part (B): 22.8 °C. For experimental details see Materials and Methods.

increase in the absorbance due to the osmotic shrinkage of the liposomes, will be identical provided the osmotic gradient is equal in all cases. It is a prerequisite that this result is obtained with liposomes above the T_c in order to explain any possible difference in shrinkage behaviour in the vicinity of the phase transition temperature.

Fig. 2A shows that this prerequisite holds for dimyristoyl phosphatidylcholine liposomes. At 34.5 °C, a temperature well above the T_c, the osmotic shrinkage of the liposomes was identical with all four alkali chlorides tested. At 22.8 °C, however, a temperature in the vicinity of the phase transition temperature [2, 17], the liposomes showed hardly any shrinkage after an osmotic shock with NaCl, KCl or RbCl, whereas there was still a substantial shrinkage after a shock with LiCl (Fig. 2B). The absence of an osmotic shrinkage after injecting concentrated NaCl, KCl or RbCl correlates very well with experiments in which the release of these alkali ions was measured (compare Fig. 1). However, Fig. 2 indicates that in the vicinity of the T_c dimyristoyl phosphatidylcholine liposomes are much more permeable to Na^+, K^+ and Rb^+ than to Li^+.

Fig. 3 gives the results of experiments with liposomes prepared from various saturated lecithins and from some mixtures exhibiting only a single phase transition because of cocrystallization [18–20]. Since the light scattering of liposome suspensions changes abruptly on passing the T_c [21, 22], this criterion could be used to determine the temperature region where the phase transition occurs. It was found that the phase transition of especially the long chain lecithins is shifted to somewhat lower temperatures, and is also slightly broadened, due to the incorporation of 4 mol % of egg

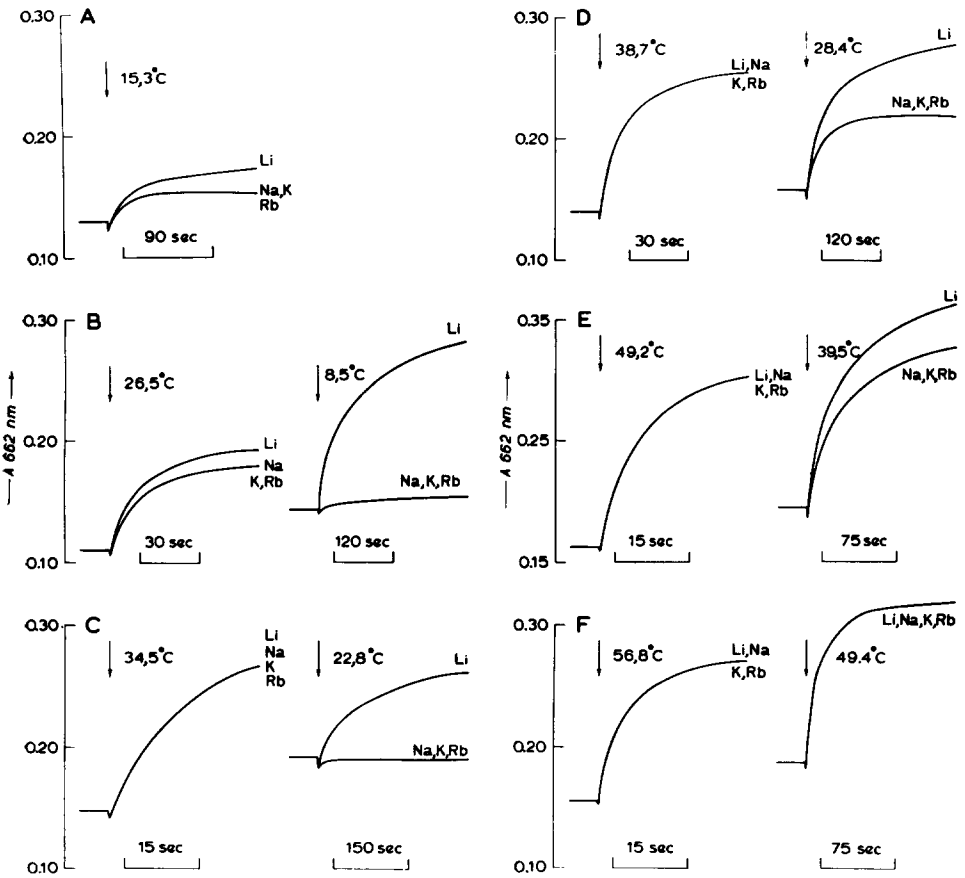

Fig. 3. Osmotic shrinkage of liposomes, prepared from synthetic lecithins with 4 mol % egg phosphatidic acid, following an osmotic shock with various alkali chlorides. A, dilauroyl phosphatidylcholine/phosphatidic acid = 96/4; B, dilauroyl phosphatidylcholine/dimyristoyl phosphatidylcholine/phosphatidic acid = 48/48/4; C, dimyristoyl phosphatidylcholine/phosphatidic acid = 96/4; D, dimyristoyl phosphatidylcholine/dipalmitoyl phosphatidylcholine/phosphatidic acid = 64/32/4; E, dipalmitoyl phosphatidylcholine/phosphatidic acid = 96/4; F, distearoyl phosphatidylcholine/phosphatidic acid = 96/4. The experiment was done above (left trace) and in the temperature region of the phase transition (right trace). The symbols in the figures refer to the alkali chlorides used to apply the osmotic gradient. Experimental conditions were as described in Fig. 2.

phosphatidic acid (Blok, M. C., de Gier, J. and van Deenen, L. L. M., unpublished).

Comparative studies on the osmotic response of the various liposome systems showed that the change in $A_{662\ nm}$ for dilauroyl phosphatidylcholine liposomes (Fig. 3A) and dilauroyl phosphatidylcholine/dimyristoyl phosphatidylcholine = 1/1 liposomes (Fig. 3B), at temperatures well above the T_c,is obviously larger after an osmotic shock with LiCl, as compared to a shock with NaCl, KCl and RbCl. This result, indicating that Na^+, K^+ and Rb^+ can penetrate the bilayers of these liposomes very easily, is not surprising since it is known that the membranes of dilauroyl phosphatidylcholine liposomes are not good permeability barriers to ions [23]. It is, however, of interest to note that, although the liposomal membrane in the liquid crystalline

state is rather permeable to ions, the increased response to a shock with LiCl suggests that also in this case the size of the permeating ion is very important. Furthermore, Fig. 3B may suggest that for liposomes prepared from equimolar mixtures of dilauroyl phosphatidylcholine and dimyristoyl phosphatidylcholine, the discrimination between Li^+ and the other alkali ions at the T_c is even more pronounced than that above the phase transition temperature.

Fig. 3 further shows that liposomes prepared from lecithins with acyl chains of 14 or more carbon atoms, all gave identical results after an osmotic shock above the T_c with the various alkali chlorides, in agreement with the view that these bilayers in the liquid crystalline state are highly permeable to water, but practically impermeable to ions. At the T_c, however, markedly different results were obtained with the various liposome preparations. Liposomes of dimyristoyl phosphatidylcholine show hardly any shrinkage after an osmotic shock at this temperature with NaCl, KCl and RbCl (Figs 2B and 3C), whereas liposomes of dimyristoyl phosphatidylcholine/dipalmitoyl phosphatidylcholine = 2/1 show an increase of $A_{662\ nm}$ after a shock with these solutes, reaching a maximum after about 150 s and followed by a slight but significant decrease of the absorbance (Fig. 3D). With the latter liposome preparation, shrinkage of the liposomes induced by an osmotic shock with LiCl was much more pronounced and did not reach a maximum within about 5 min. It is principally different compared to the shrinkage induced by a shock with the other alkali chlorides. With dipalmitoyl phosphatidylcholine liposomes there is still a difference in the osmotic shrinkage at the T_c after a shock with LiCl as compared to the other solutes, but a maximum is not reached within a short period of time (Fig. 3E). Qualitatively identical results were obtained with liposomes prepared from equimolar mixtures of dimyristoyl phosphatidylcholine with dipalmitoyl phosphatidylcholine and of dipalmitoyl phosphatidylcholine with distearoyl phosphatidylcholine (not shown). Studying the osmotic shrinkage of distearoyl phosphatidylcholine liposomes in the region of the phase

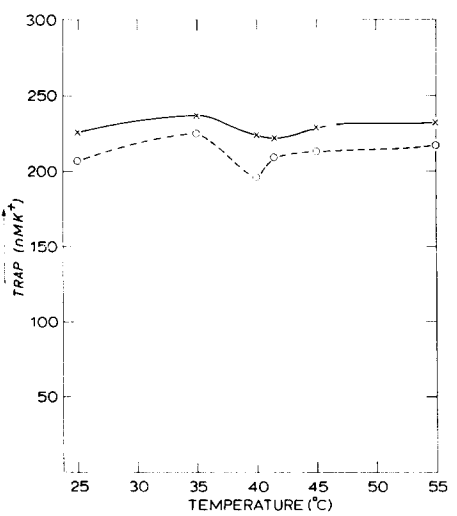

Fig. 4. Determination of the amount of potassium trapped in liposomes, prepared from dipalmitoyl phosphatidylcholine with 4 mol % egg phosphatidic acid, 30 s (×) and 5 min (○) after injection at the desired temperature. Experimental conditions were as described in Fig. 1.

transition, no significant differences were found in shrinkage behaviour after a shock with any of the four alkali chlorides (Fig. 3F).

Summarizing the results of Fig. 3, it can be concluded that the maximum of the permeability at the transition temperature is strongly determined by the size of the permeating ion and by the length of the paraffin chains of the lecithins.

To prove more directly the importance of the length of the paraffin chains of the lecithins, the release of K^+ from dipalmitoyl phosphatidylcholine liposomes was measured at various temperatures (Fig. 4). At the transition temperature, being about 40 °C for this liposome preparation, the release of K^+ is slightly elevated. The increase of the release of K^+, however, is an order of magnitude less as compared to that from dimyristoyl phosphatidylcholine liposomes (compare Fig. 1).

In addition to the series of alkali chlorides we also tested the osmotic shrinkage of liposomes after applying osmotic gradients with a series of potassium salts in which the anion varied in size (Fig. 5). Above the T_c the change in the absorbance is independent on the solute used to apply the osmotic gradient, provided the osmotic difference was equal. In the vicinity of the T_c the osmotic shrinkages, induced by the various solutes, scattered for both dimyristoyl phosphatidylcholine liposomes and dimyristoyl phosphatidylcholine/dipalmitoyl phosphatidylcholine = 2/1 liposomes, and an obvious correlation between the extent of shrinkage and the size of the hydrated anion could be observed.

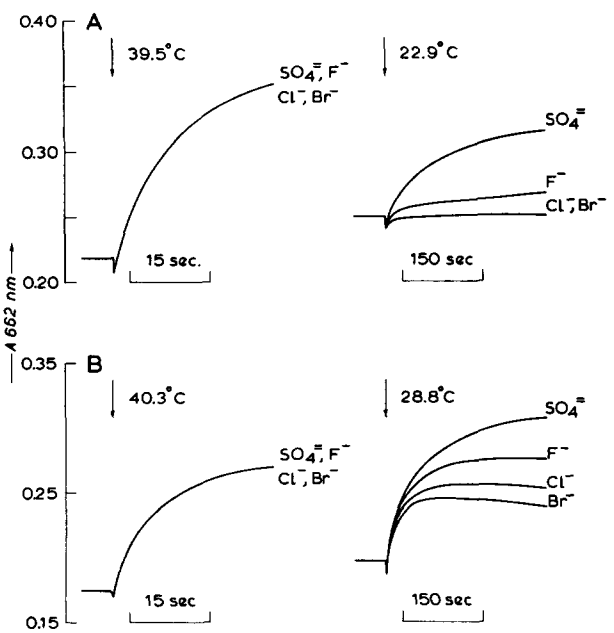

Fig. 5. Osmotic shrinkage of liposomes, following an osmotic shock with a series of potassium salts. A, dimyristoyl phosphatidylcholine/egg phosphatidic acid = 96/4; B, dimyristoyl phosphatidylcholine/dipalmitoyl phosphatidylcholine/egg phosphatidic acid = 64/32/4. The experiment was done above (left trace) and in the vicinity of the phase transition temperature (right trace). Experimental conditions were as described in Fig. 2, except that 0.5 ml 0.8 M potassium salt (for 0.66 M K_2SO_4), 10 mM Tris · HCl, pH 7.5, was injected into 9.5 ml liposome suspension.

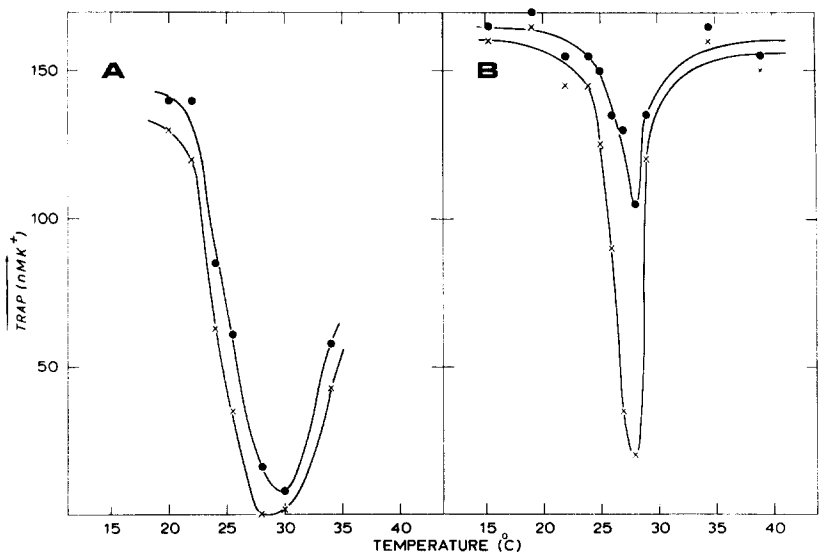

Fig. 6. Determination of the amount of potassium trapped by liposomes, prepared from dimyristoyl phosphatidylcholine/dipalmitoyl phosphatidylcholine/egg phosphatidic acid = 64/32/4, 30 s (×–×) and 5 min (●–●) after injection at the temperature indicated. A and B, liposomes prepared in 150 mM KCl and 100 mM K_2SO_4, respectively, both buffered with 10 mM Tris at pH 7.5. The external medium was 150 mM choline chloride, 10 mM Tris · HCl, pH 7.5. For experimental details see Materials and Methods.

Measurements of the release of K^+ from dimyristoyl phosphatidylcholine/dipalmitoyl phosphatidylcholine = 2/1 liposomes proved directly the permeating ion dependence of the maximum of the permeability at the T_c (Fig. 6). If KCl was trapped, the liposomes lost most of the trapped potassium during an incubation period of about 30 s at temperatures ranging from about 28 to 32 °C (Fig. 6A), being the temperature region where the lipid phase transition occurs as shown by light scattering experiments. If, however, K_2SO_4 was trapped the liposomes still contained considerable amounts of potassium 30 s after injection at these temperatures, and did not lose all their potassium content during an incubation time of 5 min (Fig. 6B) as was found if KCl was enclosed (Fig. 6A).

DISCUSSION

Earlier studies have shown that the permeation of non-electrolytes through liposomal membranes of saturated lecithins in the liquid crystalline state increases gradually with decreasing chain length [14]. However, the barrier properties of dilauroyl phosphatidylcholine membranes differ drastically from those of higher lecithin homologues, since dilauroyl phosphatidylcholine liposomes are not capable of retaining ions [23]. The osmotic shrinkage experiments, described in this paper, confirmed a high ionic permeability of these liposomes at temperatures well above the gel to liquid crystalline phase transition temperature. Based on the finding that dilauroyl phosphatidylcholine vesicles are unstable and rapidly form larger multilamellar particles, the inferior barrier properties have been ascribed to a collision-

induced rupture and resealing of the membrane [23]. Although for the strongly curved vesicles these macroscopic events may be important, the dependence of the shrinkage of dilauroyl phosphatidylcholine-multilayered liposomes on the size of the permeating ion (Fig. 3A) suggests that there is at least a large contribution of permeation through statistical "pores" of variable sizes, being formed very frequently due to the low interaction energy between the lipid molecules [1, 24].

Recent investigations showed the existence of a maximum of the permeability for both electrolytes [8, 9, 11] and non-electrolytes [9, 10] in the vicinity of the transition temperature of the membrane lipids. The experiments in this paper clearly demonstrate the dependence of this permeability increase on the size of both the permeating cations (Figs 2 and 3) and anions (Figs 5 and 6), indicating permeation through statistical pores of variable sizes. Furthermore it is shown that the increase in permeability near the phase transition temperature is strongly dependent on the length of the paraffin chain (compare Figs 1, 4 and 6A, see also Fig. 3). Since the extent of the permeability increase will depend on several factors, such as the size distribution, the number and the life time of the pores, it can be concluded that one or more of these factors are dependent on the fatty acid chain length.

With respect to the formation of the statistical pores the following qualitative suggestions can be made. Due to discontinuities in the membrane at the boundaries of liquid and solid domains the probability of pore formation is enhanced at these sites. Moreover, the existence of a dynamic phase equilibrium of lipid molecules, going from the gel to the liquid crystalline state or vice versa, may be important in the formation of the pores. As the transition of the lipid molecules from the liquid crystalline to the gel state is accompanied by a decrease in the area per molecule [1, 2, 25] the extent of the decrease and the cooperativity of the system may influence the size of the pore. The significance of the decrease in area per molecule resulting from a liquid crystalline to gel transition has been discussed earlier by Linden et al. [26]. They introduced the term "increased lateral compressibility", a phenomenon which would also be important for the insertion and penetration of proteins into membranes [26–28].

Summarizing the present study demonstrates that a compositional controlled lipid phase transition gives the lipid bilayer specific and selective properties with respect to simple diffusion of ions, which may be relevant for the functioning of biological membranes.

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