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INTERACTIONS OF GLYCEROL MONOOLEATE AND DIMETHYLSULPHOXIDE WITH PHOSPHOLIPIDS

A DIFFERENTIAL SCANNING CALORIMETRY AND ^{31}P-NMR STUDY

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1. A comparative study has been made of the effects of the fusogens glycerol monooleate and dimethylsulphoxide on the polymorphic phase behaviour of dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylethanolamine by differential scanning calorimetry and ^{31}P-NMR techniques. 2. Glycerol monooleate induces a reduction in the temperature, cooperativity and enthalpy of the gel to liquid-crystal transitions of dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylethanolamine, whereas dimethylsulphoxide induces an increase in the temperature and enthalpy and a reduction in the cooperativity of the gel to liquid-crystal transitions for those same phospholipids. 3. Glycerol monooleate induces the formation of isotropic and hexagonal (H_{II}) phases when mixed with either dipalmitoyl phosphatidylcholine or dipalmitoyl phosphatidylethanolamine. By contrast, in the presence of dimethylsulphoxide, those same phospholipids retain the lamellar configuration observed in the absence of fusogen. 4. These results are discussed in terms of the mechanisms of chemically induced cell fusion.

Introduction

Cell fusion may be induced by a wide variety of chemical agents (fusogens) of widely disparate chemical properties [1]; however, the mechanism(s) by which these agents induce fusion is unclear. Two current views of fusogen action centre upon their ability to induce either changes in membrane fluidity [1,2], or structural changes in membrane lipids [2–4].

Lucy [1] has proposed that the fusogenic activity of various lipophilic agents is related to their ability to increase membrane fluidity, whereas Lyman et al. [5] have shown that certain lipophilic fusogens actually increase the temperature of the gel to liquid-crystal transitions for various species of phospholipid, and so would be expected to reduce membrane fluidity.

Lucy [1] has suggested that the action of the fusogen lysolecithin is related to its ability to induce bilayer to micelle transformation of membrane lipids; however, this ability is not common to various other lipophilic fusogens [6]. More recently, Hope and Cullis [4] have demonstrated that various lipophilic fusogens induce hexagonal (H_{II}) phase in membrane lipids, effects that were not observed with non-fusogenic lipophilic agents.

In the present studies we have examined the effects of glycerol monooleate, a lipophilic fuso-

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Abbreviations: DPPC, Dipalmitoyl phosphatidylcholine; DPPE, dipalmitoyl phosphatidylethanolamine; DMSO, dimethylsulphoxide; DSC, differential scanning calorimetry.

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gen, and dimethylsulphoxide (DMSO), a fusogen with broad solvent capabilities, upon the polymorphic phase behaviour of dipalmitoyl phosphatidylcholine (DPPC) and dipalmitoyl phosphatidylethanolamine (DPPE), in order to gain further insight into the mechanism of action of these two fusogens. The effects of glycerol monooleate were of particular interest since Hope and Cullis [4] have demonstrated that whereas fusogens such as palmitoleic acid, oleic acid and retinol induce only the hexagonal (H_{II}) phase in reconstituted erythrocyte lipid systems, glycerol monooleate induces both hexagonal (H_{II}) and isotropic phases.

It is shown that glycerol monooleate induces changes in the thermotropic properties of both DPPC and DPPE consistent with an ability to increase membrane fluidity. In contrast, the effects of DMSO are consistent with an ability to reduce membrane fluidity. Further, glycerol monooleate is able to induce the formation of nonbilayer lipid phases when mixed with earlier DPPC or DPPE, whereas DMSO has no such effects on those same lipids.

Materials

DPPC, DPPE, glycerol monooleate and DMSO were purchased from Sigma Chemical Co. and the lipids shown to be homogeneous by thin-layer chromatography, in the case of phospholipids, fatty acid and lyso contaminants comprising less than 0.5% of total lipids. Water was doubly distilled. All other reagents were of analytical grade.

Methods

Differential scanning calorimetry

All DSC analyses were performed using a Perkin-Elmer DSC-1B calorimeter. Transition enthalpy, entropy and cooperativity were calculated as previously described [7]. Transition temperatures were taken as the temperature of maximum excess specific heat (T_m) and measured to the nearest 0.5°C. DPPC/glycerol monooleate and DPPE/glycerol monooleate mixtures were prepared from an appropriate mixture of stock solutions in chloroform, the solvent was removed by evaporation under a stream of nitrogen followed by storage under reduced pressure for 2 h. Water was added to give a lipid concentration of 20% w/w, and the lipids dispersed by heating to 50°C for DPPC/glycerol monooleate or 70°C for DPPE/glycerol monooleate mixtures, followed by extensive vortex mixing. DPPC/glycerol monooleate mixtures were scanned in triplicate over the temperature range 280–330 K, and DPPE/glycerol monooleate mixtures in triplicate over the temperature range 300–360 K, both at 8°C/min.

DSC of glycerol monooleate dispersed in excess water reveals three endothermic transitions at 282.5, 286 and 303 K, corresponding to the melting of the αL (hexagonal), β'L (orthorhombic) and βL (triclinic) crystal modifications, respectively. DSC of neat glycerol monooleate shows only one transition at 285 K, most probably due to the melting of the αL crystal form (unpublished data).

Lipid/glycerol monooleate samples were also scanned over the temperature range 250–300 K following prior analysis; in no instance were transitions detected corresponding to the melting of any glycerol monooleate crystal modification, indicating that all the glycerol monooleate was incorporated into the phospholipid matrix.

For lipid/DMSO mixtures, a known weight of lipid dispersion was added to a tared DSC pan, DMSO added, the pan then sealed and reweighed. DPPC/DMSO mixtures were scanned in triplicate over the temperature range 280–350 K, and DPPE/DMSO scanned in triplicate over the temperature range 300–360 K, both at 8°C/min. Subsequently, lipid/DMSO mixtures were scanned in triplicate over the temperature range 280–173 K at 8°C/min, in order to determine the amount of free water in the mixture. DMSO concentrations were calculated as previously described [7], based on the assumption that all the unbound water in a lipid dispersion was available for solvation of the DMSO.

Following DSC analysis, DSC pans were pierced and their contents dissolved in ethanol (DPPC/glycerol monooleate and DPPC/DMSO mixtures) or chloroform/methanol 1 : 1, v/v (DPPE/DMSO) and samples taken for phosphorus analysis [8] and thin-layer chromatography. Controls showed that neither glycerol monooleate nor DMSO interfered in the phosphorus assay. No lipid degradation was detectable over the time-course of the experiments.

Nuclear magnetic resonance

Samples for ^{31}P-NMR were prepared from an appropriate mixture of the lipids in a 10 mm NMR tube, solvent being evaporated first under nitrogen and subsequently under reduce pressure. Lipids were dispersed in 0.7 ml of buffer (10 mM Tris-HCl/100 mM NaCl/2 mM EDTA/10% v/v ^2H_2O, pH 7) by vortexing. For lipid/DMSO mixtures the buffer contained 50% v/v DMSO.

^{31}P-NMR spectra were recorded employing a Bruker WP-200 Fourier Transform spectrometer. Accumulated free induction decays were obtained for up to 1000 transients using an interpulse time of 0.8 s, a 25 μs 90°C pulse and a sweepwidth of 20 kHz, in the presence of broad band proton decoupling. Spectra were recorded at 45, 75 and 90°C, allowing 10 min equilibration at each temperature prior to accumulation.

Results

Fig. 1 shows DSC traces of DPPC in the presence of glycerol monooleate; plots of transition enthalpy and cooperativity as a function of the mol% glycerol monooleate are shown in Fig. 2. With increasing membrane concentration of glycerol monooleate there was a progressive decrease in the temperature of the DPPC gel to liquid-crystal transition from (mean ± S.D., n) 315.0 ± 0.2 (6) K for DPPC in water to 299 ± 1.8 (6) K at 50 mol% glycerol monooleate, accompanied by a reduction in the size of the cooperativity unit from 72.4 ± 3.8 (6) to 16.9 ± 3.2 (6) at 50 mol% glycerol monooleate. The enthalpy of the main transition decreased from 8.7 ± 0.2 (6) kcal · mol^{-1} for DPPC in water to 2.5 ± 0.6 (6) kcal · mol^{-1} in the presence of 50 mol% glycerol monooleate. The temperature of the DPPC pretransition decreased from 309.2 ± 0.4 (6) K for DPPC in

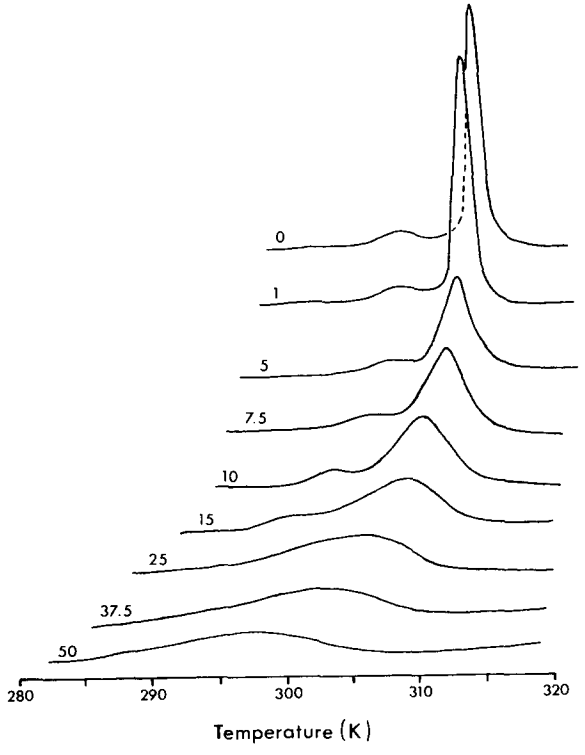

Fig. 1. DSC traces of DPPC/glycerol monooleate mixtures dispersed in water are shown. The mol% glycerol monooleate in each DPPC/glycerol monooleate mixture is indicated adjacent to each trace. The samples contained varying weights of phospholipid, hence the traces are not comparable directly in terms of peak area.

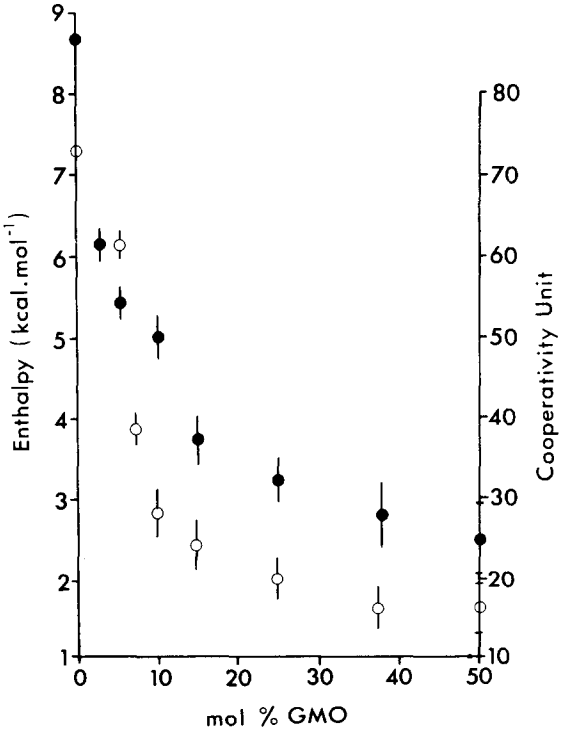

Fig. 2. The effect of glycerol monooleate (GMO) on the enthalpy (●) and cooperativity (○) of the gel to liquid-crystal transition of DPPC. Values are given as the mean ± S.D. of a sample measured in triplicate.

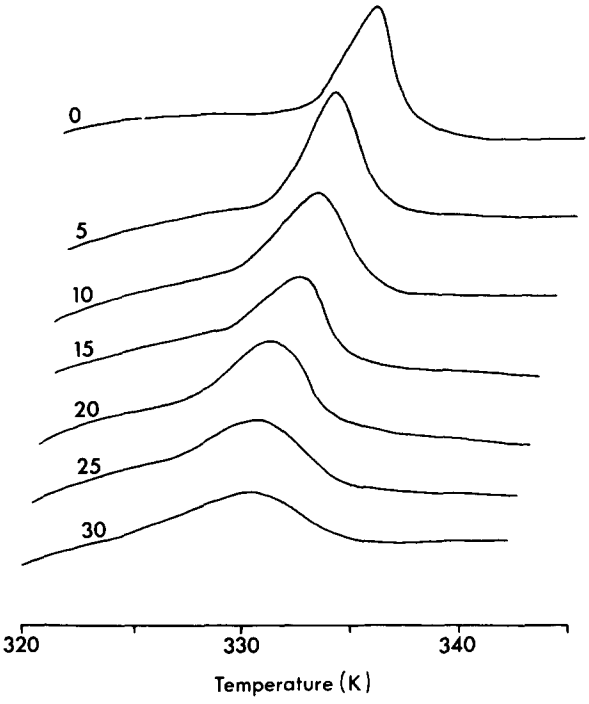

Fig. 3. DSC traces of DPPE/glycerol monooleate mixtures dispersed in water are shown. The mol% glycerol monooleate in each DPPE/glycerol monooleate mixture is shown adjacent to each trace. Samples contained varying weights of phospholipid, hence the traces are not directly comparable in terms of peak area.

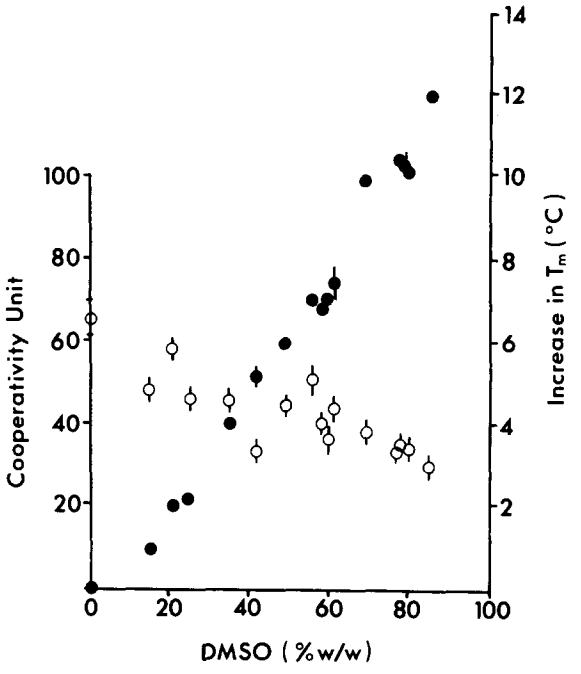

Fig. 4. The effect of DMSO on the temperature and cooperativity of the DPPC gel to liquid-crystal transition. The increase in the transition of DPPC in the presence of DMSO (●) is given as the mean ± S.D. of the difference between the transition temperature observed in the presence of DMSO and its absence, each measured in triplicate. The size of the cooperativity unit (○) is given as the mean ± S.D. for the same samples.

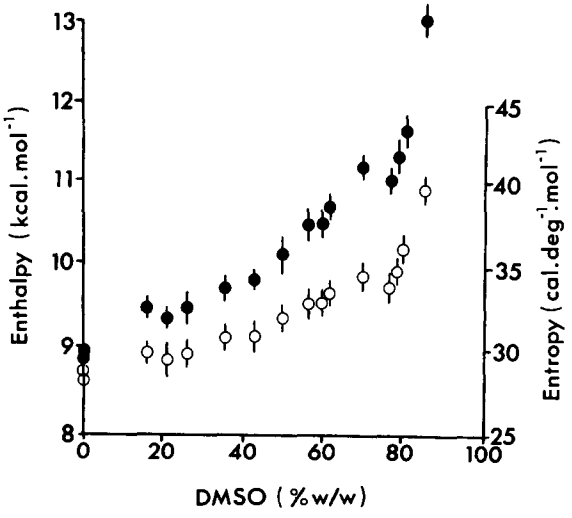

Fig. 5. The effect of DMSO on the enthalpy (●) and entropy (○) of the gel to liquid-crystal transition of DPPC. Values are given as the mean ± S.D. of a sample measured in triplicate.

water to 300 ± 0.4 (6) K at 15 mol% glycerol monooleate; at 25 mol% glycerol monooleate the pretransition was undetectable. A similar decrease in the temperature and cooperativity of the gel to liquid-crystal transition of DPPE was observed in the presence of glycerol monooleate (Fig. 3), although at equivalent molar ratios of glycerol monooleate, the decrease in the transition temperature for DPPE was less than that observed for DPPC.

Fig. 4 shows the changes in transition temperature and cooperativity for DPPC in the presence of DMSO; associated changes in transition enthalpy and entropy are shown in Fig. 5. With increasing DMSO concentration there was a linear increase in the transition temperature of DPPC from 315.0 ± 0.3 (6) K for DPPC in water to 327.0 ± 0.4 (6) K in the presence of 85% w/w DMSO. The size of the cooperativity unit decreased from

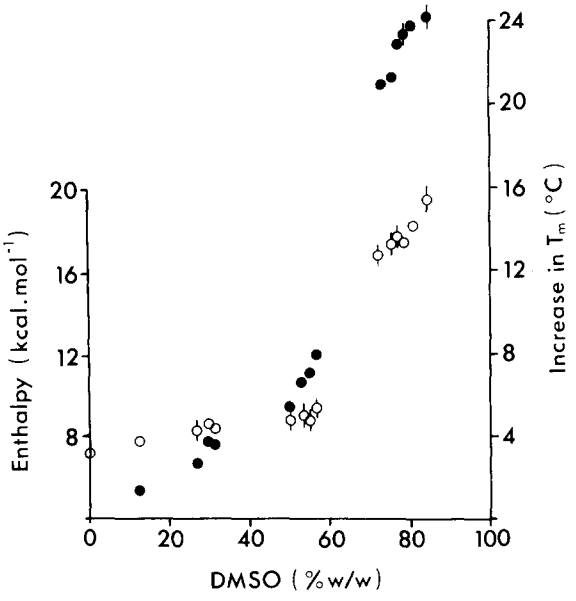

Fig. 6. The effect of DMSO on the temperature and enthalpy of the gel to liquid-crystal transition of DPPE. The increase in the transition temperature of DPPE (●) is given as the mean ± S.D. of the difference between the transition temperature observed in the presence of DMSO and its absence. The enthalpy values (○) are given as the mean ± S.D. for the same samples.

66.5 ± 4.5 (9) for DPPC in water to 38.2 ± 6.7 (27) for DPPC over the concentration range 56–85% w/w DMSO. There was an increase in the enthalpy (ΔH) and entropy (ΔS) of the DPPC main transition with increasing concentration of DMSO. Above 50% w/w and up to 85% w/w DMSO, the enthalpy and entropy of DPPC, for nine different concentrations of DMSO studied, were $\Delta H = 11.1 \pm 1.9$ (27) kcal · mol^{-1} and $\Delta S = 34.4 \pm 2.5$ (27)

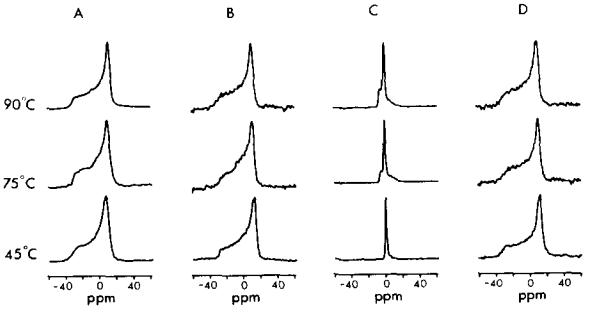

Fig. 7. ^{31}P-NMR spectra of (A) DPPC, (B) DPPC/glycerol monooleate (1 : 1), (C) DPPC/glycerol monooleate (1 : 2) and (D) DPPC in the presence of 50% v/v DMSO, at 45, 75 and 90°C.

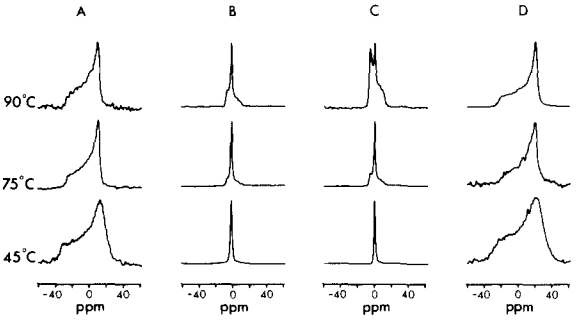

Fig. 8. ^{31}P-NMR spectra of (A) DPPE, (B) DPPE/glycerol monooleate (1 : 1), (C) DPPE/glycerol monooleate (1 : 2) and (D) DPPE in the presence of 50% v/v DMSO, at 45°C, 75°C and 90°C. Spectra were collected under the conditions as described in Methods except for DPPE/DMSO at 45°C. In this case free induction decays were collected employing a 3 s interpulse time and 50 kHz sweepwidth.

cal · deg^{-1} · mol^{-1}. These values were increased significantly ($P < 0.0002$ for enthalpy data, $P < 0.0003$ for entropy data; t-test), over those values for DPPC in water, $\Delta H = 8.7 \pm 0.2$ (6) kcal · mol^{-1} and $\Delta S = 28.4 \pm 0.9$ (6) cal · deg^{-1} · mol^{-1}.

DMSO also caused an increase in the temperature and enthalpy of the DPPE transition as shown in Fig. 6, values of transition temperature and enthalpy increasing from 336.2 ± 0.2 (6) K and $\Delta H = 7.45 \pm 0.21$ (6) kcal · mol^{-1}, respectively, for DPPE in water to 360.5 ± 0.3 (3) K and $\Delta H = 19.5 \pm 0.3$ (3) kcal · mol^{-1} for DPPE in 84% w/w DMSO.

^{31}P-NMR spectra of DPPC, DPPC/glycerol monooleate and DPPC/DMSO mixtures are shown in Fig. 7, and for DPPE, DPPE/glycerol monooleate and DPPE/DMSO mixtures in Fig. 8. In excess water, up to 90°C, both DPPC and DPPE form lamellar phases, as indicated by their characteristic ^{31}P-NMR lineshape with a low field shoulder and high field peak separated by approx. 50 ppm. For a DPPC/glycerol monooleate (1 : 1) mixture and also DPPC in the presence of 50% v/v DMSO, the lamellar phase was observed up to 90°C. For a DPPC/glycerol monooleate (1 : 2) mixture two spectral components were observed, a resonance at 0 ppm indicative of isotropic motional averaging and a second, less intense, component with reversed asymmetry as compared to the 'bilayer' spectrum and which is narrower by a factor of two; this resonance being indicative of

hexonal (H_{II}) phase structure [9].

For DPPE/glycerol monooleate 1:1 and 1:2 mixtures, the spectra exhibited resonances indicative of both isotropic and hexagonal (H_{II}) phases, the percentage hexagonal component increasing with temperature. For a DPPE/glycerol monooleate (1:2) mixture, at 75 and 90°C, the percentage hexagonal (H_{II}) phase component was greater than that observed for a DPPE/glycerol monooleate (1:1) mixture. At 90°C, the ^{31}P-NMR spectrum of a DPPE/glycerol monooleate (1:2) mixture indicated that the lipid was predominantly in the hexagonal (H_{II}) phase. For DPPE in the presence of DMSO at 45°C, a broad spectrum indicative of a gel phase was observed, whereas at 75 and 90°C, liquid-crystalline lamellar phases were indicated.

Discussion

The decrease in the gel to liquid-crystal transition temperatures for both DPPC in mixtures with glycerol monooleate reflects a decrease in acyl chain order and Van der Waal's interactions between adjacent chains. In light of the interpretation of the gel to liquid-crystal transition for DPPC in terms of *gauche* isomer formation [10], it is conceivable that the reduced enthalpy of the DPPC transition is a consequence of glycerol monooleate -induced *gauche* isomer formation, as has been suggested previously on the basis of monolayer studies [11].

In contrast, the elevation of transition temperatures and enthalpies for both DPPC and DPPE in the presence of DMSO indicates that this fusogen stabilizes phospholipid bilayers, inducing close packing of acyl chains with concommitant increased Van der Waal's interactions. Calculation of the molar ratio of DMSO to DPPC for the data presented in Fig. 4 showed that the increase in the DPPC transition temperature was independent of this ratio, suggesting that the effects of DMSO upon the thermotropic properties of DPPC arise as a consequence of perturbation of the solvent, rather than to a direct interaction between the DPPC and DMSO. The effects of DMSO upon DPPC and DPPE presented here are in general agreement with the findings of Lyman et al. [5] and inconsistent with certain results of Maggio and Lucy [12] who found that monolayers of DPPE behaved as if their transition temperatures had been depressed in the presence of DMSO.

From these results it would not be expected that glycerol monooleate and DMSO would have similar effects upon membrane fluidity; however, this cannot be taken to imply that their effects upon fluidity are irrelevant to their mode of action. By either increasing or decreasing membrane fluidity it is conceivable that fusogens may perturb the phase equilibria of different lipids within a biological membrane, thereby forming local regions of increased disorder/fluidity in one area of a membrane and regions of relatively decreased disorder/fluidity in another area of the same membrane. Such perturbations may arise because of differential interactions of a given fusogen with different lipid components of a membrane (related to miscibility properties), as well as differential effects of a given fusogen upon the thermotropic properties and phase behaviour of different lipids.

Such speculation is supported by the findings that glycerol monooleate exhibits different miscibility behaviour with DPPC and DPPE [13], raising the possibility that glycerol monooleate may cause differential changes in the fluidity of membrane that are asymmetric with respect to their distributions of phosphatidylcholine and phosphatidylethanolamine across the bilayer.

Further, as demonstrated by Lyman et al. [5], DMSO may cause differential changes in the transition temperature of acidic and neutral phospholipids. It is possible, therefore, that DMSO may induce the preferential gelling of acidic phospholipids within the plane of biological membrane, analogous to the effects of calcium [14].

From Figs. 7 and 8 it is clear that glycerol monooleate, at fusogenic membrane concentrations [4], induces both hexagonal (H_{II}) and isotropic phases in mixtures with either DPPC or DPPE. While the ^{31}P-NMR spectral lineshape associated with the hexagonal (H_{II}) phase is unique, there are a variety of lipid structures that can give rise to isotropic motional averaging, including small bilayer vesicles, micelles, inverted micelles or other lipid phases such as rhombic and, of particular relevance to the effects of glycerol monooleate, cubic phases [9].

For both DPPC/glycerol monooleate and

DPPE/glycerol monooleate mixtures, the samples appeared, by visual inspection, both viscous and aggregated, suggesting that small bilayer vesicles were not the source of the isotropic motional averaging observed by ^{31}P-NMR. Further, neither small bilayer vesicles, micelles or inverted micelles (lipidic particles) were detectable by freeze-fracture techniques (results not included).

The phase diagram of glycerol monooleate in water [15] shows that at water contents of 20–40% w/w, between approximately 40 and 80°C, a viscous isotropic phase occurs in which glycerol monooleate forms a body-centred cubic lattice consisting of lamellar bilayer units [16,17]. At water contents above 40% w/w, a mixed cubic and excess water phase is formed. Upon heating to approximately 90°C, the cubic phase is transformed into a hexagonal (H_{II}) phase [18].

In the absence of definite X-ray analysis, we propose that the resonance indicative of isotropic motion observed for DPPC and DPPE in the presence of glycerol monooleate may represent a cubic phase. With increasing temperature, the DPPC or DPPE forms a hexagonal (H_{II}) phase either as a result of glycerol monooleate itself entering the hexagonal (H_{II}) phase [18] and/or due to glycerol monooleate-induced *gauche* isomer formation in the phospholipid acyl chains, thereby effectively increasing the swept area of the chains and so promoting the phase transformation [17]. The greater percentage hexagonal (H_{II}) phase observed for DPPE/glycerol monooleate mixture as compared to DPPC/glycerol monooleate mixtures is probably a reflection of the smaller area subtended at the membrane/water interface by the phosphatidylethanolamine headgroup as compared to the phosphatidylcholine.

This would tend to favour the hexagonal (H_{II}) phase for DPPE in comparison to DPPC and would be consistent with previous findings for unsaturated phosphatidylethanolamines and phosphatidylcholines [9].

The results presented herein suggest that in addition to bilayer-micelle [3] and either bilayer-hexagonal (H_{II}) or bilayer-inverted micelle [4] transformations, the formation of hexagonal (H_{II}) and possibly cubic phases may be of relevance to the mechanism of action of glycerol monooleate. DMSO induces no such equivalent phase transformations; thus, an alternate mechanism of action for this fusogen is indicated.

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