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PHASE BEHAVIOUR IN MONOLAYERS AND IN WATER DISPERSIONS OF MIXTURES OF DIMANNOSYL DIACYLGLYCEROL WITH PHOSPHATIDYLGLYCEROL

EFFECT OF MONOVALENT AND BIVALENT CATIONS

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Mixtures of dimannosyl diacylglycerol, extracted from the membrane of *Micrococcus luteus*, with synthetic dipalmitoyl phosphatidylglycerol or with samples of phosphatidylglycerol and phosphatidylinositol, extracted from the same bacterium, have been studied. Through a monolayer (π, ΔV) study and from fluorescence polarization data relative to diphenylhexatriene embedded in vesicles of the mixed lipids, it is shown that the glycolipid interacts with the phospholipids. These interactions are independent of the structure and physical state of the phospholipid acyl chains, of the lipid molecular packing and of the nature of the cations (monovalent, bivalent) present in the aqueous phase. No phase separation was detected, either in monolayers or in water dispersions. Furthermore, the data presented demonstrated a marked influence of the glycolipid on the phase behaviour of phosphatidylglycerol, both in the presence of monovalent (Na^+, K^+) and bivalent (Ca^{2+}, Mg^{2+}) cations. This point is of particular interest with regard to the highly rigid phase this phospholipid is known to assume in the presence of bivalent cations. It is then suggested that the glycolipid could act as a regulator of the membrane fluidity by preventing a too high rigidity of the lipid phase when bivalent cations are present at the membrane surface.

Introduction

Glycolipids present in cell membranes must be subdivided into at least two distinct classes: sphingoglycolipids and glyceroglycolipids.

Whereas there is some experimental evidence suggesting that the former might be implicated in important cell functions such as recognition, contact inhibition, intercellular adhesion, differentiation or as receptors [1,2], the role of the latter in plant and bacterial membranes where they are principally located [3], is far from being understood. One of the possible approaches to solve this problem is to study their physical properties in biomembranes and in model membrane systems. Even in this field, available information at the present time is rather scarce. It essentially consists of two monolayer [4,45], three X-ray diffraction [5,6,46], one ^{2}H-NMR [46] and two differential scanning calorimetry [45,47] studies of galactosyl [4–6] and glycosyl diacylglycerol [45–47] which demonstrate the capability of these glycolipids to form well-organized phases. From another point of view, the possible physiological significance of the different molecular shapes of the mono- and diglucosyl diacylglycerol from the membrane of *Acholeplasma laidlawii* A has been investigated [48].

In the present investigation, the question was posed as to whether glyceroglycolipids can interact with acidic phospholipids and to what extent these

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interactions, if they do exist, modify the phase behaviour of the phospholipid. A dimannosyl diacylglycerol extracted from a strain of *Micrococcus luteus* [7] and different species of phosphatidylglycerols, of both synthetic and natural (*M. luteus*) origins, have been used. Surface pressure and surface potential measurements carried out on monomolecular films of the lipids at the air-water interface, as well as fluorescence polarization experiments with water dispersion of the lipids, clearly indicate strong phospholipid/glycolipid interactions, whatever the ionic conditions in the aqueous phase may be. The glycolipid proved to have a fluidizing effect on the rigid phase given by the phosphatidylglycerols when associated with the bivalent ions Ca^{2+} and Mg^{2+}. No phase separation was detected in the presence of these two cations.

Materials and Methods

Chemicals. 1,6-Diphenyl-1,3,5-hexatriene was obtained from Merck. *rac*-Dipalmitoyl phosphatidylglycerol ammonium salt was of synthetic origin [8]. Samples of phosphatidylglycerol, of phosphatidylinositol and of dimannosyl diacylglycerol were extracted from a strain of *Micrococcus luteus* (Institut Pasteur, A 270) [7] in which the phospholipids and glycolipids were more than 98% substituted by *iso* and *ante-iso* methyl-branched pentadecanoic acids (Welby, M. unpublished results). The three lipids were pure as indicated by thin-layer chromatography.

Monolayer experiments. As a general remark, the compression isotherms shown in this paper were recorder traces obtained by continuous compression of film. The ΔV data presented were obtained from another set of experiments consisting of stepwise compression of new films, ΔV being measured at each step. It was checked that each π/A couple obtained was identical with values calculated from the previously recorded compression curves. Compression isotherms were obtained with an automatic apparatus devised in our laboratory, in which the surface pressure was measured with a platinum plate.

The surface potential was measured with an apparatus using two americium electrodes, the principle of which has been already described [9]. In this case, the surface pressure was monitored with a floating barrier (paraffin-coated mica) connected to a torsion balance of our fabrication, allowing continuous recording of the film surface pressure.

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 [10].

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) whereas the reproducibility of ΔV determinations was ±10 mV. The data presented here are the averages of two to three experiments. Unless otherwise stated, the temperature was 20°C.

Surface radioactivity was measured by means of a circular low-energy β scintillation detector probe of 18 cm^2 surface area (BP4 probe, Nuclear Entreprise Ltd., U.K.) fixed above a circular trough of the same area, and connected to a ratemeter (Analyser model 45, Pale Medicoteknik, Denmark).

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 carried out with a PF1 apparatus [15].

The excitation wave length was selected by means of an interference filter centered at 536 nm. Fluorescence emission was recovered through a cut-off 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 degree celsius/min (0.5 K/min).

The lipids (10 mg) were dispersed in 1 ml of the desired aqueous salt solution (10 mM NaCl 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 10 min at 80 KHz (sonicating bath) to give stable opalescent suspensions.

Since bivalent cations are known to strongly interact with phosphatidylglycerol [9,11–14], it was important to check that the lipid suspensions obtained in the presence of Mg^{2+} were not formed of lipid aggregates. In fact, under the electron microscope, the negatively stained [16] lipid suspensions appeared to mainly consist of small multilamellar vesicles, both in the presence of Na^+ and of Mg^{2+}.

Results

Influence of monovalent cations

Monolayer experiments

A compression isotherm recorded for dimannosyl diacylglycerol on pure water (pH 5.6) is shown in Fig. 1 (curve a). The curve, which is characteristic of a lipid in a liquid-expanded state, remained unchanged

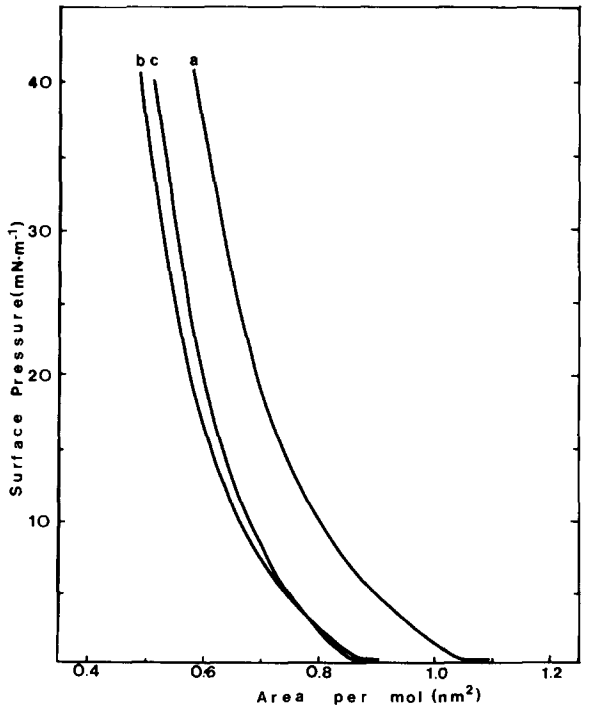

Fig. 1. Compression isotherms of lipids extracted from the membrane of *Micrococcus luteus*: a, dimannosyl diacylglycerol; b, phosphatidylglycerol; c, phosphatidylinositol. Subphase was pure water, pH 5.6, 20°C.

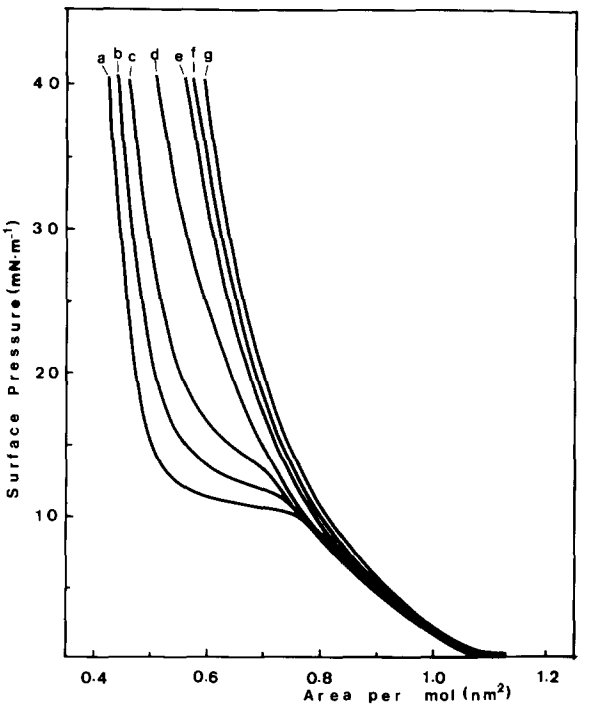

Fig. 2. Force/area curves for mixed monolayers of dipalmitoyl phosphatidylglycerol and dimannosyl diacylglycerol. Mole fraction of glycolipid: a, 0; b, 0.12; c, 0.25; d, 0.50; e, 0.75; f, 0.88; g, 1.00. Subphase was 100 mM NaCl, pH 5.6, 20°C.

after modifying either the pH, or the ionic strength or the nature of cations (monovalent, bivalent) in the subphase. It appeared nearly superimposable with that previously reported for a digalactosyl diacylglycerol [4].

Surface potentials measured for the lipid also proved insensitive to these ionic parameters and stood at values around 220 mV (ΔV = 210 and 230 mV for π = 5 and 30 mN · m^{-1}, respectively).

Since perturbations in phase transitions can be used as a probe for intermolecular associations, glycolipid/phospholipid mixtures were first studied with dipalmitoyl phosphatidylglycerol. The ionic conditions in the subphase (100 mM NaCl, pH 5.6) were chosen in order to ensure complete ionization of the phospholipid phosphate group [10] in the monolayer. As can be seen in Fig. 2, dipalmitoyl phosphatidylglycerol can undergo phase transition from liquid to gel states upon compression of the film. Mixing of the glycolipid and the lipid brought about

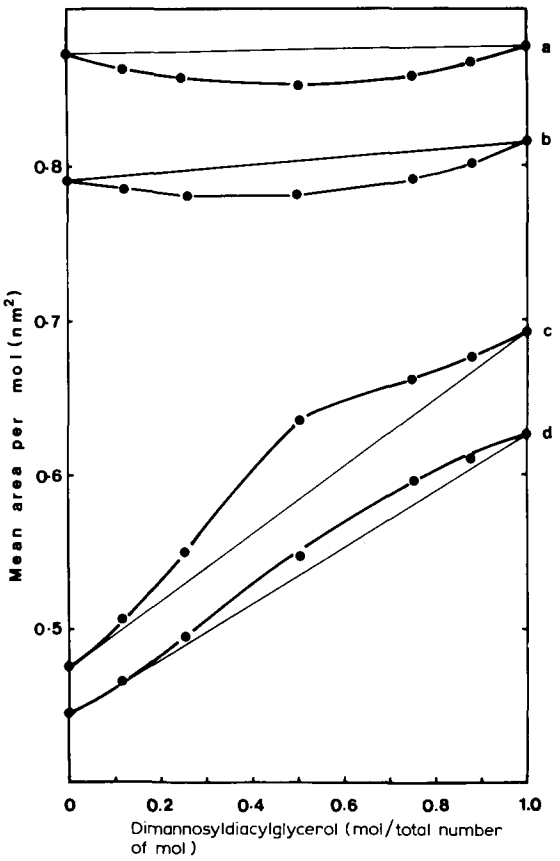

Fig. 3. Plots of mean area per mol vs. mole fraction for dimannosyl diacylglycerol mixed with dipalmitoyl phosphatidylglycerol, at various surface pressures π. π: a, 6 mN · m^{-1}; b, 9 mN · m^{-1}; c, 20 mN · m^{-1}; d, 30 mN · m^{-1}. Subphase was 100 mM NaCl, pH 5.6, 20°C.

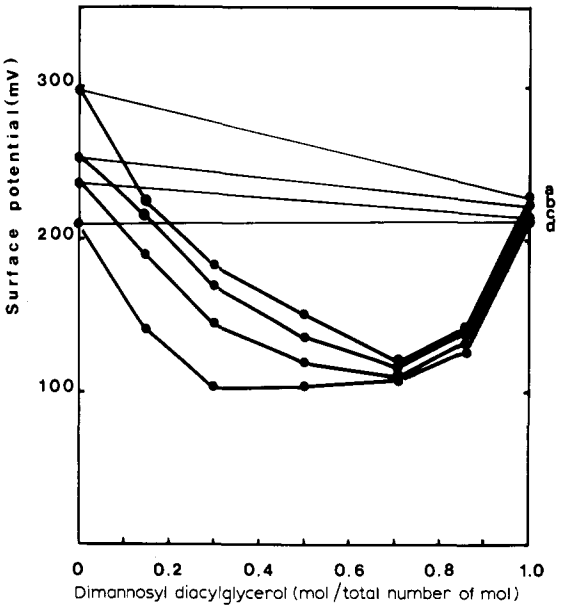

Fig. 4. Changes in surface potential (ΔV) vs. mole fraction for dimannosyl diacylglycerol mixed with dipalmitoyl phosphatidylglycerol at various surface pressures π. π: a, 30 mN · m^{-1}; b, 20 mN · m^{-1}; c, 10 mN · m^{-1}; d, 5 mN · m^{-1}. Subphase was 100 mM NaCl, pH 5.6, 20°C.

perturbations in the phospholipid phase transition which completely disappeared for glycolipid/phospholipid molar ratios higher than 1/1.

As shown in Fig. 3, these curves are well analysed by plotting the average molecular area vs. the glycolipid molar fraction for various surface pressures. In this case, any straight line is to be referred either to ideal mixing or to phase separation, while any deviation from linearity will be indicative of intermolecular interactions between the two components. As can be seen, negative deviations were observed at low surface pressures (π = 5 and 9 mN · m^{-1}) where the phospholipid is in the liquid state, whereas positive deviations were detected at high surface pressures (π = 20 and 30 mN · m^{-1}) where the phospholipid is in the gel state. Such behaviour, which accounts for glycolipid/phospholipid interactions in the monolayer was also observed in the presence of KCl (100 mM) or when using lower salt concentrations in the subphase (1 mM NaCl and KCl, pH 5.6, curves not shown). Deviations from linearity were somewhat lower with K^+ than with Na^+.

Surface potential measurements were carried out with Na^+ and K^+ at a concentration of 1 mM in the subphase (pH 5.6). Results relative to Na^+ are shown in Fig. 4 where changes in ΔV are plotted against the glycolipid molar fraction, at constant surface pressures. A considerable negative deviation from linearity should be noted, which reaches its maximum value for a glycolipid molar fraction of about 0.5 at low surface pressure (π = 5 mN · m^{-1}) and for a molar fraction of about 0.7 for higher surface pressures. ΔV similarly deviated, although to a lesser extent, in the presence of K^+. As an example, for a glycolipid molar fraction of 0.2 and for a surface pressure of 30 mN · m^{-1}, the difference in surface potential between the two cations was 50 mV.

Similar experiments were carried out by mixing the dimannosyl diacylglycerol with phosphatidylglycerol extracted from *M. luteus*. As shown in Fig. 1 (curve b) this phospholipid exists only in the liquid state and displays a compression isotherm (on pure water) which is more condensed than that recorded for the glycolipid. Mixtures of the two species were studied in the presence of NaCl, at a concentration of 1 mM in the subphase (pH 5.6). As above, a slight but systematic negative devation from linearity was observed when plotting the average molecular area versus the glycolipid mole fraction, at any surface pressure (curves not shown). Surface potential was measured in the presence of 1 mM NaCl and KCl. A large negative deviation from linearity was found, similar in shape and amplitude, to that shown in Fig. 4 for glycolipid/dipalmitoyl phosphatidylglycerol mixtures. As with dipalmitoyl phosphatidylglycerol, the deviation was less marked with K^+ than with Na^+. The difference in surface potential between the two cations was of 25 mV for a glycolipid molar fraction of 0.2 and a surface pressure $\pi = 30$ mN $\cdot$ m^{-1}.

Regardless of the structure of the phospholipid, these large negative deviations were still detected after expressing ΔV data in terms of $\Delta V/N$ which corrects for the changes in lipid surface density [14] resulting from the phospholipid/glycolipid interactions in the monolayers.

Fluorescence polarization

Changes with temperature of the fluorescence polarization rate of diphenylhexatriene embedded in lipid vesicles are shown in Fig. 5. As mentioned in Materials and Methods, the lipids were dispersed in a 10 mM NaCl water solution at pH 5.6.

Dipalmitoyl phosphatidylglycerol displayed a sharp phase transition centered at a temperature of 43°C ($\Delta T \sim 6$ K). Adding dimannosyl diacylglycerol to the phospholipid lowered its transition temperature and increased the transition width. With 20% of the glycolipid, transition was centered at 36°C with a ΔT of about 12 K. A transition was still observed near 29°C (ΔT approx. 15 K) with 40% glycolipid.

On the other hand, no phase transition was detected either for pure glycolipid or for its 1/1 mixture with dipalmitoyl phosphatidylglycerol. In these cases, the polarization rate of diphenylhexatriene varies from 0.15 to 0.06 over the temperature range

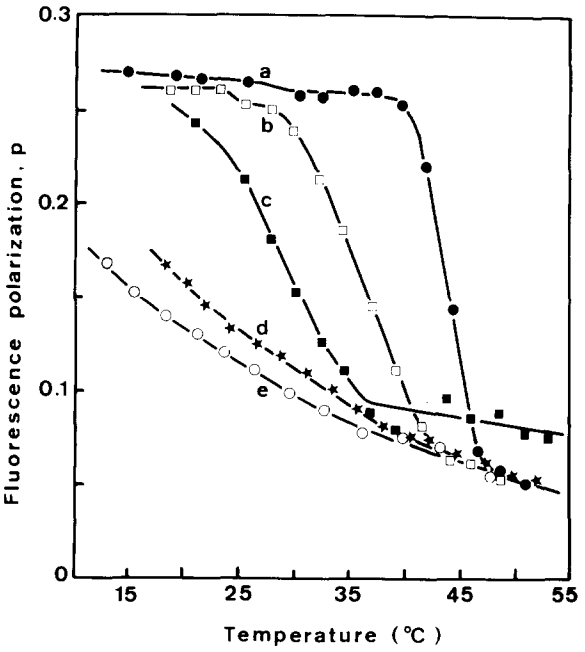

Fig. 5. Changes with temperature of the fluorescence polarization rate p of diphenylhexatriene embedded in mixed vesicles of dipalmitoyl phosphatidylglycerol and dimannosyl diacylglycerol. Mole fraction of glycolipid: a, 0; b, 0.20; c, 0.40; d, 0.50; e, 1.00; Aqueous phase was 10 mM NaCl, pH 5.6.

explored (15–20°C), these low polarization values being indicative of lipids in a rather fluid state.

It is worthwhile emphasizing the good agreement which exists between vesicle and monolayer systems. Indeed, the decrease in transition temperature and the increase in temperature width resulting, in vesicles, from the addition of glycolipid to dipalmitoyl phosphatidylglycerol both correlate well with the rising surface pressure at which the phase transition starts to be detectable in monolayers and with the steeper slope of the compression curve in the transition region (see for example curve c in Fig. 2). Similarly, for glycolipid/phospholipid molar ratios higher than 1/1, lipids found in a liquid state in vesicles were found to also exist in a liquid state in monolayers (curves e and f, Fig. 2).

Influence of bivalent cations

Monolayer experiments

Film compressions of dipalmitoyl phosphatidyl-

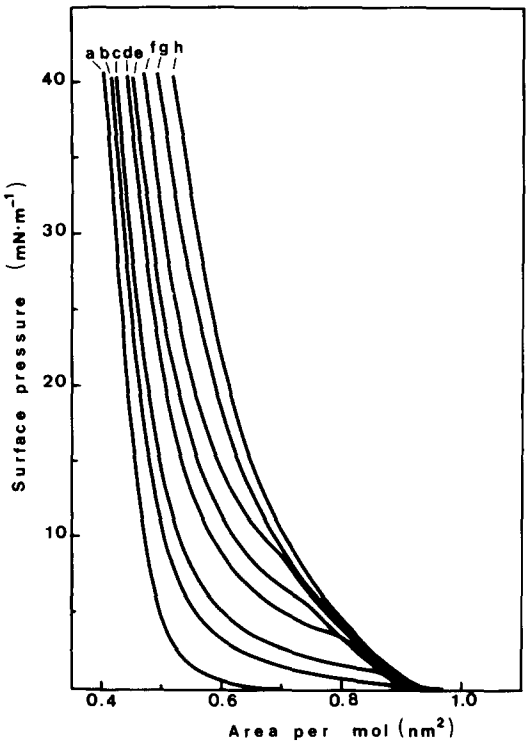

Fig. 6. Force/area curves for mixed monolayers of dipalmitoyl phosphatidylglycerol and dimannosyl diacylglycerol. Mole fraction of glycolipid: a, 0; b, 0.10; c, 0.15; d, 0.30; e, 0.40; f, 0.50; g, 0.85; h, 1.00. Subphase was 100 mM $MgCl_2$, pH 5.6, 20°C.

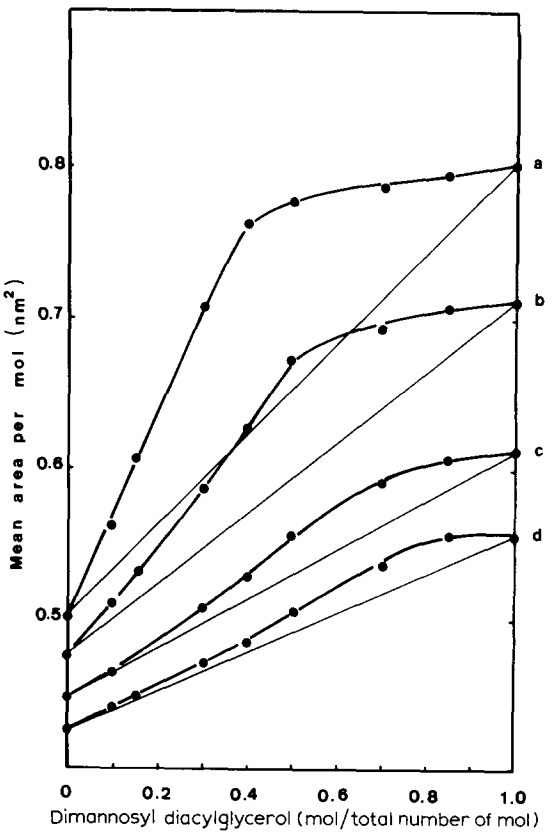

Fig. 7. Plots of mean area per molecule vs. mole fraction for dimannosyl diacylglycerol mixed with dipalmitoyl phosphatidylglycerol, at various surface pressures π. π: a, 5 mN · m^{-1}; b, 10 mN · m^{-1}; c, 20 mN · m^{-1}; d, 30 mN · m^{-1}. Subphase was 100 mM $MgCl_2$, pH 5.6, 20°C.

glycerol, dimannosyl diacylglycerol and their mixtures were performed in the presence of $MgCl_2$ at concentrations of 100 mM and 1 mM in the subphase (pH 5.6). Results concerning 100 mM Mg^{2+} are shown in Fig. 6. As can be seen, the compression curve for the phospholipid alone (curve a) is characteristic of a lipid in a gel state, whereas that of the glycolipid accounts for molecules in a liquid expanded state. Addition of glycolipid to phospholipid gave rise to progressive film expansion with the appearance of a break in the compression curves, over a certain glycolipid molar fraction domain, suggesting the existence of an acyl chain phase transition. This is illustrated in Fig. 6 by curves b and c for lipid mixtures in which glycolipid mole fractions were 0.10 and 0.40, respectively. This hypothesis was confirmed by compression experiments carried out at 25°C, which showed temperature dependence of the transition. The phase transition was detectable for glycolipid mole fractions of 0.10 to 0.60. The same film behaviour was observed in the presence of Ca^{2+} but in this case, phase transitions were detected over the range 0.31–0.60 only (curves not shown). Similar results were obtained when using the two cations at a 1 mM concentration.

Changes in mean molecular area against glycolipid mole fraction at constant surface pressures are reported in Fig. 7 for Mg^{2+}. Note that a systematic positive deviation was observed. The maximum occurred at various glycolipid mole fractions depending on the reference surface pressure. A similar phase diagram was obtained in the presence of Ca^{2+}. At a given surface pressure and compared with Mg^{2+}, the

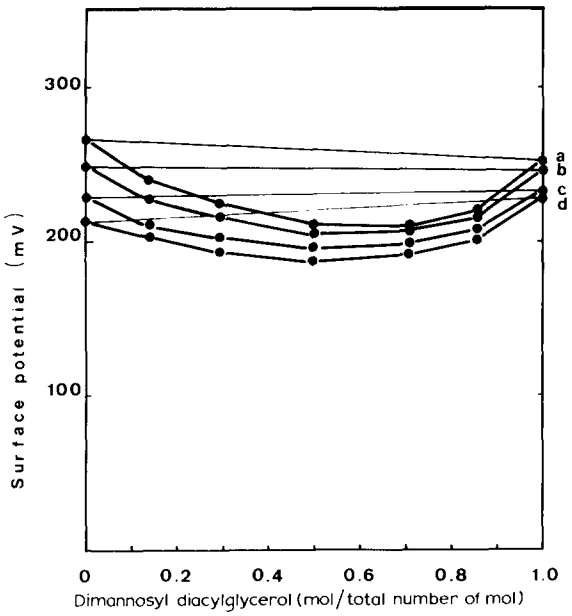

Fig. 8. Changes in surface potential (ΔV) vs. mole fraction for dimannosyl diacylglycerol mixed with dipalmitoyl phosphatidylglycerol, at various surface pressures π. π: a, 30 mN · m^{-1}; b, 20 mN · m^{-1}; c, 10 mN · m^{-1}; d, 5 mN · m^{-1}. Subphase was 1 mM $MgCl_2$, pH 5.6, 20°C.

main differences were a less pronounced deviation from linearity and a shift of the maximum deviation toward higher glycolipid mole fractions.

Owing to the insensitivity of the system towards the ionic strength, surface potential measurements were carried out with salts at the 1 mM concentration (pH 5.6). Data obtained in the presence of Mg^{2+} are shown in Fig. 8. As previously observed with monovalent cations, a marked negative deviation from linearity can be seen, which still exists when the data are expressed in terms of $\Delta V/N$. A negative deviation of similar amplitude was also observed in the presence of Ca^{2+}. Nevertheless, it is worth noting the large discrepancy between the surface potentials measured for phosphatidylglycerol alone in the presence of Mg^{2+} and Ca^{2+}: 270 and 430 mV, respectively, at $\pi = 30$ mN · m^{-1}. The surface potential found for dimannosyl diacylglycerol was unaffected by these bivalent cartions. Similar experiments were carried out by mixing dimannosyl diacylglycerol with phosphatidylglycerol and phosphatidylinositol extracted from *M. luteus*. The compression isotherm of phosphatidylinositol on pure water, pH 5.6 (curve c, Fig. 1) was similar to that recorded for phosphatidylglycerol. Both are characteristic of lipids in a liquid-expanded state. In the presence of Ca^{2+} and Mg^{2+} (1 mM, pH 5.6), the plots of mean area per molecule against glycolipid mole fraction, at constant surface pressure, were linear in the case of phosphatidylglycerol whereas a slight but positive deviation took place with phosphatidylinositol. On the other hand, surface potentials deviated negatively from linearity in both

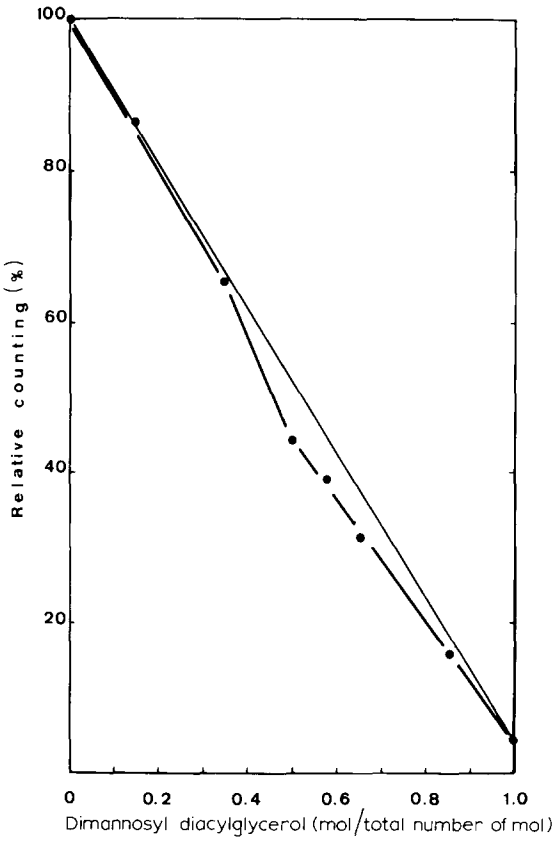

Fig. 9. Relative surface radioactivity vs. mole fraction for dimannosyl diacylglycerol mixed with dipalmitoyl phosphatidylglycerol. In these experiments, the subphase (5 ml) was 10^{-4} M $CaCl_2$, pH 5.6, 20°C. Radioactivity was monitored by addition of 30 μl of a $^{45}CaCl_2$ solution (2 mCi/ml, the Radiochemical Centre, Amersham, England) into the subphase, under magnetic stirring. Then, the lipids were spread with a molecular area of 0.62 nm^2 ($\pi = 20$ mN · m^{-1}). In these conditions and at equilibrium, the surface count of $4 \cdot 10^3$ cpm in the absence of film, reached a value of $8 \cdot 10^3$ cpm in the presence of phosphatidylglycerol alone.

cases, in a quite similar way (curves not shown). Therefore, it is once more concluded that phosphatidylglycerol and phosphatidylinositol from *M. luteus* mix and interact with dimannosyl diacylglycerol.

As a complementary experiment, it was of interest to check whether the fluidizing effect of the glycolipid upon the phospholipid might not be due to the desorption of the bivalent cations from the film surface. Such a possibility was tested by measuring the surface radioactivity for various phospholipid/glycolipid mixtures with ^{45}Ca in the subphase (see experimental part). Irrespective of a slight (but systematic) negative deviation from linearity, we can conclude from data plotted in Fig. 9, that the surface count was proportional to the phospholipid surface concentration.

Fluorescence polarization

As, in monolayers, phase transitions were detected over glycolipid mole fraction domains which were larger with Mg^{2+} than with Ca^{2+}, Mg^{2+} was preferred for fluorescence polarization experiments.

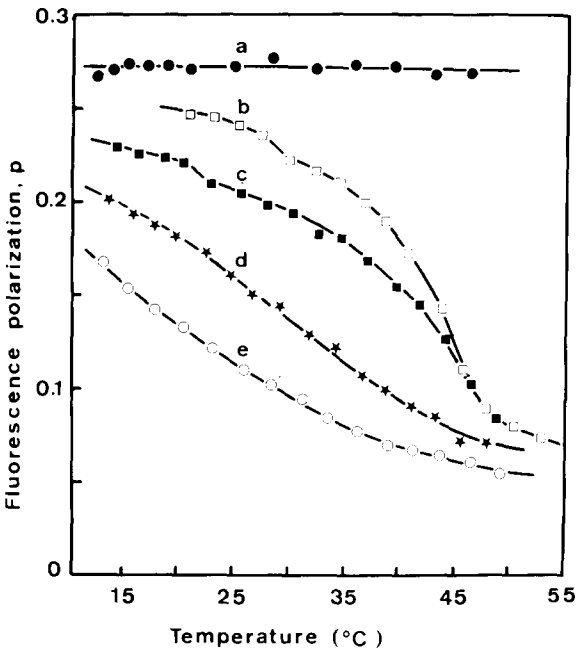

Fig. 10. Changes with temperature of the fluorescence polarization rate p of diphenylhexatriene embedded in mixed vesicles of dipalmitoyl phosphatidylglycerol and dimannosyl diacylglycerol. Mole fraction of glycolipid: a, 0; b, 0.20; c, 0.30; d, 0.50; e, 1.00. Aqueous phase was 1 mM $MgCl_2$, pH 5.6.

As can be seen in Fig. 10 and as expected [8], dipalmitoyl phosphatidylglycerol alone did not display any phase transition over the explored temperature range 14–47°C. The fluorescence polarization rate of diphenylhexatriene stood at a high and constant value of 0.27, characteristic of a lipid in a ridig state.

On the other hand, and in agreement with monolayer experiments, clear-cut phase transitions were detected for lipid mixtures with 20 and 30% glycolipid. These transitions were centered at temperatures of 44°C and 42°C, respectively (curves b and c). Phase transitions were no longer observed for glycolipid mole fractions higher than 0.5 (see curve d). As found above with Na^+, glycolipid alone existed in a liquid phase only. It is to be noted that curves e in Fig. 10 (Mg^{2+}) and in Fig. 5 (Na^+) are superimposable. In other words, the organization state of the glycolipid phase is independent of the cations present in the aqueous phase.

Discussion

It is clear from the above that dimannosyl diacylglycerol strongly interacts with phosphatidylglycerols both in monolayers and in liposomes, as well as with phosphatidylinositol in monolayers. These interactions appear to be independent of the structure and physical state of the phospholipid acyl chain, of the lipid molecular packing and of the nature of cations (monovalent or bivalent) present in the aqueous phase. Whatever the ionic conditions, surface pressure (Figs. 3 and 7) and surface potential (Figs. 4 and 8) data allow us to rule out the hypothesis of phase separation in monolayers. This conclusion also applies to the aqueous dispersions of the lipid mixtures, as ascertained by the fluorescence polarization experiments carried out in the presence of Na^+ (Fig. 5) or Mg^{2+} (Fig. 10). The same conclusion has been reached recently for mixtures of phosphatidylglycerol and diglucosyl diacylglycerol (from *Acholeplasma laidlawii* B) in the presence of Mg^{2+} [47].

Such phase behaviour is reminiscent of that previously reported for phosphatidylglycerol/phosphatidylcholine mixtures, either in monolayers [17] or in water dispersions [18,19]. In these cases, no phase separation was detected, except for high Ca^{2+} concentrations (1 M) and for high phosphatidylglycerol mole

fractions (0.9) [17–19]. Similarly, cord factors, which are toxic glycolipids from the cell walls of mycobacteria, have been shown to strongly interact with phosphatidylcholine, both in monolayers and in liposomes [20,21].

It is to be noted that in the present study, as well as in the case of phosphatidylglycerol/phosphatidylcholine mixtures [17], surface potential values plotted against the lipid mole fraction (Figs. 4 and 8) deviated negatively from linearity. It should be remembered that the experimental surface potential ΔV is the sum of two terms: a dipolar one ($4\pi\mu_1 n$) and an electrostatic one (ψ_0) [22]. The fact that non-ideality was encountered with monovalent as well as with bivalent cations eliminates the possibility of an important contribution of the electrostatic term. More likely, it is suggested that the deviations are mainly of dipolar origin. In this respect, dipolar effects within lipid films can originate either from changes in the conformation of the polar head groups or from changes in their hydration shell or even from hydrogen bonding. The systems under investigation are very complex, and at the present time, one cannot predict either the sign or the magnitude of these various possible contributions. Nevertheless, calculations have shown that, energetically, dipole-dipole interactions (such as those which could result from conformational changes) may only play a minor role in the stabilization of the molecular packing at the interface [23]. On the other hand, changes in the hydration of the polar head groups due to lipid-lipid interactions are likely to be of some importance [20, 24,25], in connection of course with intermolecular hydrogen bonding. Thus, an X-ray study of cerebroside crystals has shown the existence of a long range hydrogen bond lattice extending within each leaflet of the bilayer structure [26]. Similarly, the occurrence of hydrogen bonds between polar heads has been demonstrated for phospholipids in crystalline [27] and in hydrated [28] phases and also when dissolved in organic solvents [29]. Therefore, hydrogen bonds between phosphate and hydroxyl groups or between hydroxyl functions themselves can be reasonably expected in the glycolipid/phospholipid mixtures studied in the present work. It should be noted that the maximum of ΔV deviation occurred at the glycolipid mole fractions 0.7–0.8, a result which suggests that one phosphatidylglycerol molecule could simulatneously interact with four glycolipid molecules. In addition, it is worth noting that phase separation triggered by calcium ions has only been reported for phosphatidylcholine/phosphatidylserine [30–32] and phosphatidylcholine/phosphatidic acid [33,34] mixtures. With such lipids, only 'ionic' hydrogen bonds can exist between phosphate and amine groups [29], a kind of hydrogen bond which is broken by calcium ions [29]. Altogether, these observations lead us to suggest that non-ionic hydrogen bonds, when they form, could play an important role in stabilizing lipid mixtures in biomembranes.

From another point of view, data presented here showed a marked influence of the glycolipid on the physical state of phosphatidylglycerol, in the presence of both monovalent and bivalent cations.

This point is of particular interest if one considers the strong Ca^{2+} and Mg^{2+} binding on this lipid and the dramatic rigidification of the lipid phase which results as inferred from: (1) the film condensations which are observed in the presence of these ions compared with Na^+ or K^+ [11]; (2) the considerable increase of the lipid phase transition temperature which, in the case of didodecanoyl phosphatidylglycerol shifts from 0°C in the presence of Na^+ to 73°C in the presence of Mg^{2+} and Ca^{2+} [11–13]; (3) the large decrease in the lateral mobility of the lipid in monolayers, $D = 30 \cdot 10^{-6}$ cm$^2 \cdot$ s^{-1} for Na^+, $5 \cdot 10^{-6}$ cm$^2 \cdot$ s^{-1} for Mg^{2+} and $0.1 \cdot 10^{-6}$ cm$^2 \cdot$ s^{-1} for Ca^{2+} [36].

In the present study, data obtained with monolayers (Fig. 6) and with liposomes (Fig. 10) clearly demonstrate the occurrence of phase transition at moderate temperatures (30–40°C) as soon as a certain amount of dimannosyl diacylglycerol has been added to phosphatidylglycerol: above 10% and 35% glycolipid in the presence of Mg^{2+} and Ca^{2+}, respectively. Owing to its substitution by *iso* and *ante-iso* methyl-branched fatty acids, the glycolipid itself should display phase transition at very low temperatures only [37,38]. Therefore, the occurrence of a unique phase transition in glycolipid/phospholipid mixtures (i) confirms the above assumption of perfect miscibility of the two lipids and (ii) shows that the glycolipid has a fluidizing effect on the phospholipid, presumably by disturbing the highly organized rigid phase assumed by phosphatidylglycerol alone in the presence of bivalent cations. Indeed, bivalent cations are known to bind to carbohydrates in water solu-

tions [39,40] and also to form crystalline 'complexes' the structure of which have been determined by X-ray diffraction [41–43]. Furthermore, it has been shown that addition of Mg^{2+} and Ca^{2+} to diglucosyl diacylglycerol increases the hydration capability of this glycolipid [46]. This change in hydration capacity might be due either to the fact that divalent cations are highly hydrated or to the fact that the ions are causing a conformational change of the polar head group allowing a larger water uptake [46]. In the present case, the two glycolipid mannose residues at the water-lipid interface could modify the local distribution of Mg^{2+} and Ca^{2+} and therefore their binding with the phospholipid. Such a hypothesis is not contradictory with the slight ^{45}Ca surface concentration decrease which can be infered from the negative deviation from linearity in Fig. 9. Ca^{2+} and Mg^{2+} are very important cations, imperatively required for the stabilization of biomembranes [44]. In plant and bacterial membranes, rich in phosphatidylglycerol (and other acidic phospholipids) and in glyceroglycolipids, it is suggested that the latter could have a regulation function in the membrane 'fluidity' by preventing too high a rigidity of the lipid phase when bivalent cations are present at the membrane surface.

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