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EFFECT OF pH, MONO- AND DIVALENT CATIONS ON THE MIXING OF PHOSPHATIDYLGLYCEROL WITH PHOSPHATIDYLCHOLINE

A MONOLAYER (π, ΔV) AND FLUORESCENCE STUDY

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The mixing of various molecular species of phosphatidylglycerol and phosphatidylcholine differing in their acyl chain lengths has been studied both in monolayers (π, ΔV), and in water dispersions (fluorescence polarization) with varying pH and ionic strength of the aqueous phase and in the presence of the divalent cations Mg^{2+} and Ca^{2+}. In dilauroylphosphatidylglycerol/dipalmitoylphosphatidylcholine mixtures, both in monolayers and in water dispersions, no phase separation was detected at pH 2.9 where phosphatidylglycerol was protonated. With dipalmitoylphosphatidylglycerol/dipalmitoylphosphatidylcholine mixtures, in monolayers and at the same pH, no phase separation was detected for surface pressures below $\pi = 40$ mN·m^{-1}. In monolayers, and under ionic conditions such that phosphatidylglycerol was ionized (pH 5.6, 10 mM NaCl) miscibility was observed with dilauroylphosphatidylglycerol and dipalmitoylphosphatidylcholine and also with dipalmitoylphosphatidylglycerol and dilauroylphosphatidylcholine. Varying the ionic strength did not alter the miscibility of these lipids. The divalent cations Mg^{2+} and Ca^{2+} did not modify that of dilauroylphosphatidylglycerol with dilauroylphosphatidylcholine or with dipalmitoylphosphatidylcholine. Both in monolayers and in water dispersions, dipalmitoylphosphatidylglycerol and dilauroylphosphatidylcholine appeared to be at least partly miscible, in the presence of magnesium. Only in the presence of calcium and at high surface pressure might the monolayer data account for phase separation between these two lipids. The data presented demonstrate the existence of strong cohesive forces between phosphatidylcholine and phosphatidylglycerol with a marked influence of the former on the physical state of the latter. From an analysis of the ΔV data, it is suggested that intrafacial hydrogen bonds may play a significant role in stabilizing phosphatidylcholine/phosphatidylglycerol mixtures.

Introduction

Biological membranes invariably contain mixtures of acidic and zwitterionic phospholipids or neutral glycolipids. Zwitterionic lipids such as phosphatidylcholines or phosphatidylethanolamines are not very sensitive to pH or ions, at least over the pH range 3 to 8 [1]. This is not the case with acidic lipids, the ionization state, the molecular packing and the phase properties of which have been shown to be strongly dependent on the pH and on the ionic strength of the aqueous phase and on the presence of the bivalent cations Ca^{2+} and Mg^{2+} [2–4,25–28,45]. Various investigations have revealed that, in biomembranes, phospholipides are asymmetrically distributed between the inner and the outer layers of the lipid bilayer [5]. As yet however, little is known about the lateral

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distribution of phospholipids within each lipid leaflet. In the view of possible ionotropic lipid-mediated regulation of membrane functions, it is of great interest to study the phase behaviour of mixtures of acidic phospholipids with neutral or zwitterionic ones, in connection with the ionization state of the former. In this respect, Ca^{2+} has been shown to induce phase separation in different mixtures of phosphatidylcholine and acidic lipids, like phosphatidylserine and phosphatidic acid [6–11], and phosphatidylethanolamine/phosphatidylserine mixtures [12]. In contrast, no phase separation has been detected in the case of mixtures of phosphatidylcholine and phosphatidylglycerol with identical acyl chains. Only partial immiscibility was observed in the case of phosphatidylglycerol with longer acyl chains than phosphatidylcholine and in the presence of a large excess of calcium ions [11,13,14]. Similarly, no phase separation has been observed for mixtures of phosphatidylglycerol with a dimannosyl diacylglycerol [15] and for mixtures of phosphatidylcholine with phosphatidylinositol [16].

Only very recently has the influence of pH and ionic strength been investigated: in phosphatidylcholine/phosphatidylserine mixtures, lowering the pH or the ionic strength triggers phase separation [17].

The aim of the present communication is to report, in addition to the efect of Ca^{2+} and Mg^{2+}, on an investigation of the influence of pH and ionic strength on the mixing of phosphatidylcholine and phosphatidylglycerol. These two lipids are encountered in some bacterial membranes [18]. They are believed to play an important role in the interfacial behaviour of pulmonary surfactant [19,20]. Most of the present study was carried out by means of the monolayer technique, through surface pressure and surface potential measurements. Complementary fluorescence polarization experiments were carried out on lipid water dispersions.

Materials and Methods

Chemicals

1,6-Diphenyl-1,3,5-hexatriene was obtained from Merck. Dilauroylphosphatidylglycerol sodium salt and *rac*-dipalmitoylphosphatidylglycerol ammonium salt were both of synthetic origin [21,22].

Dilauroylphosphatidylcholine, dimyristoylphosphatidylcholine and *rac*-dipalmitoylphosphatidylcholine were purchased from Sigma. All these lipids were pure as checked by thin-layer chromatography.

Monolayer experiments

The compression isotherms shown in this paper were recorder traces obtained by continuous compression of the film. Surface potential was measured with an apparatus [23] and according to a procedure [15] which have already been described.

For both π and ΔV experiments, ultrapure water from an industrial source (Motorola, Toulouse) was used. Lipids were spread in the form of chloroform/methanol (5:1, v/v) solutions of known concentrations prepared by weighing lipid samples carefully dried under vacuum, prior to use. The experimental procedure was identical to that described elsewhere [4].

Throughout all experiments, reference surface potentials of aqueous subphases were around 20–30 mV. Film compressions were reproducible to within 1% ($\pm 5 \cdot 10^{-3}$ nm^2) and the reproducibility of ΔV determinations was ± 10 mV. The data presented here are the average of two to three experiments. The temperature was 20°C.

Fluorescence

The phase transition temperatures of the lipids were determined by following changes with temperature of the fluorescence polarization rate of diphenylhexatriene embedded in the lipid vesicles. Experiments were carreid out with a PF1 apparatus [24].

The excitation wavelength was selected by means of an interference filter centered at 365 nm. Fluorescence emission was recovered through a cutoff filter transmitting light above 430 nm (Wratten Kodak filter). Intensities were measured vertically (I_v) and horizontally (I_h) to calculate the polarization rate:

$$p = \frac{I_v - I_h}{I_v + I_h}$$

The temperature was raised stepwise at a rate of 0.5 K/min.

The lipids (10 mg) were dispersed in 1 ml of the desired aqueous salt solution (10 mM NaCl, pH 2.7 or 1 mM $MgCl_2$, pH 5.6) in the presence of the fluorescent probe at a concentration of $4.6 \cdot 10^{-6}$ M. In these conditions, the final probe/lipid molar ratio was about 2/100. The lipids were stirred by Vortex for 2 or 3 min, then sonicated for 10 min at 80 kHz (sonicating bath) and at room temperature to give stable opalescent suspensions.

Since bivalent cations are known to strongly interact with phosphatidylglycerol [4,22,25–28], it was checked that the lipid suspensions obtained in the presence of Mg^{2+} were not formed of lipid aggregates. Under the electron microscope, the negatively stained [29] lipid suspensions appeared to consist mainly of small multilamellar vesicles, in the presence of either Na^+ or of Mg^{2+}.

Results

Influence of acidic pH

Monolayer experiments. Consequences of lowering the pH were first studied on mixtures of dilauroylphosphatidylglycerol and dipalmitoylphosphatidylcholine spread on a 10 mM NaCl subphase at pH 2.9. Under these ionic conditions, phosphatidylglycerol has been shown to be protonated [4].

The addition of dilauroylphosphatidylglycerol to dipalmitoylphosphatidylcholine caused an increase of the phase transition pressure of the latter and a decrease of the mean molecular area which, in the gel state, reached a nearly constant value of 0.44 nm^2 (Fig. 1). It is clear from these results that dipalmitoylphosphatidylcholine has a strong condensing effect on protonated dilauroylphosphatidylglycerol. That the two lipids interact together is also clearly demonstrated in Fig. 2 where the mean area per molecule is plotted versus the lipid mole fraction, at constant surface pressure. In such phase diagrams, any straight line is to be considered as representing either ideal mixing or phase separation, while any deviation from linearity is indicative of intermolecular interactions between the two lipids.

Surface potentials measured on the same mixed films also negatively deviated from linearity (Fig. 2). It should be stressed that the linar plot observed at high surface pressures ($\pi = 30$ mN $\cdot$ m^{-1}) for dilauroylphosphatidylglycerol mole fractions greater than 0.5 turned out to deviate negatively after expressing the surface potential in terms of $\Delta V/n$ which corrects for changes in lipid surface density [33].

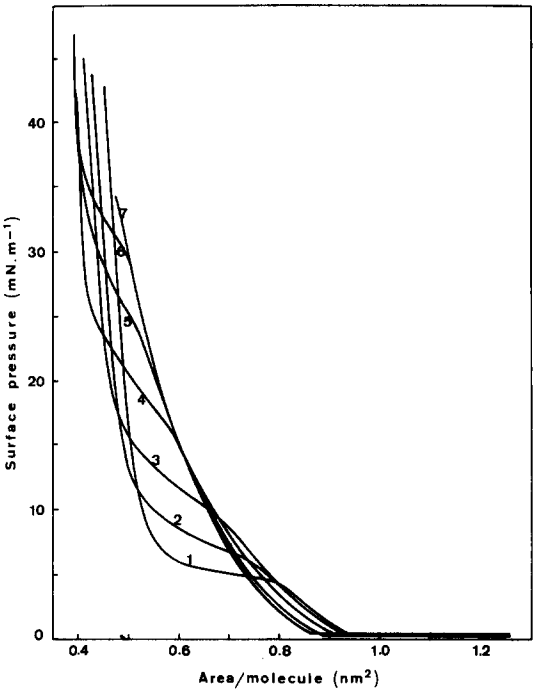

Fig. 1. Compression isotherms for mixed monolayers of dilauroylphosphatidylglycerol and dipalmitoylphosphatidylcholine. Mole fraction of phosphatidylglycerol: 0 (1); 0.10 (2); 0.25 (3); 0.50 (4); 0.75 (5); 0.90 (6); 1.00 (7). Subphase was 10 mM NaCl, pH 2.9.

The mixing of dipalmitoylphosphatidylglycerol and dilauroylphosphatidylcholine in monolayers at pH 2.9 (10 mM NaCl) was also studied. For surface pressures below $\pi = 40$ mN $\cdot$ m^{-1}, a positive deviation from linearity was observed in the mean area versus lipid mole fraction phase diagrams. At and above this pressure, linearity was obeyed.

Fluorescence polarization. As shown in Fig. 3, dipalmitoylphosphatidylcholine (curve a) displayed a phase transition centered at a temperature of $T_t = 42°C$ ($\Delta T \sim 8$ K) which is the usual value reported for this lipid in neutral conditions [2]. This means that even at the acidic pH 2.7, the polar head still possessed a zwitterion structure. Dilauroylphosphatidylglycerol (curve d) showed a

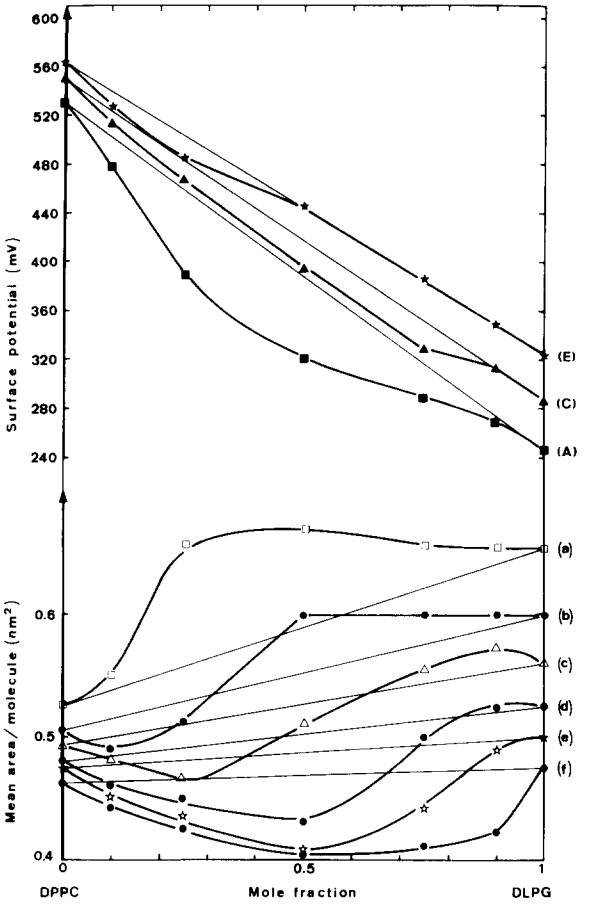

Fig. 2. Mean area per molecule (lower part) and surface potential (upper part) versus mole fraction for dilauroylphosphatidylglycerol (DLPG) mixed with dipalmitoylphosphatidylcholine (DPPC) at various surface pressures π. π in $mN \cdot m^{-1}$: 10 (a, A); 15 (b); 20 (c, C); 25 (d); 30 (e, E); 35 (f). Subphase was 10 mM NaCl, pH 2.9.

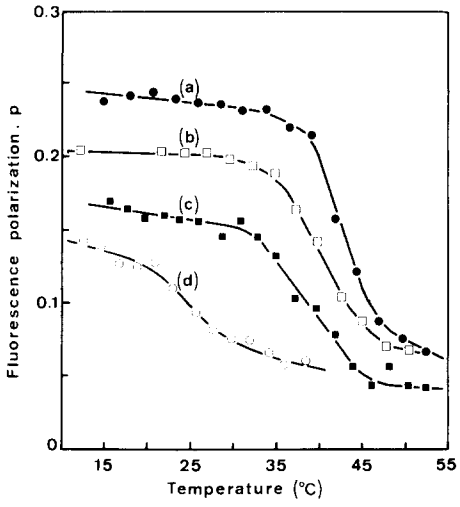

Fig. 3. Changes with temperature of the fluorescence polarization rate p of diphenylhexatriene embedded in mixed vesicles of dilauroylphosphatidylglycerol and dipalmitoylphosphatidylcholine. Mole fraction of phosphatidylglycerol: 0 (a); 0.20 (b); 0.50 (c); 1.00 (d). Aqueous phase was 10 mM NaCl, pH 2.7.

phase transition centered at $T_t = 25°C$ ($\Delta T \sim 9$ K) as expected for the protonated form of this lipid [22]. Adding dilauroylphosphatidylglycerol to dipalmitoylphosphatidylcholine lowered the phase transition temperature. T_t was 39°C ($\Delta T \sim 10$ K) and 38°C ($\Delta T \sim 13$ K) for phosphatidylglycerol mole fractions of 0.20 and 0.50, respectively.

Influence of monovalent cations and of the ionic strength

Monolayer experiments. In a first set of experiments, mixing of dilauroylphosphatidylglycerol and dipalmitoylphosphatidylcholine was studied on pure water and in the presence of NaCl at various concentrations in the subphase, at pH 5.6. It should be remembered that phosphatidylglycerols, on pure water, give condensed films presumably due to nearly complete protonation of the lipid molecules, an increase of the subphase ionic strength giving rise to a progressive film expansion originating from the ionization of the phosphate groups at the interface [4].

Compression isotherms and phase diagrams obtained for the lipids alone and their mixtures on pure water (curves not shown) were similar to those recorded on acidic subphases (presented in Figs. 1 and 2).

The compression isotherms and the corresponding phase diagrams obtained on 1 mM, 10 mM and 100 mM NaCl subphases were similar. As illustrated in Figs. 4 and 5, relative to the 10 mM NaCl subphase, at moderate dilauroylphosphatidylglycerol mole fractions, all π/A curves tended to converge at high surface pressures to reach a molecular packing characteristic of lipids in a condensed state (mean molecular area ~ 0.45 nm^2). This means that dipalmitoylphosphatidylcholine still has a strong condensing effect on dilauroylphosphatidylglycerol, even when this lipid

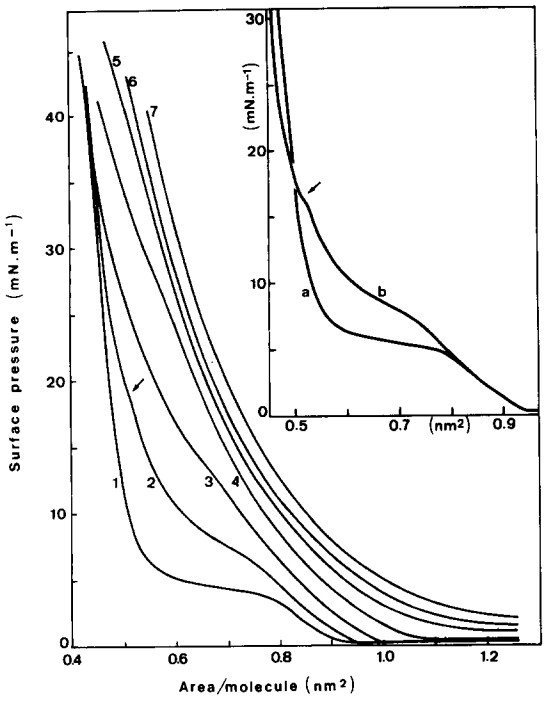

Fig. 4. Compression isotherms for mixed monolayers of dilauroylphosphatidylglycerol and dipalmitoylphosphatidylcholine. Mole fraction of phosphatidylglycerol: 0 (1); 0.10 (2); 0.25 (3); 0.50 (4); 0.75 (5); 0.90 (6); 1.00 (7). Subphase was 10 mM NaCl, pH 5.6. Insert: mole fraction of phosphatidylglycerol: 0 (a); 0.10 (b). Subphases were either pure water, pH 5.6 or 10 mM $CaCl_2$, pH 5.6.

is ionized. At a surface pressure of about $\pi = 19$ $mN \cdot m^{-1}$ (arrow, curve 2), a shoulder was visible on the compression isotherm related to a dilauroylphosphatidylglycerol mole fraction of 0.10. A shoulder was also observed on the compression curve which was recorded for the same lipid mixture on pure water (curve b, insert, Fig. 4). Dipalmitoylphosphatidylcholine being assumed to be in the gel state for surface pressures above $\pi \sim 6$ $mN \cdot m^{-1}$, this shoulder might be assigned to a phase transition of dilauroylphosphatidylglycerol from the liquid-expanded to the condensed state. Such a hypothesis is supported by the phase diagrams shown in Fig. 5 where, for a dilauroylphosphatidylglycerol mole fraction of 0.10, a positive deviation from linearity was observed up to a surface pressure $\pi = 20$ $mN \cdot m^{-1}$ (curves a, b and c) whereas a negative deviation, characteristic of phase condensation, was detected for surface pressure above $\pi = 25$ $mN \cdot m^{-1}$ (curves d, e, f and g).

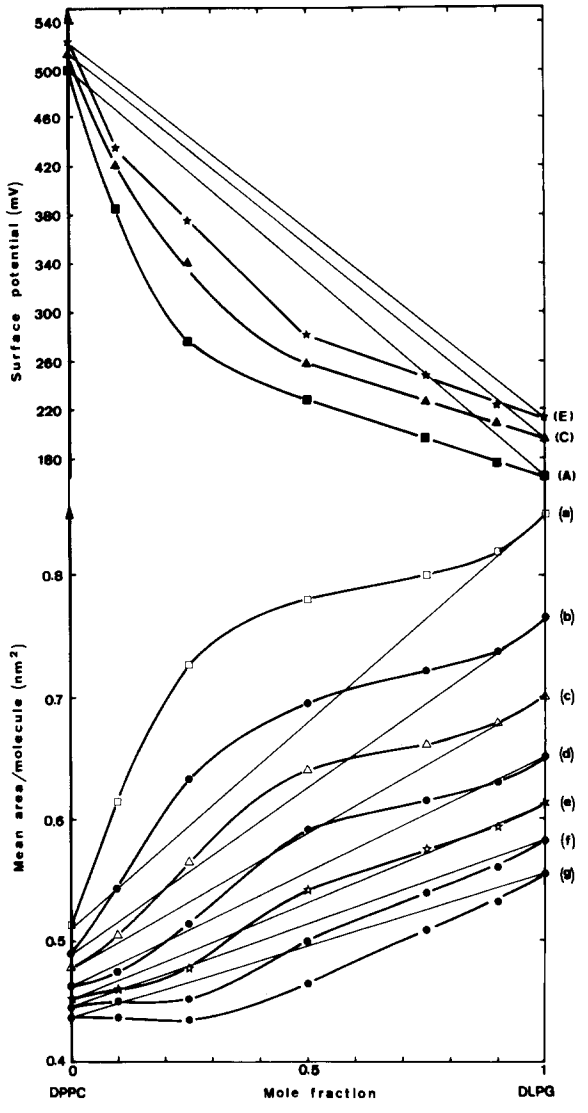

Fig. 5. Mean area per molecular (lower part) and surface potential (upper part) versus mole fraction for dilauroylphosphatidylglycerol (DLPG) mixed with dipalmitoylphosphatidylcholine (DPPC) at various surface pressures π. π in $mN \cdot m^{-1}$: 10 (a, A); 15 (b); 20 (c, C); 25 (d); 30 (e, E); 35 (f); 40 (g). Subphase was 10 mM NaCl, pH 5.6.

In terms of surface potential (Fig. 5), large negative deviations from linearity were observed at any surface pressure, even after correcting for lipid surface density changes ($\Delta V/n$).

In a second set of experiments, the influence of the acyl chain length was tested by mixing di-

palmitoylphosphatidylglycerol with dilauroylphosphatidylcholine, on a 10 mM NaCl subphase (pH 5.6). In this case, no film condensation was detected. Instead, film expansion was observed (Fig. 6). This can be seen in Fig. 7 where all the mean area versus mole fraction plots positively deviate from linearity. Large negative deviations from linearity were also found with the surface potential (Fig. 7).

Influence of bivalent cations. The influence of Ca^{2+} was first studied by mixing dilauroylphosphatidylglycerol and dipalmitoylphosphatidylcholine in monolayers. Compression isotherms and mean area versus mole fraction phase diagrams found on a 10 mM $CaCl_2$ subphase at pH 5.6 (curves not shown) were remarkably similar to these obtained on pure water. For example, the π/A curve for 10% dilauroylphosphatidylglycerol is shown in Fig. 4 (insert, curve b). This curve was found to be the same on pure water and on a 10 mM $CaCl_2$ subphase. In the presence of calcium, plots of the surface potentials against the phospholipid mole fractions negatively deviated from linearity. Similar results were obtained on a 10 mM $MgCl_2$ subphase.

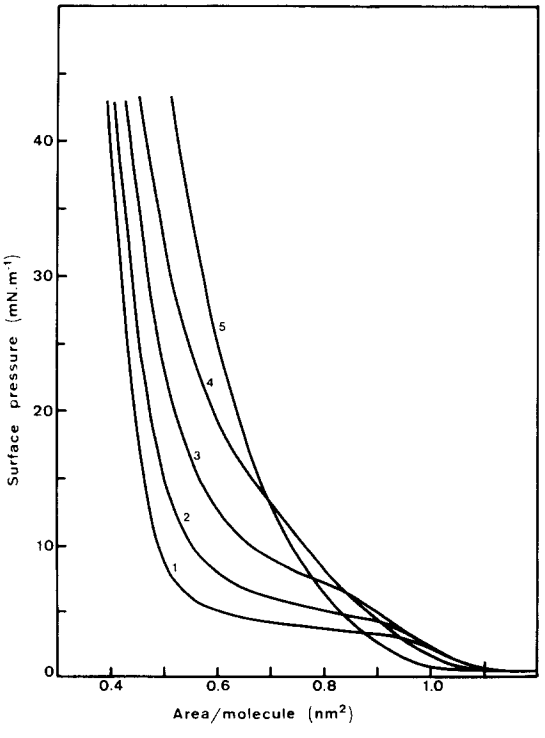

Fig. 6. Compression isotherms for mixed monolayers of dipalmitoylphosphatidylglycerol and dilauroylphosphatidylcholine. Mole fraction of phosphatidylglycerol: 1.00 (1); 0.90 (2); 0.75 (3); 0.50 (4); 0 (5). Subphase was 10 mM NaCl, pH 5.6.

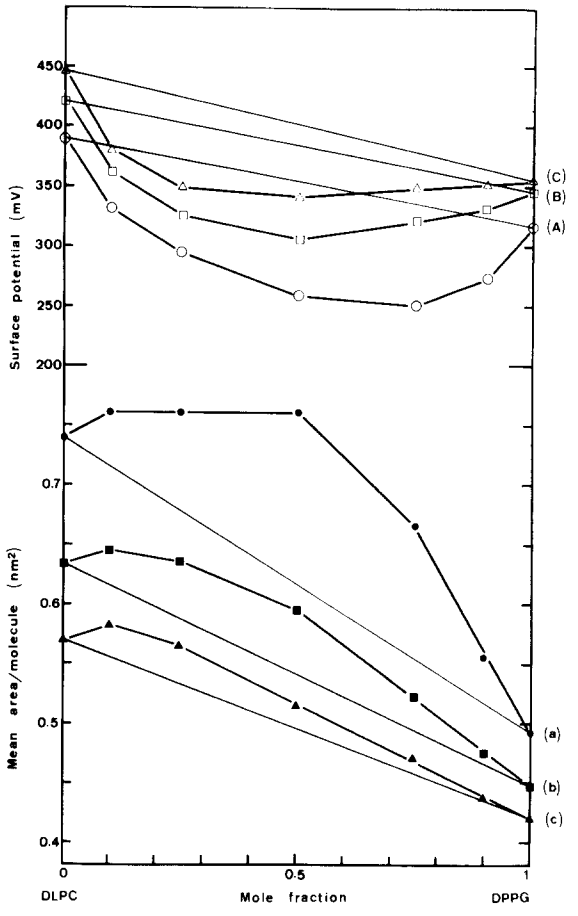

Fig. 7. Mean area per molecule (lower part) and surface potential (upper part) versus mole fraction for dipalmitoylphosphatidylglycerol (DPPG) mixed with dilauroylphosphatidylcholine (DLPC) at various surface pressures π. π in $mN \cdot m^{-1}$: 10 (a, A); 20 (b, B); 30 (c, C). Subphase was 10 mM NaCl, pH 5.6.

The influence of bivalent cations was also studied on mixtures of dipalmitoylphosphatidylglycerol and dilauroylphosphatidylcholine. Adding the latter to the former resulted in progressive film expansion at any pressure (Fig. 8). As shown in Fig. 9, mean area versus mole fraction plots positively deviated from linearity at low and moderate film surface pressures, while the corresponding surface potential negatively deviated. On the other hand, the additivity rule was found to be obeyed both in terms of mean area and surface potential,

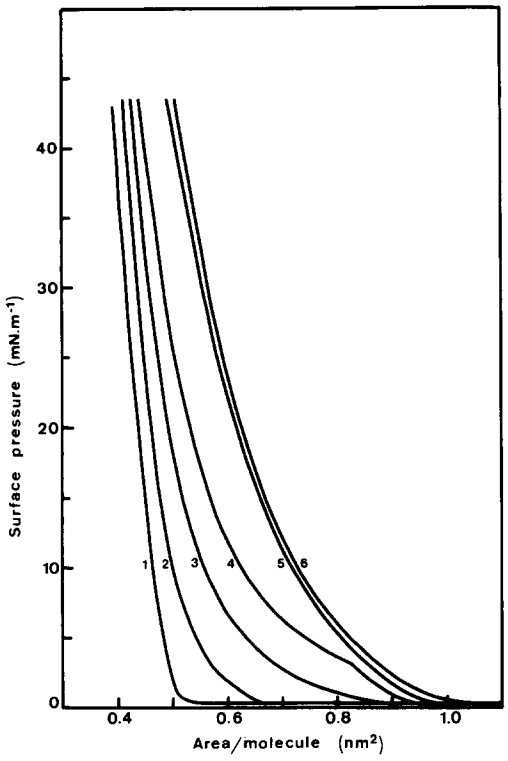

Fig. 8. Compression isotherms for mixed monolayers of dipalmitoylphosphatidylglycerol and dilauroylphosphatidylcholine. Mole fraction of phosphatidylglycerol: 1.00 (1); 0.90 (2); 0.75 (3); 0.50 (4); 0.10 (5); 0 (6). Subphase was 10 mM $CaCl_2$, pH 5.6.

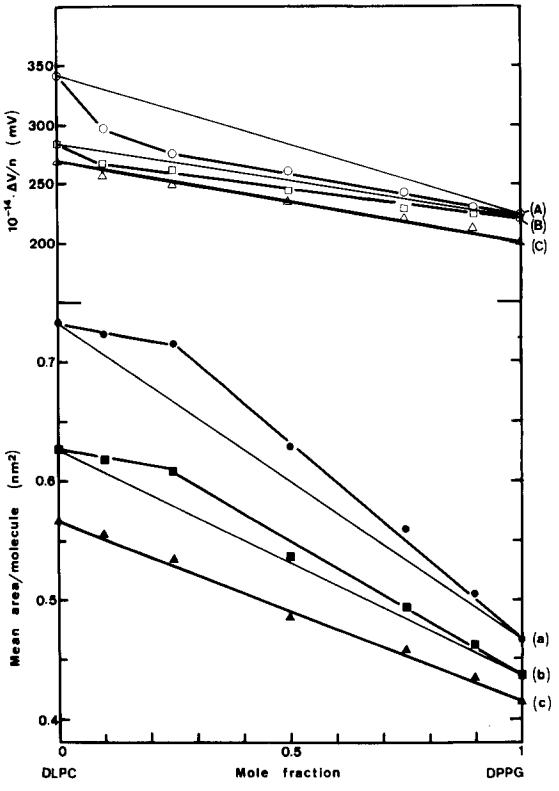

Fig. 9. Mean area per molecule (lower part) and surface potential (upper part) versus mole fraction for dipalmitoylphosphatidylglycerol (DPPG) mixed with dilauroylphosphatidylcholine (DLPC) at various surface pressures π. π in $mN \cdot m^{-1}$: 10 (a, A); 20 (b, B); 30 (c, C). Subphase was 10 mM $CaCl_2$, pH 5.6.

for high surface pressures ($\pi = 30$ $mN \cdot m^{-1}$, curves c and C in Fig. 9).

Another interesting feature was the occurrence of a break in the compression isotherms recorded for lipid mixtures containing about 30–60% dilauroylphosphatidylcholine, which can be accounted for by a liquid-expanded to liquid-condensed phase transition [15]. This is exemplified by curve 4 in Fig. 8, corresponding to an equimolecular lipid mixture.

The influence of Mg^{2+} (1 mM $MgCl_2$, pH 5.6) was also tested. The compression isotherms and mean area versus mole fraction phase diagrams (curves not shown)) resembled those obtained with Ca^{2+} (Figs. 8 and 9) except that linear plots were not observed at high surface pressures. Phase transition in the compression curves started to be detectable at a lower dilauroylphosphatidylcholine mole fraction with Mg^{2+} ($x \sim 0.10$) than with Ca^{2+} ($x \sim 0.30$).

The ability of Mg^{2+} to induce or not a phase separation within dipalmitoylphosphatidylglycerol/dilauroylphosphatidylcholine mixtures was tested on water dispersions of these lipids. In the presence of magnesium (and calcium), phase transition of dipalmitoylphosphatidylglycerol is known to occur at very high temperature ($T_t > 75°C$) [22]. Accordingly, the fluorescence polarization rate of diphenylhexatriene stood at a high and constant value of 0.27 for the lipid alone (Fig. 10, curve a). Consistent with the phase transition temperature of $-2°C$ reported for dilauroylphosphatidylcholine [35,36], a low polarization rate of about 0.05 was measured for this lipid (curve c). In agreement with monolayer experiments, lipid mixture containing 10% dilauroylphosphatidylcholine displayed a phase transition centered around 50°C (curve b).

The influence of Ca^{2+} was also checked on

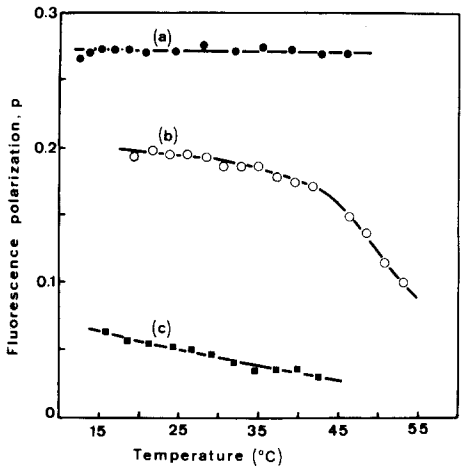

Fig. 10. Changes with temperature of the fluorescence polarization rate p of diphenylhexatriene embedded in mixed vesicles of dipalmitoylphosphatidylglycerol and dilauroylphosphatidylcholine. Mole fraction of phosphatidylglycerol: 1.00 (a); 0.90 (b); 0 (c). Aqueous phase was 1 mM $MgCl_2$, pH 5.6.

dilauroylphosphatidylglycerol/dilauroylphosphatidylcholine mixtures in monolayers. The compression isotherms recorded for the two lipids alone on a 10 mM $CaCl_2$ subphase (pH 5.6) were similar (curves not shown). Mean area versus mole fraction phase diagrams revealed that the mixing of the two lipids proceeded with a slight but systematic film condensation.

Discussion

The present study shows that phosphatidylglycerol and phosphatidylcholine exhibit a marked tendency to be miscible both in monolayers and in water dispersions, regardless of their acyl chain length, of their molecular packing and of the ionization state of the phosphatidylglycerol. No phase separation was detected either at pH 2.9 (Figs. 1–3) where phosphatidylglycerol is protonated [4], or at pH 5.6 (Figs. 4–7) where the lipid is ionized [4] at least up to surface pressures as high as 40 $mN \cdot m^{-1}$. Varying the ionic strength of the aqueous phase did not modify the mutual miscibility of the two lipids. Divalent cations such as Mg^{2+} and Ca^{2+} had no effect on the mixing of dilauroylphosphatidylglycerol with dilauroylphosphatidylcholine or with dipalmitoylphosphatidylcholine. In the case of dipalmitoylphosphatidylglycerol/dilauroylphosphatidylcholine mixtures, both the molecular area and surface potential versus lipid concentration plots became linear, but only at high surface pressures ($\pi = 30$ $mN \cdot m^{-1}$) and in the presence of calcium ions in the subphase (Fig. 9). Such linear plots are not devoid of ambiguity since, as already mentioned, they can indicate either ideal mixing or lateral phase separation. In fact, most of the monolayer data presented in this work account for non-ideal mixing of the various phosphatidylglycerol/phosphatidylcholine couples studied and, for that reason, the linear plots of Fig. 9 are likely to be accounted for by phase separation. At surface pressures lower than 30 $mN \cdot m^{-1}$, the phase diagrams are composed of two straight lines intersecting at a dipalmitoylphosphatidylglycerol mole fraction of $x = 0.25$. As breaks in the slopes can be understood as phase boundaries [31,32], this profile suggests partial immiscibility of the two lipids. Similar profiles were obtained when studying the mixing, in monolayers (Tocanne, J.F., unpublished data), of dimyristoyl- and dipalmitoylphosphatidylcholine, two lipids the mixing of which in bilayers is to be regarded as definitely non-ideal, with a miscibility gap in the solid state [30,34–37]. In bilayer structures and in the presence of magnesium, the occurrence of a phase transition in a 10%-dilauroylphosphatidylcholine in dipalmitoylphosphatidylglycerol mixture (Fig. 10) at a temperature which is quite different of that of the lipids alone is indicative of at least partial miscibility of the two lipids. Such an observation is not contradictory with the above monolayer data.

Alltogether, these results confirm and extend the previous calorimetric data of Findlay and Barton [14] and of Van Dijck et al. [11,13]. It can be stated that phase separation or at least partial immiscibility are to be expected only in mixtures where phosphatidylglycerol has longer acyl chains than phosphatidylcholine and in the presence of a strong phosphatidylglycerol-complexing ion such as calcium.

The strong cohesive forces which appear to exist between phosphatidylglycerol and phosphatidylcholine were also found to occur between phosphatidylglycerol and a dimannosyl diacylglycerol [15]. In this case, no phase separation was detected in the presence of divalent cations,

whatever the acyl chain length of the phospholipid. Similarly, it has been recently reported that the miscibility of phosphatidylcholine and phosphatidylinositol was not affected by the presence of calcium ions [16].

It is to be noted that in the present study, as well as in the case of phosphatidylglycerol/dimannosyl diacylglycerol mixtures [15], surface potential values plotted against the lipid mole fraction (Figs. 2, 5, 7 and 9) deviated negatively from linearity. It should be remembered that the experimental surface potential ΔV is the sum of two terms: a dipolar one ($4\pi\mu_{\perp}n$) and an electrostatic one (ψ_0) [38]. The fact that deviation from linearity was encountered with monovalent cations and with bivalent cations or with the protonated form and the ionized form of phosphatidylglycerol leads the possibility of an important contribution of the electrostatic term to be eliminated. More likely, and as already discussed in the case of phopshatidylglycerol/glycolipid mixtures [15,39–41], it is suggested that the deviations are mainly of dipolar origin, with a marked contribution of intermolecular hydrogen bonding. The occurrence of hydrogen bonds between polar heads has been demonstrated for phospholipids in crystalline [42] and in hydrated [43] phases and also when dissolved in organic solvents [44]. Therefore, hydrogen bonds between phosphate and hydroxyl groups can be reasonably expected in phosphatidylglycerol/phosphatidylcholine mixtures. In addition, it is worth noting that clear-cut phase separations triggered by calcium ions have only been reported for phosphatidylcholine/phosphatidylserine [6–8,11], phosphatidylcholine/phosphatidic acid [9–11] and phosphatidylethanolamine/phosphatidylserine [12] mixtures. With such lipids, only 'ionic' hydrogen bonds can exist between phosphate and amine groups [44], a kind of hydrogen bond which is broken by calcium ions [44]. All these observations lead us to suggest that non-ionic hydrogen bonds, when they form, could play a significant role in stabilizing lipid mixtures in biomembranes.

From another point of view, it is worth stressing the consequences of these strong interactions, which appear to exist between phosphatidylglycerol and phosphatidylcholine, capable of preventing lateral phase separation and of modifying their respective physical states. In particular, one should note the ability of the short-chained dilauroylphosphatidylglycerol to assume a condensed state when mixed in small amounts with the long-chained dipalmitoylphosphatidylcholine (Figs. 1–4). One should note also the occurrence of phase transition, both in monolayers and in liposomes, as soon as a given amount of dilauroylphosphatidylcholine has been added to dipalmitoylphosphatidylglycerol: above 10% and 30% phosphatidylcholine in the presence of Mg^{2+} and Ca^{2+}, respectively. Such a fluidifying effects of phosphatidylcholine on phosphatidylglycerol, similar to that recently reported in the case of phosphatidylglycerol/glycolipid mixtures [15], is of great interest if one considers the strong Ca^{2+} and Mg^{2+} binding on phosphatidylglycerol and the dramatic rigidification of the lipid phase which results, as inferred from the effect of these cations on the phase transition temperature [25–27] and on the lateral diffusion [45] of this lipid.

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