INFLUENCE OF Ca^{2+} AND Mg^{2+} ON THE THERMOTROPIC BEHAVIOUR AND PERMEABILITY PROPERTIES OF LIPOSOMES PREPARED FROM DIMYRISTOYL PHOSPHATIDYLGLYCEROL AND MIXTURES OF DIMYRISTOYL PHOSPHATIDYLGLYCEROL AND DIMYRISTOYL PHOSPHATIDYLCHOLINE

P. W. M. VAN DIJCK, P. H. J. Th. VERVERGAERT*, A. J. VERKLEIJ, L. L. M. VAN DEENEN and J. DE GIER

*Laboratory of Biochemistry and *Biological Ultrastructure Research Unit, State University of Utrecht, University Centre "De Uithof", Padualaan 8, Utrecht, (The Netherlands)*

SUMMARY

1. Calorimetric experiments showed a marked effect of Ca^{2+} and Mg^{2+} on the thermotropic behaviour of dimyristoyl phosphatidylglycerol.

2. Concentrations of Ca^{2+} and Mg^{2+} lower than 1 ion to 2 molecules of phosphatidylglycerol produced a shift of the phase transition to higher temperatures and an increase in the enthalpy change which is consistent with a closer packing of the lipid molecules in the liposomes.

3. Above the 1 : 2 ratio, freeze-fracture electron microscopy demonstrated typical "crystal" structures both in the presence of Ca^{2+} and Mg^{2+}. In the presence of Mg^{2+} a metastable behaviour was noticed in the calorimetric experiments.

4. A Ca^{2+}- and Mg^{2+}-induced shift in the transition temperature and an increase in the enthalpy change was also observed in a 1 : 1 mixture of dimyristoyl phosphatidylglycerol and dimyristoyl phosphatidylcholine. However, these mixed samples remained liposomal in structure at any concentration of the divalent ions.

5. Liposomes prepared from a 1 : 1 mixture of dimyristoyl phosphatidylglycerol and dimyristoyl phosphatidylcholine in the absence of divalent cations are permeable in the range 10–50 °C. Bilayers of mixtures neutralized by Ca^{2+} or Mg^{2+} were demonstrated to be completely impermeable to K^+, except in the vicinity of the phase transition.

6. The leak of ions from liposomes of a 1 : 1 mixture of dimyristoyl phosphatidylglycerol and dimyristoyl phosphatidylcholine in the vicinity of the phase transition temperature was considerably less in the presence of Ca^{2+} than in the presence of Mg^{2+}.

7. It is concluded that there is a correlation between the calorimetric data and the permeability properties of dimyristoyl phosphatidylglycerol-containing bilayers with respect to the influence of Ca^{2+} and Mg^{2+}.

INTRODUCTION

Temperature-induced phase transitions in phospholipids are studied intensively in order to understand more about the architecture and function of biomembranes. From the available evidence it is known that the phase transition temperature of phospholipids is strongly dependent on chain length and degree of unsaturation of the fatty acid chains and on the nature of the polar head group [1]. Furthermore, in charged phospholipids, the transition temperature can be triggered by pH, ionic strength and divalent ions present in the environment [2–8].

Recently, the influence of pH and the divalent cations Ca^{2+} and Mg^{2+} on the thermotropic behaviour of dilauroyl phosphatidylglycerol and mixtures of dilauroyl phosphatidylglycerol and dilauroyl phosphatidylcholine has been described [3, 9]. In combination with results from a monolayer study [10], the calorimetric data could easily be interpreted in terms of molecular packing.

Our interest was to study the consequences of these findings for the barrier properties of model membranes prepared from such a synthetic phosphatidylglycerol. However, liposome studies with lecithins showed that lecithin bilayers containing lauroyl or shorter chains exhibit insufficient chain-chain interaction to form stable lipid bilayers which act as efficient permeability barriers [11]. Therefore we extended our studies to phosphatidylglycerols with longer paraffin chains and in the present paper we report on the thermotropic behaviour of dimyristoyl phosphatidylglycerol, pure and in combination with dimyristoyl phosphatidylcholine, and also on the permeability properties of derived liposomes.

MATERIALS AND METHODS

Lipids

1,2-Dimyristoyl sn-glycero-3-phosphorylcholine was synthesized as described before [12]. 1,2-Dimyristoyl sn-glycero-3-phosphatidyl-1'-sn-glycerol was obtained from dimyristoyl phosphatidylcholine by treatment with a phospholipase-D preparation [13] in the presence of glycerol, as described by Papahadjopoulos et al. [14]. After incubation, the lipids were extracted according to Bligh and Dyer [15]. The extract contained mainly dimyristoyl phosphatidylglycerol and dimyristoyl phosphatidylcholine and hardly any phosphatidic acid. The dimyristoyl phosphatidylglycerol was obtained as the Ca^{2+}-salt, due to the presence of 1 M $CaCl_2$ during the Bligh and Dyer extraction [16], and purified on a silicic acid column by elution with a gradient from 3 to 30 % (v/v) of methanol in chloroform. The purified dimyristoyl phosphatidylglycerol was finally converted to the Na^+-salt [16]. The final yield of the base-exchange procedure was nearly 50 %. Both dimyristoyl phosphatidylcholine and dimyristoyl phosphatidylglycerol were pure as considered by thin-layer chromatography.

Differential scanning calorimetry

Calorimetric experiments were performed on a Perkin Elmer DCS-2B apparatus as described previously [24]. The apparatus was operating at range 1 at a heating rate of 5 °C/min. It was calibrated with distilled water, cyclohexane and naphthalene. An empty gold sample pan was used as a reference. 6 μmol of dimyristoyl phosphati-

dylglycerol or a dimyristoyl phosphatidylglycerol/dimyristoyl phosphatidylcholine mixture was brought to complete dryness by evaporation followed by overnight high vacuum storage. The lipids were dispersed in 50 μl of a 40 mM Tris · acetate/ethylene-glycol (1 : 1, v/v) solution at the desired pH values and concentrations of ions. The lipids were dispersed by agitating on a vortex mixer for 1 min, above the transition temperature. Each sample was scanned at least four times to be certain of complete reproducibility of the observed transitions. The amount of lipid sealed in a sample pan was determined, after scanning, by a phosphorus determination [17, 18]. The area under the peak, needed to calculate the ΔH value, was determined using millimeter graph paper. The standard deviation in the experiments was of the order of 0.1–0.2 kcal/mol.

Freeze-fracture electron microscopy

In the freeze-fracture experiments the same samples as in the differential scanning calorimetry experiments were used. The dispersed lipid was equilibrated for at least 1 h at the desired temperature. Samples were transferred to specimen holders and rapidly quenched. Specimens were fractured in a Denton machine. Replicas were floated off on water and cleaned with a hypochlorite solution (2 % active chlorine) and, when necessary, with chromic acid. Electron micrographs were made on a Siemens Elmiskop I A and a Philips EM 200.

Permeability experiments

Multilayered liposomes were prepared by dispersion of 29 μmol phospholipid into solutions of 150 mM Tris · HCl, pH 7.2, with or without Ca^{2+} or Mg^{2+}. The Ca^{2+}: or Mg^{2+}: phospholipid ratios indicated in the legend of the figure refer to the bulk situation and are not necessarily equivalent to the actual binding ratios at the bilayer surface. The outside K^+ was removed by dialysis of the liposomal suspension against an isotonic 150 mM cholinechloride/10 mM Tris · HCl pH 7.2 solution with or without Ca^{2+} or Mg^{2+} or by Sephadex G-50 gel filtration [19]. The amount of K^+ trapped inside the liposomes was measured by a K^+-sensitive glass electrode connected with a Radiometer type PHM 26 pH meter, after lysis of the liposome structure by addition of a small amount of Triton X-100.

RESULTS AND DISCUSSION

In general the calorimetric and freeze-fracture results on dimyristoyl phosphatidylglycerol or dimyristoyl phosphatidylglycerol/dimyristoyl phosphatidylcholine mixtures agree well with results obtained with dilauroyl phosphatidylglycerol [3, 9], and in the following discussion we will emphasize mainly the typical deviations in behaviour of dimyristoyl phosphatidylglycerol compared to dilauroyl phosphatidylglycerol.

Calorimetric data on pure dimyristoyl phosphatidylglycerol

Fig. 1 shows that dimyristoyl phosphatidylglycerol liposomes in a medium of 120 mM NaCl at pH 7 undergo a thermotropic phase transition centered at 23 °C as revealed by differential scanning calorimetry. The transition temperature does not seem to be significantly altered when the pH is lowered to 3, where the phosphate
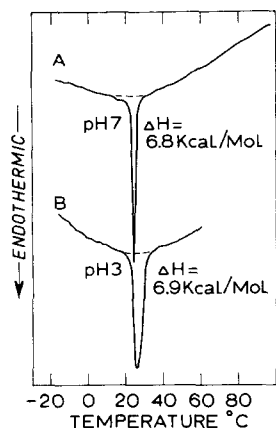
Fig. 1. Effect of pH on the phase transition of dimyristoyl phosphatidylglycerol. (A) Lipid dispersed in a medium of pH 7; 120 mM NaCl. (B) Lipid dispersed in a medium of pH 3; 120 mM NaCl.

group of dimyristoyl phosphatidylglycerol is not fully ionized. This is in contrast to dilauroyl phosphatidylglycerol, where a considerable increase in the transition temperature was observed [3]. However, a small shift of a few degrees upwards cannot be excluded because this is within experimental error. The energy content of the transition is the same at both pH 7 and 3, although the peak at pH 3 is broader. The broadening of this peak may be caused by the coexistence of both charged and neutralized dimyristoyl phosphatidylglycerol molecules.
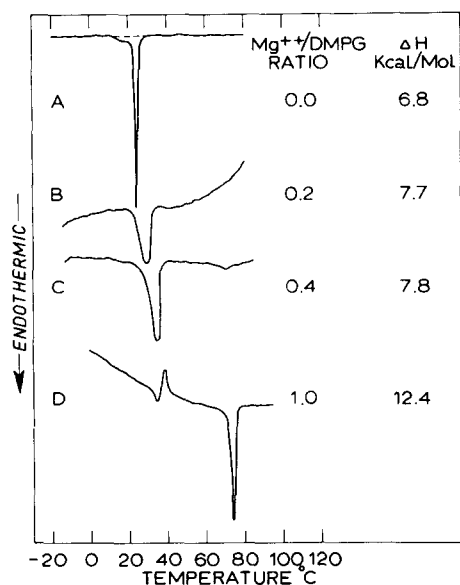

Fig. 2. Effect of Ca^{2+} on the phase transition of dimyristoyl phosphatidylglycerol. The same scan as in Fig. 1A, with baseline linearized (A). Dimyristoyl phosphatidylglycerol dispersed in a medium of pH 7 at Ca^{2+} concentrations of (B) 24 mM; (C) 48 mM; (D) 120 mM.
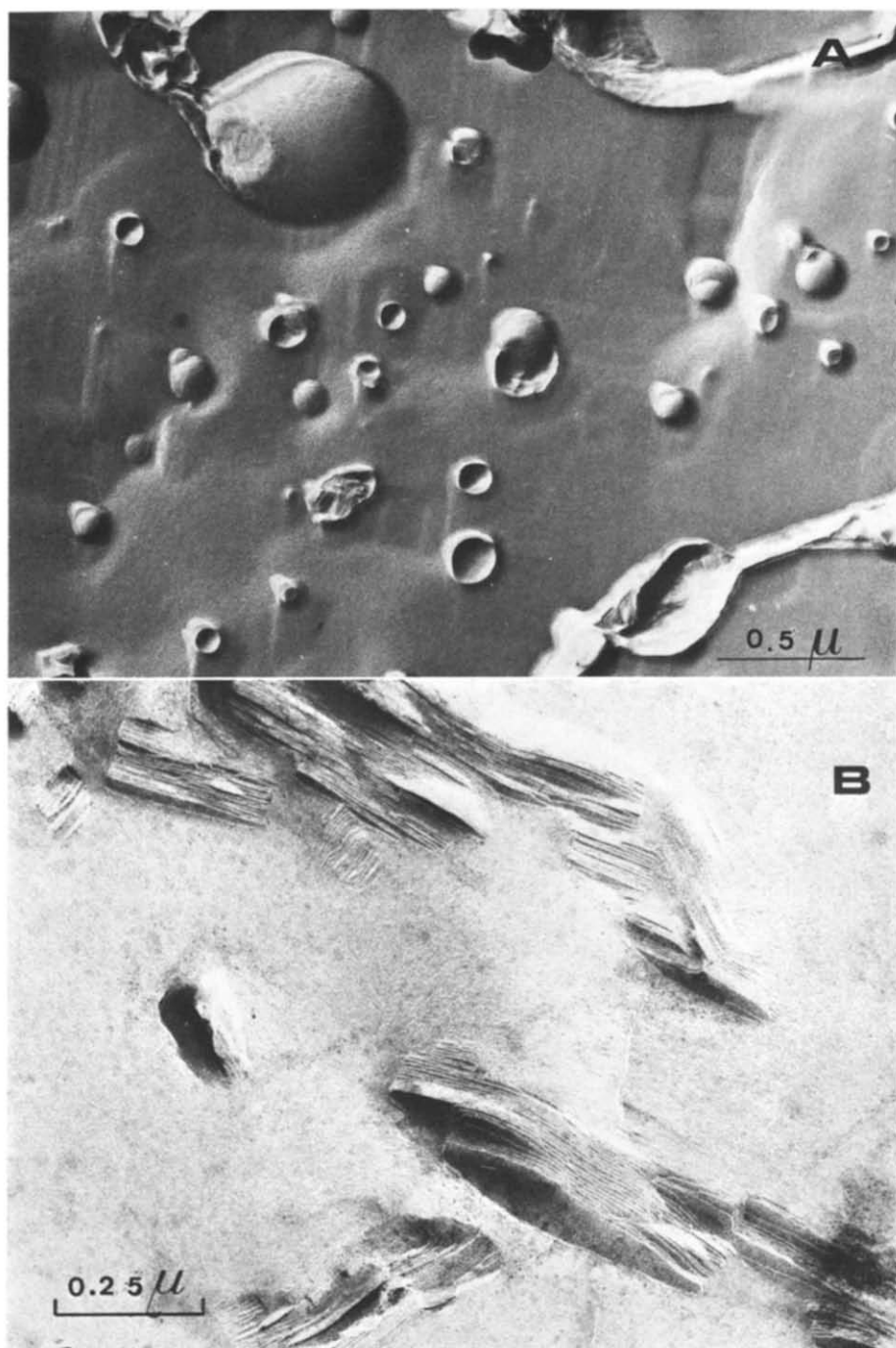
Fig. 3. Freeze-fracture faces of dimyristoyl phosphatidylglycerol. (A) Dimyristoyl phosphatidyl-glycerol at concentrations of divalent ions below a ratio of 1:2; quenched from 20 °C. (B) Dimyristoyl phosphatidylglycerol at Ca^{2+} or Mg^{2+} concentrations above a ratio of 1:2; quenching temperature: Ca^{2+}, 20 °C; Mg^{2+}, 40 °C.

When Ca^{2+} is introduced, there is, in the range of Ca^{2+} : dimyristoyl phosphatidylglycerol ratios lower than 0.5, a gradual shift in the midpoint of the peak with increasing amounts of Ca^{2+}, to a maximum of about 15 °C. This shift is accompanied by a rise of the ΔH value from 6.8 to 7.9 kcal/mol (Fig. 2). This increase indicates a more condensed packing, caused by surface charge neutralization of the bilayers due to the presence of the divalent Ca^{2+}. At low concentrations of Ca^{2+}, the electron micrographs (Fig. 3a) showed a morphology of globules and shells which might represent liposomes. In some areas a few lamellar stacks could be seen. At higher concentrations of Ca^{2+} a completely different morphology was observed. With freeze-fracture electron microscopy discrete piles of lamellae are found, suggesting a high crystallographic ordering (Fig. 3b). Although these lamellae sometimes seemed to be wrapped in cylinders, there is a marked difference with the pictures obtained for dilauroyl phosphatidylglycerol in the presence of high concentrations of Ca^{2+}. The latter preparation showed exclusively cylindrical structures. A straightforward elucidation of the molecular organization of the oriented lamellae ("crystals") might be achieved by means of X-ray crystallography. The change in morphology as a function of the Ca^{2+} concentration could also be observed by the naked eye as a precipitate instead of the milky dispersion seen at low concentrations of Ca^{2+}. The "crystals" melt at 89 °C with an enthalpy change of 15.2 kcal/mol (Fig. 2). This high value is consistent with a very condensed packing of the phosphatidylglycerol molecules in these structures. The melting peak of the "crystals" is preceded by two minor transitions, one endothermic, the other exothermic of unknown origin.

At low concentrations the influence of Mg^{2+} on dimyristoyl phosphatidylglycerol is comparable to that of Ca^{2+}, an upward shift in the transition midpoint temperature of 10–15 °C, an increase in the ΔH value and a broadening of the peak.
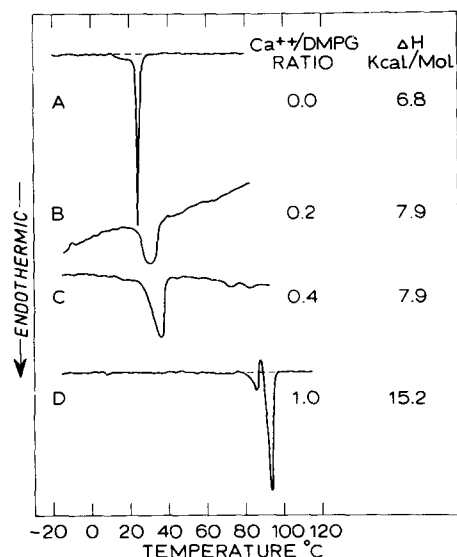

Fig. 4. Effect of Mg^{2+} on the phase transition of dimyristoyl phosphatidylglycerol. (A) The same as Fig. 2A. Dimyristoyl phosphatidylglycerol dispersed in a medium of pH 7 at Mg^{2+} concentrations of (B) 24 mM; (C) 48 mM; (D) 120 mM.
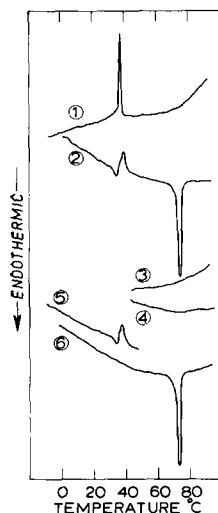
Fig. 5. The metastable transition behaviour of dimyristoyl phosphatidylglycerol in the presence of high concentrations of Mg^{2+}. (1) Complete cooling curve; (2) complete heating curve; (3) cooling to 50 °C, followed by (4) heating; (5) heating to 40 °C, followed by cooling and (6) complete reheating. The cooling curves were obtained at range 1 at a cooling rate of 5 °C/min.

Above the Mg^{2+} : dimyristoyl phosphatidylglycerol ratio of 0.5, some differences exist compared to Ca^{2+}. First, both the transition temperature (71 °C) and the heat content of the transition ΔH (12.4 kcal/mol) are lower than when Ca^{2+} is present (Fig. 4). Secondly, in these samples with a high Mg^{2+} concentration one observes a metastable transition behaviour, as has been noted also with dilauroyl phosphatidyl-glycerol [9]. The calorimetric experiment which proves this metastability is shown in Fig. 5. In the cooling curve (1) only one peak could be seen (around 40 °C). In the heating curve (2) the major transition was found at 71 °C, but there was also an endothermic peak at 32 °C directly followed by an exothermic peak. With freeze-fracture electron microscopy below 30 °C liposomal structures, and between 40 °C and 70 °C "crystal" structures could be demonstrated. Correlating the freeze-fracture faces with the calorimetric results, we can divide the heating scan (2) into three areas. (a) Below 30 °C, the lipid structures exist as gel-phase liposomes. (b) Upon heating above 32 °C, these liposomes undergo a gel to liquid-crystalline phase transition (endothermic peak at 32 °C). However, at this temperature this phase is not stable, so it forms (exothermic peak) the stable "crystal" structure which is found in heating curves between 40 and 70 °C. (c) At 71 °C the acyl chains of the lipid melt; this melting is accompanied by a simultaneous formation of (liquid-crystalline) liposomes. The lipid dispersion could be cooled from above 70–40 °C (3) and reheated (4) without any phase change. Heating to 50 °C (5) followed by cooling and reheating showed the absence of the double transition around 30–40 °C (6). Thus both the liquid-crystalline liposomes and the "crystals" demonstrated a substantial supercooling; during this cooling no intermediate "crystal" formation occurs. The supercooling was used as another proof to identify the structure in region c as liposomal. Normally the cooling rate for freeze-fracture electron microscopy is not sufficiently high to fix the situation
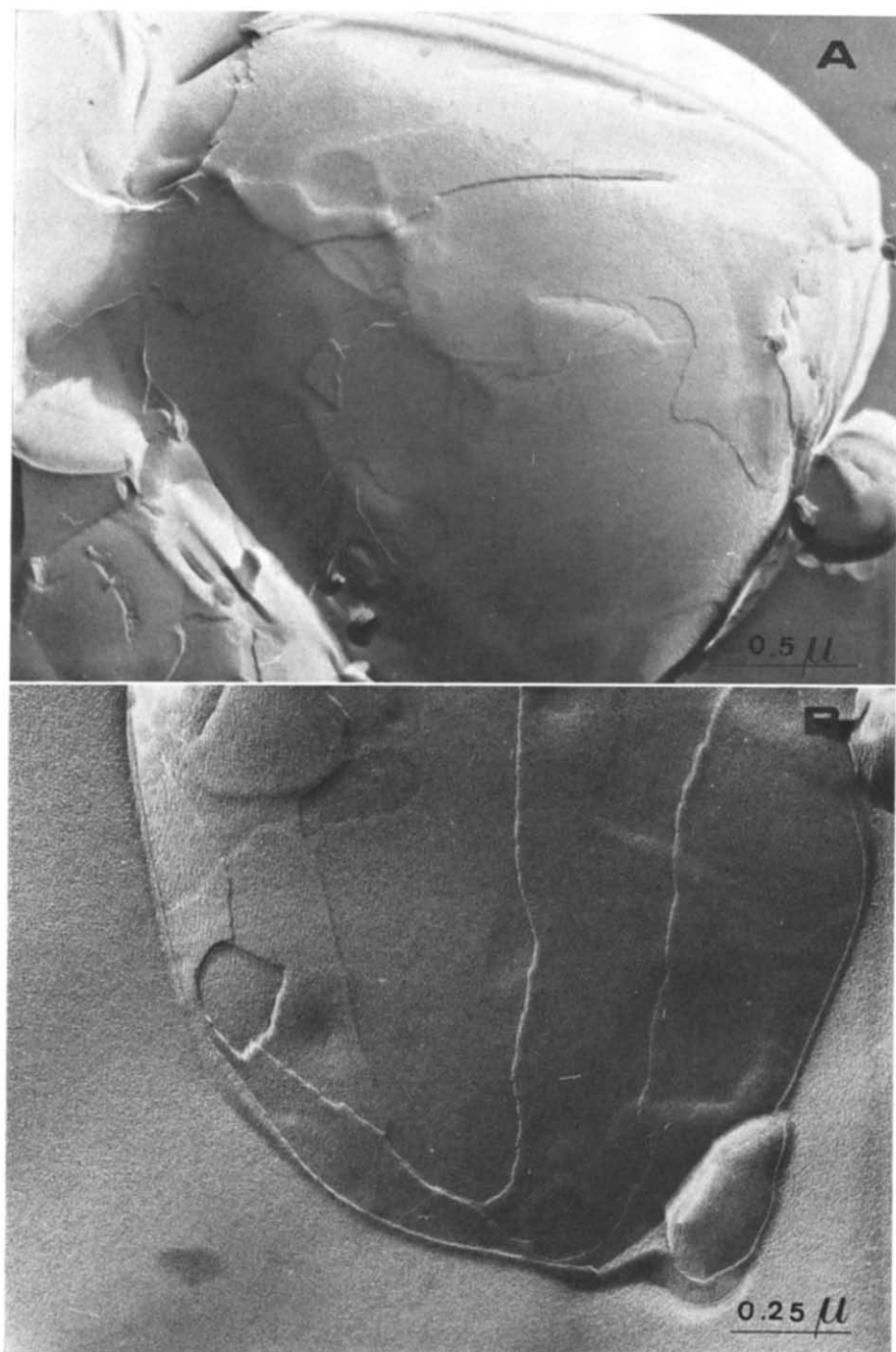
Fig. 6. Freeze-fracture faces. (A) Dimyristoyl phosphatidylglycerol in the presence of a high concentration of Mg^{2+}. The sample was heated to above 71 °C and cooled to 40 °C at which temperature it was quenched. This picture is also representative for the same sample quenched at 20 °C; (B) A mixture of dimyristoyl phosphatidylglycerol and dimyristoyl phosphatidylcholine (1 : 1) in the presence of a high concentration of Ca^{2+}. In the presence of Mg^{2+}, similar pictures were obtained.

at such a high temperature, but here the structure present at above 71 °C could be quenched from 40 °C after heating to above 71 °C (Fig. 6a). Liquid-crystalline liposomes form directly gel-phase liposomes upon cooling. As with dilauroyl phosphatidylglycerol (de Kruyff, B., personal communication) the ratio of the energy content of the two peaks involved in the transition from the gel phase liposome to the "crystal" structure (around 36 °C) varies from sample to sample but remains constant in one sample. The calorimetric data on dimyristoyl phosphatidylglycerol show, more clearly than with dilauroyl phosphatidylglycerol, that in the "crystals" the complex formed with Ca^{2+} is stronger than the one formed with Mg^{2+}.

These results are also relevant with respect to the puzzling thermograms of the effects of Ca^{2+} and Mg^{2+} on dipalmitoyl phosphatidylglycerol reported very recently by Jacobson and Papahadjopoulos [20]. As the authors scanned only to about 70 °C, it is not surprising that they did not observe a transition peak when Ca^{2+} was present. The gradual disappearance of the peak at about 50 °C in the presence of Mg^{2+} may be explained by the formation of a metastable phase analogous to the findings of dilauroyl phosphatidylglycerol [9] and dimyristoyl phosphatidylglycerol reported in this paper.

Calorimetric data on mixtures of dimyristoyl phosphatidylglycerol and dimyristoyl phosphatidylcholine

Phosphatidylethanolamines or phosphatidic acids with defined acyl chains have been shown to differ from their lecithin analogues with respect to the phase transition temperature [2, 8, 21, 22]. It is therefore remarkable that the transitions of dimyristoyl phosphatidylglycerol and dimyristoyl phosphatidylcholine occur at the same temperature. In both cases a sharp transition can be observed at 23–24 °C, with comparable
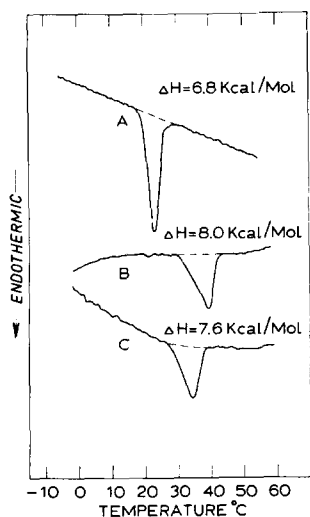
Fig. 7. Effect of Ca^{2+} and Mg^{2+} on the phase transition of a mixture of dimyristoyl phosphatidylglycerol and dimyristoyl phosphatidylcholine (1 : 1). (A) Lipid dispersed in a medium of pH 7; 120 mM NaCl; (B) lipid dispersed in a medium of pH 7; 120 mM $CaCl_2$; (C) lipid dispersed in a medium of pH 7; 120 mM $MgCl_2$.

enthalpy change values of about 6.8 kcal/mol for dimyristoyl phosphatidylglycerol (Fig. 1) and 6.6–6.7 kcal/mol for dimyristoyl phosphatidylcholine [23, 24]. Fig. 7 shows the transition of the 1 : 1 mixture which is found again around 23 °C but some broadening of the peak can be noticed. Both Ca^{2+} and Mg^{2+} are able to shift the transition temperature of the mixture, but this upward shift is limited to about 15 °C for Ca^{2+} and to 10 °C for Mg^{2+} at high concentrations of these divalent ions.

Freeze-fracture electron microscopy shows the absence of cylinders or piles of lamellae at all concentrations of the ions; only smooth liposomes can be observed (Fig. 6b). It is noteworthy that Ca^{2+} and Mg^{2+} shift the transition temperature of the whole mixture and that no phase separation is observed, as was deduced from ESR studies on phosphatidylserine/lecithin [25] and phosphatidic acid/lecithin mixtures [26]. The greater shift by Ca^{2+} compared to Mg^{2+} and the greater ΔH value indicate, as was also obvious from the effect on pure phosphatidylglycerol, that introduction of Ca^{2+} leads to a closer packing than does the introduction of Mg^{2+}.

Barrier properties

In order to test the barrier properties of the dimyristoyl phosphatidylglycerol bilayers under various conditions, liposomes were prepared in KCNS and the amount of K^+ trapped was measured after replacing the outside medium by extensive dialysis against isotonic choline chloride or by Sephadex G-50 gel filtration. Dispersions of pure dimyristoyl phosphatidylglycerol showed under polarized light microscope very small vesicular structures with birefringence. They appeared to be incapable of trapping K^+ at any temperature. Apparently the repulsive force resulting from the negative charge destabilizes the bilayer to such an extent that the membrane is freely permeable to ions at all temperatures. Studies with synthetic saturated lecithins showed that dimyristoyl phosphatidylcholine bilayers are good permeability barriers above and below the phase transition temperature, whereas they become highly permeable to ions in the vicinity of the phase transition temperature [19, 27]. Liposomes prepared from dimyristoyl phosphatidylglycerol in the absence of divalent ions behave like liposomes prepared from synthetic lecithins with less than 14 carbon atoms, which are also incapable of retaining trapped solutes [11]. In this respect it is of interest to note that Papahadjopoulos [14] found that, apart from the region of the phase transition temperature, liposomes of dipalmitoyl phosphatidylglycerol (in the absence of divalent ions) form efficient permeability barriers for $^{22}Na^+$, as do dipalmitoyl phosphatidylcholine bilayers.

As Papahadjopoulos and Ohki [28] noticed that bilayers of phosphatidylserine were stabilized by Ca^{2+} only when it was present on both sides of the membrane, we studied the permeability properties of our synthetic phospholipids with Ca^{2+} and Mg^{2+} already present in the medium used for dispersion. Dispersions of pure dimyristoyl phosphatidylglycerol in the presence of excess Ca^{2+} did not trap K^+ to a significant extent. This may confirm the open structure of the "crystals" as suggested by the freeze-fracture electron microscopy (Fig. 3B), although it should be realized that the trapped water volume of liposomes prepared from phospholipids without charge is normally very low [29].

Studies on dispersions of mixtures of dimyristoyl phosphatidylglycerol and dimyristoyl phosphatidylcholine led to the light-microscopic observation that with a decreasing dimyristoyl phosphatidylglycerol/dimyristoyl phosphatidylcholine ratio
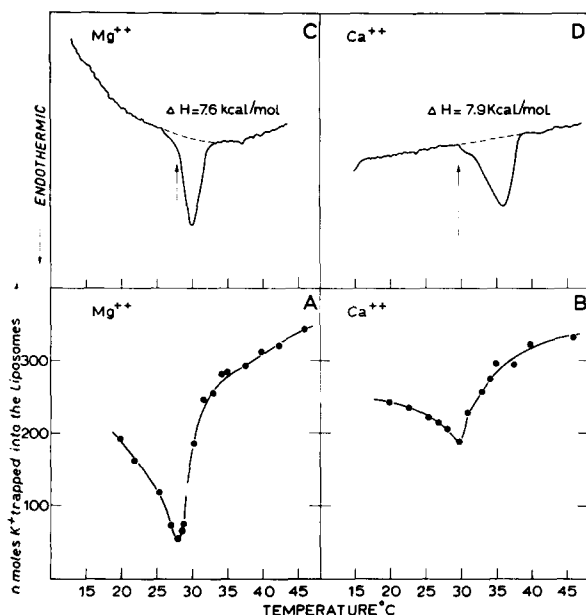
Fig. 8. Effect of Ca^{2+} and Mg^{2+} on the barrier properties of a mixture of dimyristoyl phosphatidylglycerol and dimyristoyl phosphatidylcholine. (A) 14.5 μmol dimyristoyl phosphatidylglycerol and 14.5 μmol dimyristoyl phosphatidylcholine codispersed in 1.0 ml 150 mM KCNS/10 mM Tris–HCl, pH 7.2., containing 7.0 μmol $MgCl_2$. Dispersed at 45 °C, cooled to 4 °C and dialysed for 90 min against isotonic choline chloride/7 mM $MgCl_2$ (portions of 250 ml, renewed every 15 min). Samples were pipetted into the incubation vessel at various temperatures. The K^+ leak was monitored for 3 min and then the remaining K^+ in the liposomes was liberated by the addition of 100 μl Triton X-100. (B) The same experiment in the presence of Ca^{2+}. (C) Differential scanning calorimetry scan of mixture A. (D) Differential scanning calorimetry scan of mixture B.

there is a gradual increase in the mean diameter of the liposomes. In the K^+-leak experiments we had to use a dimyristoyl phosphatidylglycerol/dimyristoyl phosphatidylcholine ratio of 1 : 4 to be able to measure significant amounts of K^+ trapped inside the structures after dialysis for 90 min. Liposomes prepared of a 1 : 1 mixture of dimyristoyl phosphatidylglycerol and dimyristoyl phosphatidylcholine are completely permeable to K^+ at all temperatures tested. However, as can be expected from the calorimetric data, the barrier properties of these model membranes were strongly improved by the addition of divalent ions. In Fig. 8 a typical experiment is given. Liposomes were prepared in a KNCS medium containing an amount of Ca^{2+} or Mg^{2+} such that it could neutralize a maximum of 95 % of the negative charges of the dimyristoyl phosphatidylglycerol. Excess Ca^{2+} (Mg^{2+}) was purposely not used, in order to leave enough negative charge on the bilayers to obtain liposomes with a suitable amount of water between the concentric bilayers. The liposomes were prepared at 45 °C, cooled to 4 °C and subsequently dialysed against isotonic choline chloride, containing divalent ions. In samples of the dialysed liposomes the amount of K^+ enclosed by the lipid bilayers was measured after 3 min incubation at various temperatures. From the results it can be concluded that the liposomes of dimyristoyl phosphatidylglycerol/dimyristoyl phosphatidylcholine (1 : 1) in the presence of Ca^{2+}

or Mg^{2+} are good permeability barriers for K^+ both below and above the transition temperature. However, a marked leak of the K^+ content of the liposomes was noted in the vicinity of the transition temperature. The maximum in this increased K^+ permeability coincided with the onset of the phase transition in these samples as measured by differential scanning calorimetry (Fig. 8). This loss of K^+ in the region of the phase transition temperature was more pronounced in the Mg^{2+} sample than in the case of Ca^{2+}. The improvement of the barrier properties in the presence of Ca^{2+} or Mg^{2+} confirms the idea about the molecular packing of dimyristoyl phosphatidylglycerol molecules arising from the calorimetric experiments. An increased permeability at the phase transition temperature has also been demonstrated for liposomes prepared from dimyristoyl phosphatidylcholine [19, 27] and dipalmitoyl phosphatidylglycerol [14].

Summarizing the results of this study, it can be concluded that divalent ions are extremely important for the packing and permeability properties of phosphatidylglycerol-containing bilayers. Bilayers containing 50 mol% of dimyristoyl phosphatidylglycerol are freely permeable to K^+ in the absence of divalent ions. Addition of Ca^{2+} or Mg^{2+} increases the packing, and consequently the bilayer becomes an efficient permeability barrier. However, at the phase transition temperature, which is dependent on the concentration and type of divalent ion added, the liposomes are more or less permeable to K^+.

As we have seen, bilayers of phosphatidylglycerol and bilayers of lecithin with the same acyl chain length exhibit totally different barrier properties. This is despite the fact that both the ΔH and ΔS values for their phase transitions (Table I) are in good agreement with each other. This indicates, as the ΔS value represents the relative amount of disorder introduced into the hydrocarbon chains as they undergo a

TABLE I

Sample	Enthalpy change ΔH (kcal $\cdot$ mol^{-1})	Entropy change ΔS (cal $\cdot$ mol^{-1} $\cdot$ deg^{-1})
Dilauroyl phosphatidylcholine*	4.3	15.8
Dilauroyl phosphatidylglycerol*		
100 mM NaCl	4.5	16.3
50 mM CaCl$_2$ or MgCl$_2$	5.0–5.5	17.2–19.0
100 mM CaCl$_2$ or MgCl$_2$	10.0	28.8
Dimyristoyl phosphatidylcholine	6.7	22.6
Dimyristoyl phosphatidylglycerol		
120 mM NaCl	6.8	22.9
60 mM CaCl$_2$	7.9	26.3
60 mM MgCl$_2$	7.8	26.0
120 mM CaCl$_2$	15.2	43.1
120 mM MgCl$_2$	12.4	36.0
Dimyristoyl phosphatidylglycerol/dimyristoyl phosphatidylcholine (1 : 1)		
120 mM NaCl	6.8	23.2
120 mM CaCl$_2$	7.9	25.9
120 mM MgCl$_2$	7.6	25.2

* Data derived from refs 3 and 9

phase transition, that the physical state above and below the lipid phase transition for lecithin is different compared to phosphatidylglycerol.

In saturated lecithins 14 carbon atoms per chain appears to be a minimum requirement for an ion-impermeable bilayer [11, 22, 26]. In saturated phosphatidylglycerols this limit appears to be more than 14 carbon atoms. However, in the presence of Ca^{2+} and Mg^{2+} this threshold value for an impermeable bilayer is reduced again to 14 carbon atoms.

Avoiding extensive discussion on the possible biological significance at the moment, it can be concluded briefly that this unusual bilayer behaviour should be considered in analysing permeability properties of biological membranes containing considerable quantities of phosphatidylglycerols.

REFERENCES

1 Ladbrooke, B. D. and Chapman, D. (1969) Chem. Phys. Lipids 3, 304–356
2 Traüble, H. and Eibl, H. J. (1974) Proc. Natl. Acad. Sci. U.S. 71, 214–219
3 Verkleij, A. J., de Kruyff, B., Ververgaert, P. H. J. Th., Tocanne, J. F. and van Deenen, L. L. M. (1974) Biochim. Biophys. Acta 339, 432–437
4 Hegner, D., Schummer, U. and Schnepel, G. H. (1973) Biochim. Biophys. Acta 307, 452–458
5 Kimelberg, H. K. and Papahajopoulos, D. (1974) J. Biol. Chem. 249, 1071–1080
6 Chapman, D. and Urbina, J. (1971) FEBS Lett. 12, 169–172
7 Papahadjopoulos, D., Cowden, M. and Kimelberg, H. (1973) Biochim. Biophys. Acta 330, 8–26
8 Chapman, D., Urbina, J. and Keough, K. M. (1974) J. Biol. Chem. 249, 2512–2521
9 Ververgaert, P. H. J. Th., de Kruyff, B., Verkleij, A. J., Tocanne, J. F. and van Deenen, L. L. M. (1975) Chem. Phys. Lipids 14, 97–101
10 Tocanne, J. F., Ververgaert, P. H. J. Th., Verkleij, A. J. and van Deenen, L. L. M. (1974) Chem. Phys. Lipids 12, 201–219
11 Hauser, H. and Barrat, M. D. (1973) Biochem. Biophys. Res. Commun. 2, 399–405
12 van Deenen, L. L. M. and de Haas, G. H. (1964) Adv. Lipid Res. 2, 168–229
13 Davidson, F. M. and Long, C. (1958) Biochem. J. 69, 458–466
14 Papahadjopoulos, D., Jacobson, K., Nir, S. and Isac, T. (1973) Biochim. Biophys. Acta 311, 330–348
15 Bligh, E. G. and Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37, 911–918
16 Tocanne, J. F., Verheij, H. M., Op den Kamp, J. A. F. and van Deenen, L. L. M. (1974) Chem. Phys. Lipids 13, 389–403
17 Fiske, C. H. and SubbaRow, Y. (1925) J. Biol. Chem. 66, 375–379
18 Bartlett, G. R. (1959) J. Biol. Chem. 234, 466–468
19 Haest, C. W. M., de Gier, J., van Es, G. A., Verkleij, A. J. and van Deenen, L. L. M. (1972) Biochim. Biophys. Acta 288, 43–53
20 Jacobson, K. and Papahadjopoulos, D. (1975) Biochemistry 14, 152–161
21 Shimshick, E. J. and McConnell, H. M. (1973) Biochemistry 12, 2351–2360
22 Blume, A. and Ackermann, Th. (1974) FEBS Lett. 43, 71–74
23 Phillips, M. C., Williams, R. M. and Chapman, D. (1969) Chem. Phys. Lipids 3, 234–244

24 de Kruyff, B., van Dijck, P. W. M., Demel, R. A., Schuyff, A., Brants, F. and van Deenen, L. L. M. (1974) Biochim. Biophys. Acta 356, 1–7
25 Onishi, S. and Ito, T. (1974) Biochemistry 13, 881–887
26 Ito, T. and Onishi, S. (1974) Biochim. Biophys. Acta 352, 29–37
27 Blok, M. C., van der Neut-Kok, E. C. M., van Deenen, L. L. M. and de Gier, J. (1975) Biochim. Biophys. Acta, in the press
28 Papahadjopoulos, D. and Ohki, S. (1969) Science 164, 1075–1077
29 Bangham, A. D., de Gier, J. and Greville, G. D. (1967) Chem. Phys. Lipids 1, 225–246