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EFFECTS OF LYSOPHOSPHATIDYLCHOLINES ON PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLCHOLINE/CHOLESTEROL LIPOSOME SYSTEMS AS REVEALED BY ^{31}P-NMR, ELECTRON MICROSCOPY AND PERMEABILITY STUDIES

C.J.A VAN ECHTELD [a], B. DE KRUIJFF [b], J.G. MANDERSLOOT [a] and J. DE GIER [a]

[a] *Laboratory of Biochemistry and* [b] *Institute of Molecular Biology, State University of Utrecht, Transitorium 3, Padualaan 8, NL-3584 CH Utrecht (The Netherlands)*

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(1) The effect of incorporation of different lysophosphatidylcholine species on the structure, barrier properties and dynamics of bilayers made of various phosphatidylcholines both the the presence and absence of cholesterol have been investigated by ^{31}P-NMR, freeze-fracture electron microscopy and K^+-permeability measurements. (2) In a dispersion of lysophosphatidylcholine : cholesterol (1 : 1) the lipids are organized in extended bilayers. Upon cooling a micellar solution of 1-palmitoyllysophosphatidylcholine below the chain-melting temperature a transition to a lamellar, most likely interdigitating organization is observed. ^{31}P-NMR shows in both situations a marked decrease in effective chemical shift anisotropy. (3) 1-Palmitoyllysophosphatidylcholine can be incorporated up to 30 mol% into liquid crystalline bilayers of dipalmitoylphosphatidylcholine and up to 35 mol% into dioleoylphosphatidylcholine bilayers. Above this concentration micellization of the bilayers occurs. In the gel state, bilayer structure is maintained up to 60 mol% of the lysocompound. (4) 1-Oleoyllysophosphatidylcholine can be incorporated to higher concentrations into liquid crystalline phosphatidylcholine bilayers than the palmitoyl analogue, which can be explained by the more cylindrical shape of the 1-oleoyllysophosphatidylcholine. (5) In marked contrast, incorporation of only 1 mol% of 1-oleoyllysophosphatidylcholine into gel state dipalmitoylphosphatidylcholine already destabilizes bilayer structure and makes the membranes completely permeable for K^+. These results are discussed with respect to the mixing properties of the various lysophosphatidylcholines. (6) In general these effects are accompanied by a loss of the K^+-permeability barrier, which however occurs at lower lysophosphatidylcholine concentrations than needed for the start of micellization. (7) Cholesterol incorporation counteracts the bilayer destabilizing role of lysophosphatidylcholines. (8) ^{31}P-NMR demonstrates with increasing lysophosphatidylcholine concentrations in the bilayers of phosphatidylcholines a decrease in the effective chemical shift anisotropy. As the rigid lattice spectra of lysophosphatidylcholine and phosphatidylcholine are identical, this reflects a change in the conformational and/or motional properties of the phospholipid head groups. This phenomenon might play a role in the observed permeability changes.

Introduction

Lysophospholipids are found in most biological membranes [1] and although the concentrations are normally low these components are of considerable interest as their physico-chemical properties are quite different from other membrane lipids. In purified form dispersed in water the lysophopholipids do not adopt a bilayer configuration but organize in micellar structures [2]. Hence elevated concentrations of lysophospholipids are expected to destabilize the membrane structure. As a consequence the membrane becomes more sensitive to fusion processes [3], can undergo morphological changes [4], its per-

meability properties may be affected [5] and eventually lysis or membrane disruption occurs [6–8].

The action of lysophosphatidylcholines in membrane model systems is only partially understood. Drastic changes in permeability characteristics seem not to be correlated with micellinization of the bilayers [9]. Lysophosphatidylcholines with different paraffin chains are known to have different effects [6]. Furthermore there are indications that other lipid components in the membrane such as cholesterol [8,10] and free fatty acids [11] can largely influence the rearrangements brought about by the lysocomponents. It also can be expected that lateral separations as a consequence of immiscibility of the components in the bilayer will largely affect the result [12,13]. In order to gain further insight into these aspects of the behaviour of lysophosphatidylcholines in model systems, we systematically studied different lysophosphatidylcholine-phosphatidylcholine (-cholesterol) mixtures. Results from K^+-entrapment experiments were combined with structural information obtained by freeze-fracture electron microscopy and ^{31}P-NMR measurements. As described in detail elsewhere [14] ^{31}P-NMR is sensitive to the motional properties of membrane phospholipids, such as local motion and orientation of the phosphate region In addition the macroscopic environment of the whole molecule determines the line shape of the ^{31}P resonance spectrum. This phase sensitivity makes ^{31}P-NMR a very suitable tool to follow the structural transitions that are expected to occur in liposomal phosphatidylcholine membranes with varying lysophosphatidylcholine content.

Materials and Methods

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine were synthesized as described in Ref. 15. 1-Palmitoyl-*sn*-glycero-3-phosphocholine and 1-oleoyl-*sn*-glycero-3-phosphocholine were obtained by enzymic hydrolysis of the corresponding diacyl compounds using phospholipase A_2 from pig pancreas (a gift from Dr. A. Slotboom). Egg phosphatidylcholine isolated from hen eggs was converted to egg phosphatidic acid using phospholipase D from Brussels sprouts. Cholesterol was obtained from Merck. The lipids were >99% pure as judged by HPTLC and GC. All other reagents used were of analytical grade.

Lipids were mixed in chloroform solutions, evaporated to dryness under nitrogen and stored overnight under vacuum. For K^+-entrapment measurements liposomes containing 4 mol% egg phosphatidic acid were prepared by dispersing the lipid in a 150 mM KCNS. 10 mM Tris-HCl, pH 7.5 buffer in concentrations ranging from 12.5 to 16 mM. To remove non-trapped K^+, the dispersions were dialyzed against a 150 mM $MgSO_4$, 10 mM Tris-HCl, pH 7.5 buffer. After refreshing the dialysis buffer every 10 min, the trap inside 100 μl of the liposomal dispersions was determined in 5 ml $MgSO_4$ buffer after 26 min from the start of the dialysis by addition of Triton X-100 with a K^+-selective glass electrode (Schott and Gen, Jena Glaswerk, Mainz, F.R.G.) at the desired temperature.

High power proton noise (input power 18 watt) decoupled ^{31}P-NMR spectra of the lipid dispersions were recorded on a Bruker WH 90 spectrometer operating at 36.4 MHz using a spectral width of 12 kHz, a 45°C pulse angle and a 0.17-s interpulse time Accumulated free induction decays were obtained from 10000 to 20000 transients on 1.2 ml samples in 10-mm tubes containing 50-100 μmol lipid in a 100 mM NaCl, 25 mM Tris-HCl, 0.2 mM EDTA pH 7.5 buffer, containing 25% ^2H_2O. To increase the signal to noise ratio all accumulated free induction decays were exponentially filtered resulting in a 50 Hz line broadening. The amount of 'bilayer' signal, arising from large lamellar structures and characterized by an asymmetric spectrum with a low-field shoulder and a high-field peak, has been determined by planimetry and comparison with simulated spectra with an estimated accuracy ranging from 1 to 5%. The effective chemical shift anisotropy ($\Delta\sigma$) has been determined by taking the distance between the isotropic resonance position and the high-field peak as 1/3 $\Delta\sigma$ [16], since the low-field shoulder was often poorly resolved. For phospholipids in the liquid crystalline state the estimated accuracy of $\Delta\sigma$ was ±2 ppm. In the case of gel state phospholipids due to residual ^{31}P-^{1}H couplings the lineshape of the ^{31}P-NMR spectrum is slightly distorted making accurate determinations of $\Delta\sigma$ more difficult. The error in the reported $\Delta\sigma$ values in this case is estimated to be ±4 ppm. High power proton noise decoupled ^{31}P-NMR

spectra (input power 20 watt) of dry lipid powder were recorded on a Bruker WP 200 spectrometer operating at 81.0 MHz using a spectral width of 50 kHz, a 90°C pulse angle and 10-s delay time. Accumulated free induction decays were obtained from 1000 transients on 140–200 μmol dry lipid in 10-mm tubes, and were exponentially filtered resulting in a 50 Hz line broadening. Freeze-fracture electron microscopy was performed as outlined before [17]. 25% (v/v) glycerol was added to the samples to prevent freeze damage.

Results

1. ^{31}P-NMR spectra of 1-palmitoyllysophosphatidylcholine

Fig. 1 shows the results of ^{31}P-NMR measurements of 1-palmitoyllysophosphatidylcholine and dipalmitoylphosphatidylcholine under various conditions. The rigid lattice spectra given in Fig. 1A and B are obtained from dry powder samples of the two lipids. The principal values of the ^{31}P shielding tensor σ_{11}, σ_{22} and σ_{33} of dipalmitoylphosphatidylcholine are −89, −31 and 124, respectively. These values fall in between those previously reported for anhydrous dipalmitoylphosphatidylcholine and the corresponding monohydrate [18–20]. σ_{11}, σ_{22} and σ_{33} for 1-palmitoyllysophosphatidylcholine were found to be −91, −30 and 122, respectively. Considering the experimental errors these values are identical to those of the dipalmitoylphosphatidylcholine.

Dispersion of dipalmitoylphosphatidylcholine in water at a temperature above the phase transition results in the spontaneous formation of large, multilayered liposomes. In the liquid crystalline state the chemical shift anisotropy is partially averaged, mainly as a consequence of rapid axial rotation. The result is a spectrum with a low-field shoulder and a high-field peak (compared Fig. 1C), which is characteristic for the bilayer organization [16,21]. When the lysophosphatidylcholine is dispersed in water, micelles are formed, which due to fast tumbling cause effective motional averaging resulting in an isotropic ^{31}P-NMR signal as shown in Fig. 1D.

From differential scanning calorimetric measurements it is known that 1-palmitoyllysophosphatidylcholine in excess water undergoes a gel to liquid crystalline phase-transition at around 3°C [13]. Therefore we thought it of interest to measure the ^{31}P-NMR spectrum also at −10°C in a supercooled buffer. The resulting spectrum given in Fig. 1E can be considered as a combination of a typical bilayer spectrum with an isotropic signal on top. Therefore

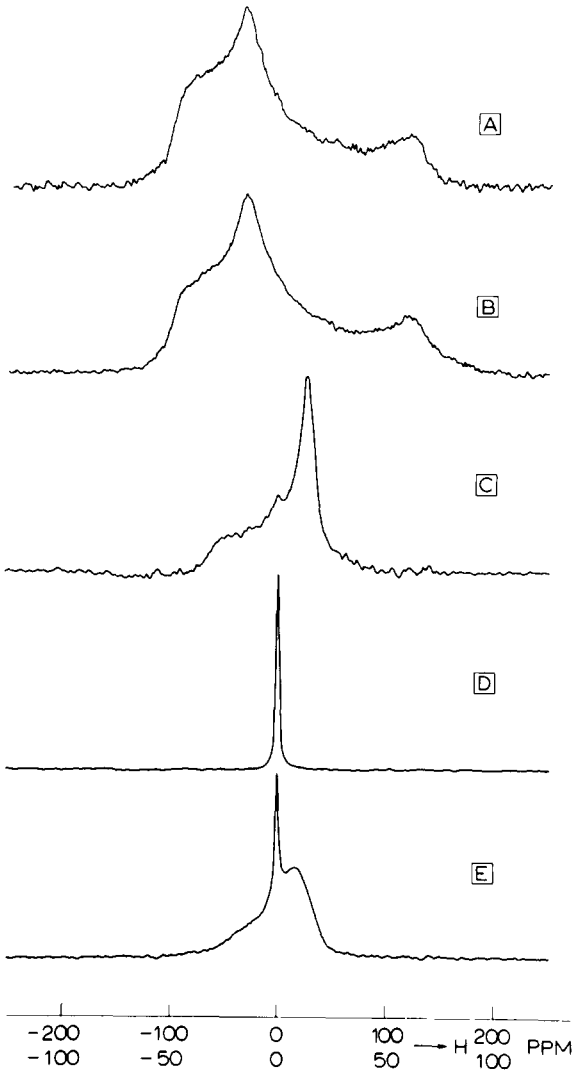

Fig 1. Dry powder 81.0 MHz ^{31}P-NMR spectra of· (A) dipalmitoylphosphatidylcholine at 25°C and (B) 1-palmitoyllysophosphatidylcholine at 25°C, corresponding with the upper scale. 36.4 MHz ^{31}P-NMR spectra of aqueous dispersions of (C) dipalmitoylphosphatidylcholine at 45°C and (D) 1-palmitoyllysophosphatidylcholine at 25°C and (E) 1-palmitoyllysophosphatidylcholine at −10°C, corresponding with the lower scale. 0 ppm corresponds with the resonance position of diacylphosphatidylcholine undergoing isotropic motion.

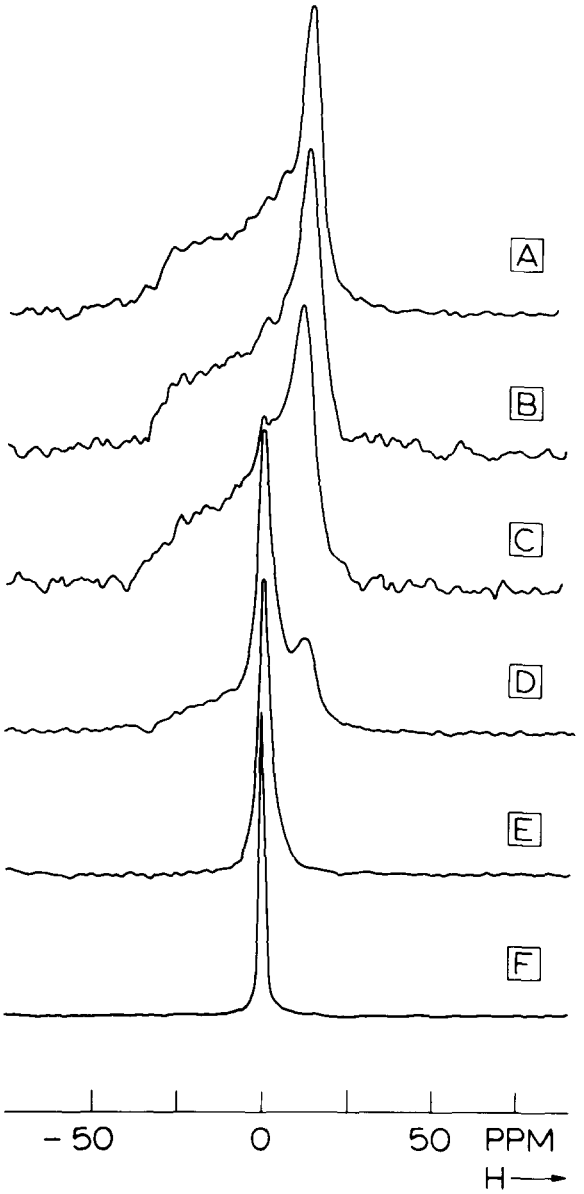

Fig 2. 36.4 MHz ^{31}P-NMR spectra of aqeous dispersions of mixtures of dioleoylphosphatidylcholine and 1-palmitoyl-lysophosphatidylcholine with molar ratios of respectively: (A) 100/0; (B) 90/10; (C) 70/30; (D) 50/50; (E) 30/70 and (F) 10/90.

it can be concluded that below the transition temperature 1-palmitoyllysophosphatidylcholine is able to form extended bilayers. Furthermore it can be seen that the $\Delta\sigma$ of the bilayer of the lysocompound is only 25 ppm as compared to ~40 ppm typical for diacylphospholipids (see Fig. 1C and Refs. 16 and 21). Freeze-fracture electron microscopic experiments on the lysophosphatidylcholine system failed to show extended smooth fracture faces as is typical for diacylphosphatidylcholine bilayer systems.

2. 1-Palmitoyllysophosphatidylcholine in mixtures with diacylphosphatidylcholines

Representative ^{31}P-NMR spectra of dispersions of mixtures of dioleoylphosphatidylcholine and increasing amounts of 1-palmitoyllysophosphatidylcholine are illustrated in Fig. 2. In agreement with former X-ray studies on an egg phosphatidylcholine system [9] the bilayer configuation is preserved up to high molar concentrations of the lyso component. The quantitative interpretation of the spectra given in Fig. 3A demonstrates that up to 35 mol% all the lipid molecules are in a bilayer orientation. Above 40 mol% of 1-palmitoyllysophosphatidylcholine a rapidly increasing isotropic signal can be noticed and beyond 60% apparently all the molecules undergo isotropic motion. This behaviour was found to be insensitive to temperature variation in the range from 5 to 45°C. Together with this relatively sharp transition the appearance of the suspension changes from

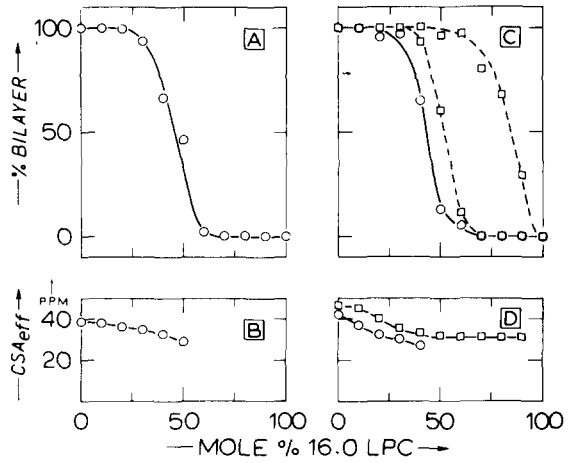

Fig. 3. Amount of ansiotropic ^{31}P-NMR signal from phospholipid molecules, in a bilayer organization and effective chemical shift ansiotropy ($\Delta\sigma$, CSA_{eff}) as derived from 36.4 MHz spectra of aqueous dispersions of mixtures of (A) and (B). dioleoylphosphatidylcholine and 1-palmitoyllysophosphatidylcholine at 25°C (○); (C) and (D): dipalmitoylphosphatidylcholine and 1-palmitoyllysophosphatidylcholine at 45°C (○) and 25°C (□) LPC, lysophosphatidylcholine

cloudy to totally clear, indicating that the bilayers are converted into mixed micelles. This is confirmed also by freeze-fracture electron microscopy which shows multilayered smooth fracture faces only in the cloudy systems.

When 1-palmitoyllysophosphatidylcholine is mixed with dipalmitoylphosphatidylcholine in the liquid crystalline state (at 45°C) a similar result can be noticed although comparison of Fig. 3A and C shows that the transition to the micellar state in the case of the saturated phosphatidylcholine occurs at a somewhat lower concentration of the lysocompound. When the phosphatidylcholine is in the gel state the bilayer organization can be retained up to much higher concentrations of the palmitoyllysophosphatidylcholine. When the mixtures are dispersed at 45°C, subsequently cooled to 0°C and afterwards measured at 25°C a full bilayer spectrum is obtained up to 60 mol% of the lysophosphatidylcholine. When the mixtures are measured at 25°C immediately after the dispersion the amount of bilayer organization is less but still more than in the liquid crystalline state. As will be discussed this phenomenon is probably related to a hysteresis effect also noted in differential scanning calorimetry experiments [13].

Fig. 3B and D show the result of measurement of the effective chemical shift anisotropy in the bilayer signal of the mixtures. It can be noticed that these $\Delta\sigma$ values gradually decrease with increasing concentrations of the lyso component. In the gel situation $\Delta\sigma$ reaches a plateau at 50 mol% of the lysocompound.

3. 1-Oleoyllysophosphatidylcholine in mixtures with diacylphosphatidylcholines

The experiments described in the previous section have been repeated with 1-oleoyllysophosphatidylcholine and the quantitative measurements on the spectra are presented in Fig. 4. The results seem to be more complicated. Already at low concentrations of the 1-oleoyllysophosphatidylcholine there is a rapid increase of the isotropic signal but with increasing concentrations this isotropic signal decreases and at around 50 mol% again a nearly complete bilayer spectrum can be observed. Above this concentration there is a rapid increase in isotropic signal due to the formation of mixed micelles. The decrease in $\Delta\sigma$ in these

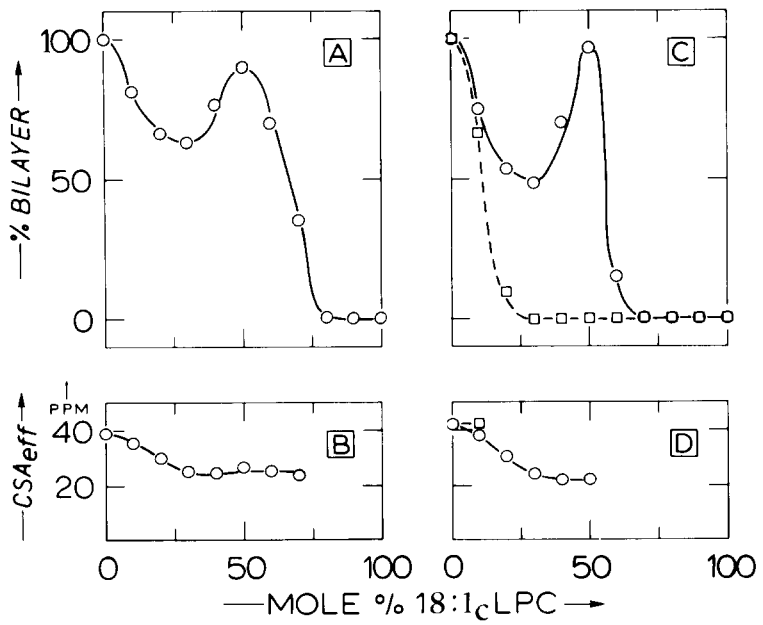

Fig. 4. Amount of anisotropic ^{31}P-NMR signal from phospholipid molecules in a bilayer organization, and effective chemical shift anisotropy ($\Delta\sigma$, CSA_{eff}) as derived from 36.4 MHz spectra of aqueous dispersions of mixtures of (A) and (B) dioleoylphosphatidylcholine and 1-oleoyllysophosphatidylcholine at 25°C (○); (C) and (D): dipalmitoylphosphatidylcholine and 1-oleoyllysophosphatidylcholine at 45°C (○) and 25°C (□). LPC, lysophosphatidylcholine

experiments is much faster and a plateau is reached at 30 mol% of the lyso compound.

The first increase in isotropic signal is not due to the formation of micelles but a consequence of the formation of small vesicles in which tumbling and lateral diffusion in the curved bilayers cause isotropic motion of the phospholipid molecules. To further investigate this anomalous behaviour samples with low lysophosphatidylcholine concentration have been centrifuged at 35 000 × *g*. After resuspending the pellets, ^{31}P-NMR spectra have been recorded once again, as well as from the supernatants. The pellets showed bilayer type of spectra with less than 5% isotropic signal, while the supernatants produced somewhat broadened isotropic signals. This indicates that the lipid responsible for the isotropic signal was not associated with the larger structures. Additionally, both pellets and supernatants were capable of trapping K^+ indicating that the supernatants also consisted of closed bilayer structures. A typical freeze-fracture picture of the non-centrifuged sample is given in Fig. 5 and it can be noticed that very small vesicles occur next to large multilayered liposomes. The 1-oleoyllysophosphatidylcholine apparently possesses unique structural features, so that at low concentrations an organization in a more curved bilayer is preferred. At lysophosphatidylcholine concentrations of approx. 50 mol% other mechanisms prevail to accomodate the lysocompound in the bilayer as appears from the extended fracture planes found by freeze-fracturing.

When the micellization by 1-oleoyllysophosphatidylcholine as shown in Fig. 4 is compared with that by 1-palmitoyllysophosphatidylcholine given in Fig. 3 it can be concluded that the unsaturated lysophosphatidylcholine is less potent in destabilizing the

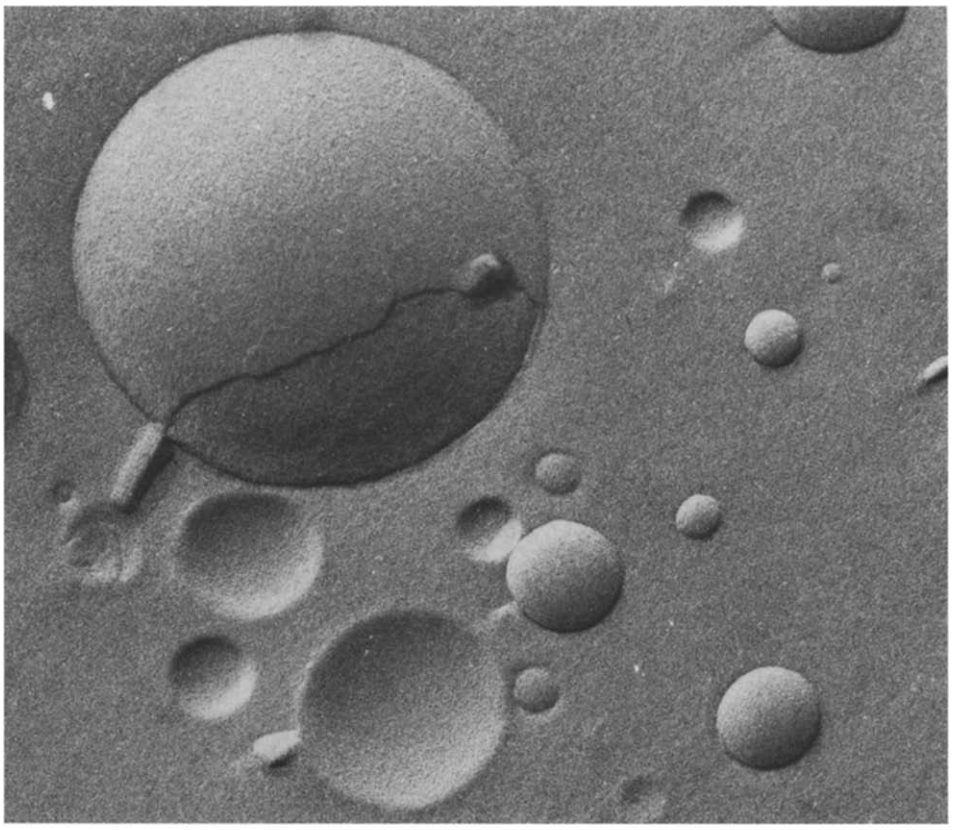

Fig 5 Freeze-fracture electron micrograph of an aqueous dispersion of a mixture of 10 mol% 1-oleoyllysophosphatidylcholine and 90 mol% dioleoylphosphatidylcholine. The sample was quenched from 25°C Magnification: ~100 000×.

bilayer structure. However, in the gel state bilayer the 1-oleoyllysophosphatidylcholine has a completely different effect. Already at low concentrations the bilayer spectrum is changed completely into an isotropic signal.

4. Permeability changes

To test the effect of the lysophosphatidylcholines on the barrier function we have measured the ability of the various mixtures to trap K^+. Fig. 6 shows that with increasing concentrations of the lysophosphatidylcholines the permeability barrier is abolished. In the liquid crystalline bilayers the 1-palmitoyllysophosphatidylcholine is more potent than the 1-oleoyllysophosphatidylcholine, whereas both lysophosphatidylcholines are more effective in the saturated bilayer. These results are in agreement with the findings concerning the potency of the lysophosphatidylcholines to destroy the bilayer. However, it can be noticed that the permeability barrier disappears at

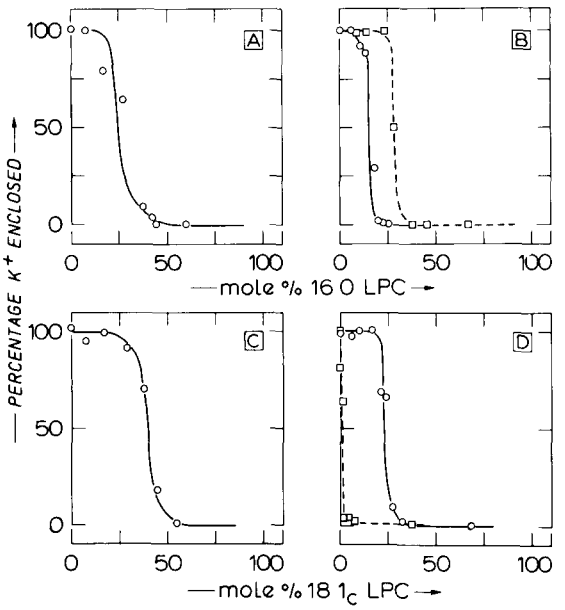

Fig. 6. K^+-trapping ability of aqeous dispersions of mixtures of: (A) dioleoylphosphatidylcholine and 1-palmitoyllysophosphatidylcholine at 25°C (○); (B)dipalmitoylphosphatidylcholine and 1-palmitoyllysophosphatidylcholine at 55°C (○) and 25°C (□); (C) dioleoylphosphatidylcholine and 1-oleoyllysophosphatidylcholine at 25°C (○); (D) dipalmitoyllysophosphatidylcholine and 1-oleoyllysophosphatidylcholine at 55°C (○) and 25°C (□). LPC, lysophosphatidylcholine.

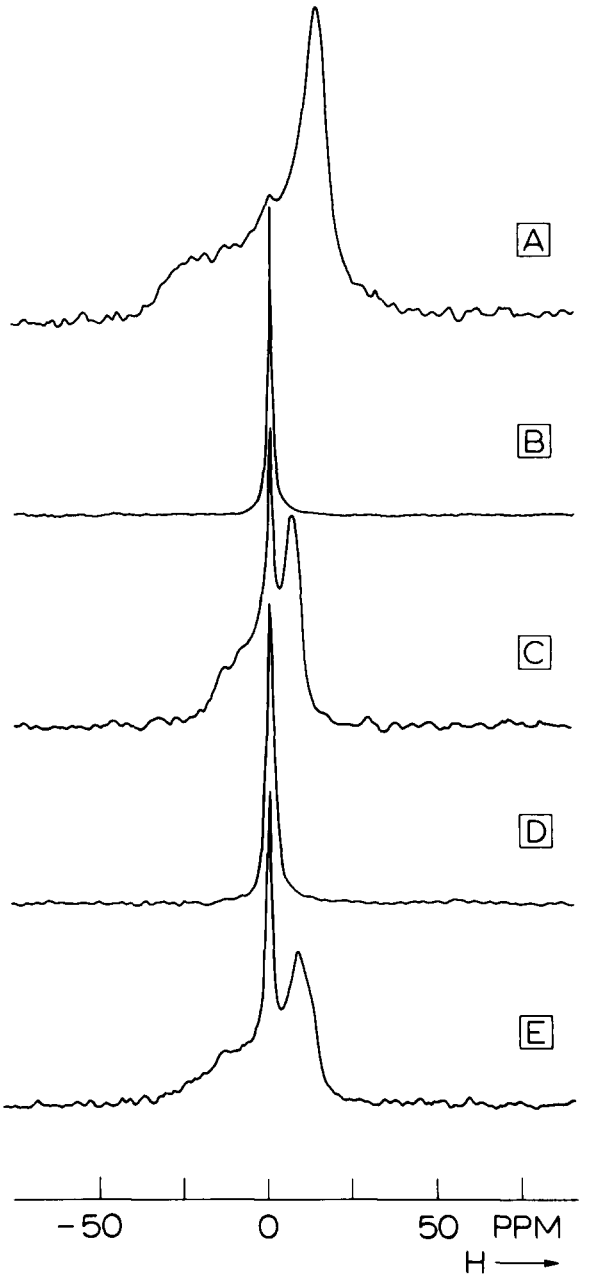

Fig. 7. 36.4 MHz ^{31}P-NMR spectra of aqeous dispersions of. (A) dipalmitoylphosphatidylcholine at 45°C; (B) 1-palmitoyllysophosphatidylcholine at 25°C; (C) a mixture of 1-palmitoyllysophosphatidylcholine and cholesterol with a molar ratio of 1 : 1 at 25°C; (D) a mixture of dipalmitoylphosphatidylcholine and 1-palmitoyllysophosphatidylcholine with a molar ratio of 2 . 3 at 45°C; (E) a mixture of dipalmitoylphosphatidylcholine, 1-palmitoyllysophosphatidylcholine and cholesterol with a molar ratio of 2 . 3 : 3 at 45°C.

much lower concentrations than the bilayer organization.

In the gel state bilayer, 1-palmitoyllysophosphatidylcholine is less active than in the liquid crystalline membrane. On the other hand a concentration as low as 1 mol% of 1-oleoyllysophosphatidylcholine in the gel state membrane makes the bilayer completely permeable to K^+.

5. The antagonistic action of cholesterol

Previous observations have indicated that cholesterol may counteract the action of the lysophosphatidylcholine. Such conclusions have been drawn from X-ray measurements [10] and the ability of lysophosphatiydylcholine-cholesterol mixtures to trap glucose [22]. These observations are confirmed by ^{31}P-NMR results. The spectrum in Fig. 7C is obtained from a dispersion of an equimolar mixture of 1-palmitoyllysophosphatidylcholine and cholesterol and shows a bilayer organization with a small amount of phospholipid undergoing isotropic motion. When Fig. 7A and C are compared it can be seen that for the mixture the $\Delta\sigma$ is largely reduced and amount to 20 ppm. Essentially the same spectrum was obtained with the oleoyl analogue. Furthermore it has been found that the spectra are insensitive to temperature variations in the range 0–45°C. Fig. 7D gives a spectrum of a mixture of dipalmitoylphosphatidylcholine and 1-palmitoyllysophosphatidylcholine in a ratio 2 . 3 which shows a complete isotropic spectrum as a consequence of the presence of mixed micelles. The presence of increasing amounts of cholesterol in this mixture causes increasing bilayer organization of the lipid molecules. When cholesterol is equimolar with the lysocompound a nearly full bilayer organization results as is shown in Fig. 7E. Again the $\Delta\sigma$ is largely reduced. By varying the fatty acids of the lysophosphatidylcholine or the phosphatidylcholine, or by changing the composition of the mixtures or the temperature, the antagonistic action of cholesterol is evident throughout. This effect is not always obvious in gel state mixtures because cholesterol also exerts its fluidizing properties. The minor isotropic peaks seen in Fig. 7C and E may originate from compositional inaccuracy, slight sample inhomogeneity or imperfect structural organization, leaving lipid molecules in a micellar organization.

Discussion

The comparative experiments in this study demonstrate that the action of a lysophosphatidylcholine on the permeability and organization of a bilayer depends on: (i) the nature of the paraffin chain of the lysophosphatidylcholine, (ii) the nature of the lipids constituting the bilayer and (iii) the physical state of the bilayer.

To understand the measured effects it is of interest to take into account the dynamic shape of the molecules. Phosphatidylcholines forming a stable bilayer can be considered as cylindrical molecules. In the lysophosphatidylcholines, missing one fatty acid, the size of the apolar and polar part is out of balance and therefore they prefer as conical shaped molecules a micellar organization in the liquid crystalline state. In mixtures of the two lipids there is apparently a rather critical concentration below which the lysomolecules are accommodated in the bilayer organization and above which the phosphatidylcholines fit into the micelles. The shape concept also explains some details. When in the lysophosphatidylcholine molecule the saturated palmitoyl chain is replaced by the unsaturated oleoyl chain some volume is added to the hydrophobic part which may explain the decrease of bilayer destabilizing ability noticed in our experiments. The same trend has been found in the lytic action on erythrocytes being less for the oleoyl compound than for the palmitoyl compound [6]. In a similar way it can be accounted for that a bilayer of dioleoylphosphatidylcholine can accommodate a higher concentration of lyso molecules than a bilayer of liquid crystalline dipalmitoylphosphatidylcholine before they break up into micelles. Also the cholesterol effect can be explained in terms of a shape concept. Cholesterol with its small polar head and large hydrophobic part has a dynamic shape which is complementary to that of lysophosphatidylcholine and consequently stable bilayers can be formed by an equimolar mixture of these two lipids.

A striking result coming from our measurements is the finding that 1-palmitoyllysophosphatidylcholine by itself in excess water is able to form bilayers at low temperatures. It is comprehensive that the lysophosphatidylcholine molecule with a gel state paraffin chain will not fit in a micellar organization. However, also in the gel state there is no balance in

size of polar headgroup and paraffin part of the molecule and therefore the bilayer organization indicated by the ^{31}P-NMR spectrum (Fig. 1E) must be of a special type in which the paraffin chains are strongly tilted with respect to the plane of the bilayer or in which the paraffin chains are interdigitating. The latter possibility is strongly supported by freeze-fracture results showing that this bilayer is not easily split into its two halves. On the base of X-ray analysis such an interdigitating bilayer has been described by Hauser et al. [23] for the crystal structure of deoxylysophosphatidylcholine monohydrate.

From differential scanning calorimetric experiments on mixtures of 1-palmitoyllysophosphatidylcholine on dipalmitoylphosphatidylcholine we reached the conclusion that in the gel state this lyso component is homogeneously distributed in the bilayer, but hysteresis effects indicated that differential organizations may be possible [13]. Also the present ^{31}P-NMR measurements indicate two different gel state organizations at 25°C. It can be suggested that the less stable organization is formed by mixed bilayers in which the paraffins are highly tilted. In view of the above discussion it seems possible that upon cooling the bilayer may be stabilized by interdigitation of the paraffin chains and in this organization the bilayer is stable also in the presence of high concentrations of 1-palmitoyllysophosphatidylcholine. Confirmation of this hypothesis will require detailed X-ray analysis.

If in the gel state mixtures with dipalmitoylphosphatidylcholine 1-palmitoyllysophosphatidylcholine is replaced by 1-oleoyllysophosphatidylcholine a completely different result is obtained as is shown in Fig. 4B. The calorimetric experiments have already indicated that upon transition from the liquid crystalline to the gel state there is a lateral separation of the 1-oleoyllysophosphatidylcholine [13]. The formation of pore-like structures by the segregated component may in this particular system explain the disappearance of the K^+-permeability barrier at very low concentrations of the lyso component and the disappearance of the bilayer signal also at much lower concentrations when compared to the liquid crystalline conditions.

It is noteworthy that the reflections of compositional differences can be seen both in the micellinization of the bilayer and in the disappearance of the K^+-permeability barrier. The permeability effects, however, become apparent well below the concentrations at which the mixed micelles are formed. The same observation has been made in a former study on a system of egg phosphatidylcholine [9]. In this study the permeability measurements are correlated with X-ray results that show a decreasing bilayer thickness with increasing lysophosphatidylcholine concentrations. At a critical concentration and corresponding bilayer thickness the K^+-permeability barrier is lost. In this respect it may be of relevance that a dispersion of dilauroylphosphatidylcholine has a bilayer thickness less than the critical lysophosphatidylcholine/phosphatidylcholine mixture and is incapable of trapping ions [9]. Yet ^{31}P-NMR shows a complete bilayer organization for this system with a $\Delta\sigma$ slightly less than for dipalmitoylphosphatidylcholine [24]. On the other hand, the equimolar mixture of lysophosphatidylcholine and cholesterol [10] has also a bilayer thickness less than the critical one mentioned but is perfectly capable of trapping ions (results not shown). As shown this mixture gives a bilayer type of ^{31}P-NMR spectrum with a $\Delta\sigma$ of 20 ppm.

This reduced $\Delta\sigma$ is a common feature in all lysophosphatidylcholine-containing mixtures. As the principal values of the chemical shielding tensor of 1-palmitoylphosphatidylcholine are identical to those of dipalmitoylphosphatidylcholine it can be ruled out that the differences in the effective chemical shift ansiotropy originate from tensor differences. The size of the structure may also influence the $\Delta\sigma$ [25]. This most likely plays a role in phosphatidylcholine mixtures with oleoyllysophosphatidylcholine, but is not a satisfying explanation when freeze-fracturing shows large extended fracture planes. When lysolecithin is the only phospholipid constituent of a membrane, alone (Fig. 1E), or mixed with free fatty acids [11] or cholesterol (Fig. 7C) the reduced $\Delta\sigma$ is most prominent.

The conformation of the phosphocholine moiety of different lysophosphatidylcholine, deoxylysophosphatidylcholines and phosphatidylcholines has been found closely comparable, although actual information on the phosphodiester conformation of lysophosphatidylcholines is lacking [26–31]. It seems to be primarily governed by intramolecular interactions. So there is no apparent reason to suppose the reduced

$\Delta\sigma$ to originate from conformational differences in the phosphocholine part of the molecule.

The glycerol backbone of the molecule is a more likely candidate for conformational differences. The preferred conformations for phosphatidylcholines and lysophosphatidylcholines in micelles are those which allow intra- and intermolecular chain stacking, although less restricted for the lysophosphatidylcholine [29,30]. For hydrated lysophosphatidylcholine in a bilayer organization no detailed conformations are known.

Although conformational differences could result in the $\Delta\sigma$ found we favour the idea that the decreased $\Delta\sigma$ is primarily governed by an increased motional freedom, e.g. in the glycerol backbone or even of the whole molecule. Unfortunately, the order parameter for the C_1-C_2 glycerol segment in lysophosphatidylcholine is not known. However, from the presented results it is obvious that the different conformational or motional properties of the lysophosphatidylcholine and phosphatidylcholine molecules influence each other. In these mixtures such an increased motional freedom or different conformation, which more statistically may be described by or correlated with bilayer thickness, may explain the increased permeability towards K^+. In those mixtures where the other membrane constituents have complementary shapes to that of lysophosphatidylcholine, other properties than bilayer thickness and decreased $\Delta\sigma$ apparently govern permeability behaviour.

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