Biochimica et Biophysica Acta, 507 (1978) 207—218
© Elsevier/North-Holland Biomedical Press

BBA 77931

POLYMORPHIC PHASE BEHAVIOUR OF LIPID MIXTURES AS DETECTED BY ^{31}P NMR

EVIDENCE THAT CHOLESTEROL MAY DESTABILIZE BILAYER STRUCTURE IN MEMBRANE SYSTEMS CONTAINING PHOSPHATIDYLETHANOLAMINE

P.R. CULLIS [a,*] and B. DE KRUIJFF [b]

[a] *Biochemistry Department and* [b] *Institute of Molecular Biology, State University of Utrecht, Transitorium 3, Padulaan 8, "De Uithof", Utrecht (The Netherlands)*

(Received July 7th, 1977)

Summary

1. ^{31}P NMR is a useful analytical technique for the study of the polymorphic phase behaviour of hydrated phospholipids in excess water. Such possibilities arise due to lateral diffusion of phospholipids which, in non-bilayer phases, produce additional motional averaging mechanisms. This results in distinctive ^{31}P NMR spectra for lipids in the bilayer phase, hexagonal (H_{II}) phase, or phases such as the inverted micellar, cubic or rhombic.

2. The polymorphic phase behaviour of soya phosphatidylethanolamine and mixtures of soya phosphatidylethanolamine with saturated and unsaturated phosphatidylcholines has been investigated. As the temperature is decreased below −10°C soya phosphatidylethanolamine tends to enter the bilayer phase. At higher temperatures (0—50°C) the hexagonal (H_{II}) phase is observed. The addition of 50 mol% egg yolk or (liquid-crystalline) 16 : 0/16 : 0 phosphatidylcholine is sufficient to stabilize the bilayer phase.

3. The addition of equimolar cholesterol to 18 : 1_c/18 : 1_c phosphatidylethanolamine results in the bilayer-hexagonal (H_{II}) transition moving to lower temperatures. Similarly, the addition of equimolar cholesterol to soya phosphatidylethanolamine/35 mol% egg yolk phosphatidylcholine mixtures destabilizes the component in the bilayer phase. Alternatively, the addition of cholesterol to mixtures containing 30 mol% 16 : 0/16 : 0 phosphatidylcholine stabilizes the bilayer configuration. These results are discussed with regard to the observation that biological membranes containing high concentrations of phosphatidylethanolamine contain little or no cholesterol. It

* Present address: Biochemistry Department, University of British Columbia, Vancouver V6T 1W5, Canada.

is suggested that cholesterol may be excluded because its presence would disrupt bilayer structure.

4. It is proposed that the bilayer structure of lipids in biomembranes may be in dynamic equilibrium with other available phases. In the light of such possibilities a mechanism of "flip-flop" phenomena in biological membranes is suggested.

Introduction

The fluid mosaic model of biological membranes [1] has as one of its basic tenets that a large proportion of the lipid constituents are arranged in a bilayer configuration. That such a configuration exists in nature is supported by many results obtained from diverse biological membranes. In the case of the erythrocyte membrane, for example, it has been shown that at least 97% of the membrane phospholipids have motional properties consistent with bilayer structure [2], and the proposal that such bilayer structure is a basic property of the lipid component is supported by the bilayer arrangement of hydrated preparations of the extracted lipids.

There is, however, strong evidence that particular species of lipid found in significant quantities in biological membranes have a preference for non-bilayer configurations such as the hexagonal (H_{II}), cubic or inverted micellar phases. Two examples include cardiolipin (in the presence of Ca^{2+}) [3] and various species of phosphatidylethanolamine from natural [4,5] and synthetic [6] sources. The preference of unsaturated phosphatidylethanolamines for the hexagonal (H_{II}) phase is intriguing, as such species of phospholipid are found in high concentrations in the membranes of gram positive bacteria (e.g. *Escherichia coli*, where phosphatidylethanolamine makes up 85% of the outer membrane phospholipid [7]) and are a major component of most other bacterial and mammalian cell membranes. The presence of such lipids which may not naturally assume a bilayer configuration must be expected to have profound effects on the dynamic structural and functional properties of biological membranes.

In this work the polymorphic phase behaviour of aqueous dispersions of lipid consisting of soya and $18:1_c/18:1_c$ phosphatidylethanolamine in the presence of various species of phosphatidylcholine and cholesterol has been examined, with a view to determining the ability of various lipid components to stabilize or destabilize bilayer structure. Particular use is made of ^{31}P NMR, which is employed in a new way in this study. It has previously been shown that ^{31}P NMR is sensitive to the motional properties of phospholipids in model [6,8–11] and biological [2,11–13] membranes and it has been noted that in most situations this motion is consistent with the restricted anisotropic motion expected for lipids in a bilayer configuration [11]. However, as briefly indicated previously [6], and explained in greater detail here, the motion experienced by the phospholipid phosphorus may be sensitive to its macroscopic environment, particularly to the occurrence of hexagonal (H_{II}) phases and cubic or inverted micellar phases, due to the possibility of additional motional averaging in these structures arising from lateral diffusion processes. This results in distinctive ^{31}P

NMR signals arising from phospholipids in each phase, and, in situations where two or more phases may be present, allows the amount of lipid in each phase to be estimated from the spectra.

Materials and Methods

Phosphatidylethanolamine was prepared from the total lipids of soya beans by silicic acid column chromatography, where the column was eluted stepwise with increasing amounts of methanol in chloroform. The lipid was further purified employing carboxymethyl cellulose column chromatography [14] arriving at a final purity of at least 98% as judged by thin-layer chromatography. It is important that the phosphatidylethanolamine be as pure as possible, as relatively minor amounts of impurities, particularly charged lipid species, can cause large changes in the polymorphic phase behaviour (Cullis, P.R., unpublished). 1,2-Dioleoyl-*sn*-glycero-3-phosphorylethanolamine (18 : 1_c/ 18 : 1_c phosphatidylethanolamine) was synthesized as described previously [6]. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphorylcholine (16 : 0/16 : 0 phosphatidylcholine) was synthesized as described previously [15] and egg yolk phosphatidylcholine was isolated according to well established procedures. Cholesterol was obtained from Fluka (Buchs, Switzerland).

Liposomes were prepared from mixtures of lipid dissolved in chloroform (100—150 mg total phospholipid) and the chloroform was evaporated under nitrogen. The samples were stored under high vacuum overnight and hydrated in 1 ml of ^2H_2O (2 mM EDTA, 10 mM Tris/acetic acid, p^{2}H 7.0) by vortexing. In cases where the lipids assumed the hexagonal (H_{11}) phase exhaustive vortex mixing was required. In order to obtain reproducible temperature-dependent phase behaviour it was often necessary to heat the lipid dispersions to 50°C for 15 min prior to experimentation. To obtain ^{31}P NMR spectra at low (~ -5°C) temperatures 15 vol% ethylene glycol was added to prevent complete freezing of the ^2H_2O.

A Bruker WH-90 Fourier Transform NMR spectrometer operating at 36.4 MHz for ^{31}P was employed, which had facilities for temperature control, a deuterium "lock" and proton decoupling. All spectra were obtained in the presence of high power proton decoupling (18 W). Accumulated free induction decays were obtained from up to 10 000 transients, employing a 0.17 s interpulse time and a 45° r.f. pulse. Differential scanning calorimetry was performed as indicated elsewhere [16].

Results

Various phospholipid phases and the simulated ^{31}P NMR spectra expected from them on the basis of the theoretical considerations of the Appendix (in the absence of dipolar broadening effects) are illustrated in Fig. 1. It is known that the "effective chemical shift anisotropy" $\Delta\sigma_{CSA}^{EFF}$ (the chemical shift between the high field peak and the low field shoulder) of phospholipids in the bilayer phase is on the order of —40 ppm for phosphatidylcholines [19] and phosphatidylethanolamines [6] and therefore such lipids in the hexagonal H_{11} phase exhibit lineshapes with a high field shoulder where $\Delta\sigma_{CSA}^{EFF} \simeq 20$ ppm.

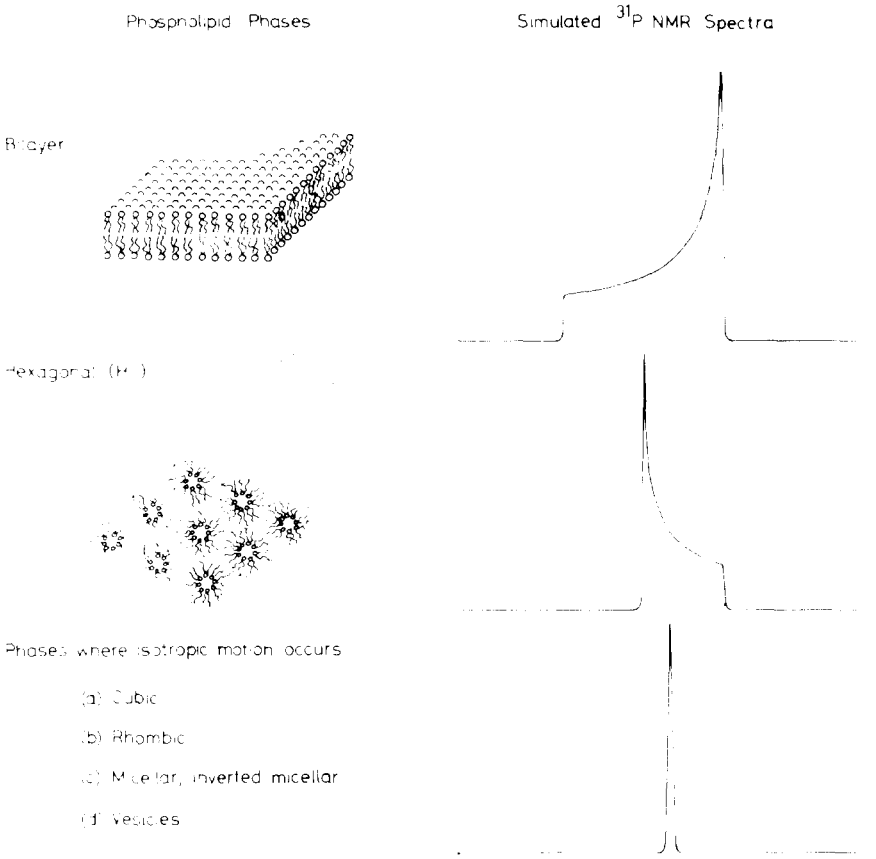


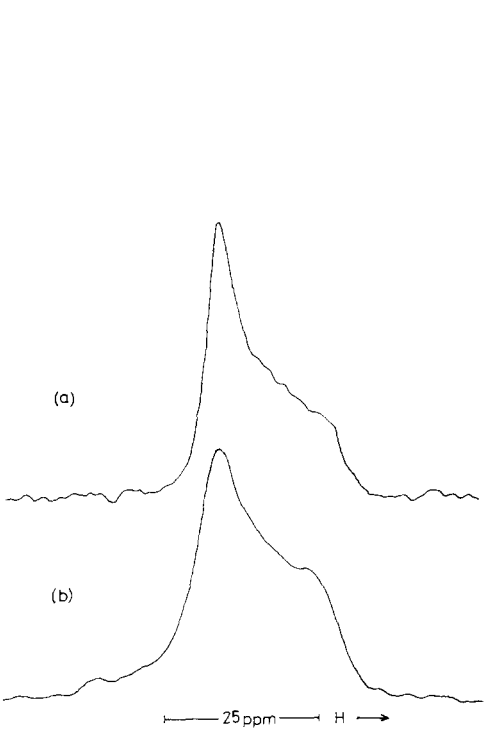
Fig. 1. The polymorphic phases available to hydrated phospholipids and the simulated ^{31}P NMR spectra expected, in the absence of dipolar broadening effects (i.e. in the presence of proton decoupling).

Finally the cubic or inverted micellar phases exhibit very narrow, symmetric ^{31}P NMR spectra which have a chemical shift characteristic of diesterified phosphates which may undergo rapid isotropic averaging motion. This narrow line has the chemical shift characteristic of sonicated vesicles, and it may be suggested that the observation of such spectra may reflect the presence of a population of such vesicles or small micelles in solution. That this is not the case in the work reported here is indicated by the fact that when such lineshapes were observed the phospholipid-water dispersion formed a visible two phase system, where the hydrated phospholipids floated in the ^2H_2O. Also, in most situations it was found that the linewidth of the ^{31}P NMR spectra increased with increasing temperature, in marked contrast to the behaviour observed in vesicle systems [10,17].

The ^{31}P NMR spectra obtained from aqueous dispersions of soya phosphatidylethanolamine at −10°C and 0°C are illustrated in Fig. 2. The 0°C spectra has the characteristic asymmetry (a high field shoulder) and chemical shift anisotropy ($\Delta\sigma_{CSA}^{EFF} \simeq 21$ ppm) we associate with the hexagonal (H_{11}) phase. Alternatively, a small component with a low field shoulder is visible for the −10°C spectra (Fig. 2(b)) suggesting a tendency for soya phosphatidylethanolamine to

enter the bilayer phase at these lower temperatures. However, even at temperatures as low as −30°C spectra very similar to that of Fig. 2(b) were obtained. A precise estimate of the bilayer-hexagonal (H_{II}) phase transition temperature (T_{BH}) for soya phosphatidylethanolamine is therefore not available, but the data show that $T_{BH} \leqslant -10°C$. This temperature is much lower than that observed for 18 : 1_c/18 : 1_c phosphatidylethanolamine [6] ($T_{BH} = 12 \pm 5°C$). In order to ascertain whether such differences could arise from different fatty acid compositions, a fatty acid analysis of the soya phosphatidylethanolamine was made: 16 : 0, 23.5 wt.%; 18 : 0, 2.8 wt.%; 18 : 1_c, 9.9 wt.%; 18 : 2_c, 59 wt.%, unidentified, 5 wt.%. It may be observed that this phospholipid is therefore very unsaturated, containing 59% linoleic acid.

The ability of egg yolk phosphatidylcholine to stabilize the bilayer phase in aqueous dispersions of egg yolk lecithin/soya phosphatidylethanolamine mixtures, at 30°C, is illustrated in Fig. 3. The addition of 15 mol% egg yolk phosphatidylcholine inhibits the hexagonal (H_{II}) phase, converting the lipid to a phase which is characterized by a narrow, symmetric ^{31}P NMR lineshape indicating rapid isotropic motional averaging. It may be noted that such a lineshape does not arise from small micelles or vesicles in solution as preliminary



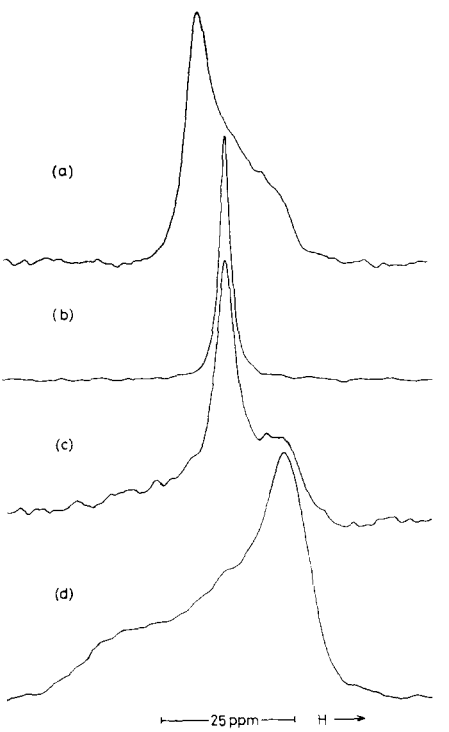


Fig. 2. 36.4 MHz ^{31}P NMR spectra of aqueous dispersions of soya phosphatidylethanolamine at 0°C and −10°C. All dispersions contained 25 mM Tris/acetic acid (p^2H 7.0) and 2 mM EDTA.

Fig. 3. 36.4 MHz ^{31}P NMR spectra of aqueous dispersions of soya phosphatidylethanolamine/egg yolk phosphatidylcholine mixtures at 30°C. The mol% phosphatidylcholine (with respect to total phospholipid) contained in the various samples are (a) 0 mol%, (b) 15 mol%, (c) 35 mol% and (d) 50 mol%. All dispersions contained 25 mM Tris/acetic acid (p^2H 7.0) and 2 mM EDTA.

freeze fracture studies do not reveal such structures (Verkleij, A.J., unpublished). Also, if this sample was heated above 50°C, the lipid was increasingly converted to the hexagonal (H_{II}) phase as indicated by ^{31}P NMR. It would appear that this phase is an intermediary between the hexagonal (H_{II}) and the bilayer phase. The addition of 35 mol% egg yolk phosphatidylcholine causes a decrease in the amount of lipid in the intermediary phase, and the appearance of a substantial bilayer component (70%). Finally, the addition of 50% egg yolk phosphatidylcholine results in complete stabilization of the bilayer phase. Very similar results were obtained (at 40°C) when 16 : 0/16 : 0 phosphatidylcholine was incorporated (above the hydrocarbon transition temperature of the 16 : 0/16 : 0 component as detected by differential scanning calorimetry). Alternatively, at 0°C, the addition of only 15 mol% 16 : 0/16 : 0 phosphatidylcholine was sufficient to stabilize 73% bilayer phase. Differential scanning calorimetry measurements indicated that conditions of lateral phase separation existed at 0°C with the 16 : 0/16 : 0 component in the gel phase (data not shown).

The influence of equimolar concentrations of cholesterol on the polymorphic phase behaviour of mixtures of soya phosphatidylethanolamine with egg phosphatidylcholine and 16 : 0/16 : 0 phosphatidylcholine are illustrated in Fig. 4. As shown in Figs. 4(a) and 4(b) the presence of cholesterol in the system containing 35 mol% egg yolk lecithin destabilizes the component in the bilayer phase, causing a total reversion to the "intermediate" phase. (At higher temperatures (50°C) the sample of Fig. 4(b) revealed ^{31}P NMR spectra characteristic of the hexagonal (H_{II}) phase). This is in marked contrast to the situation when 30 mol% 16 : 0/16 : 0 phosphatidylcholine is present (Fig. 4(c) where, at 40°C (well above the transition temperature of the phosphatidylcholine component as detected by differential scanning calorimetry (data not

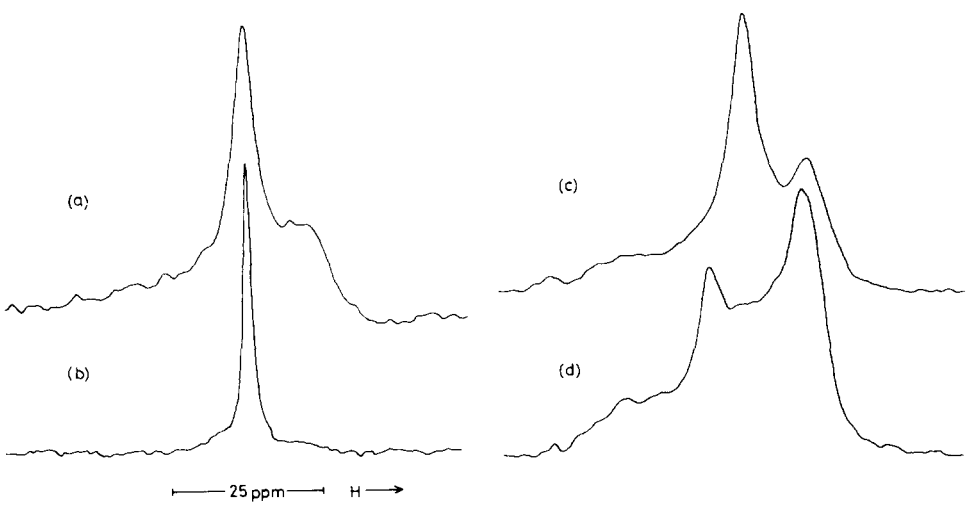


Fig. 4. 36.4 MHz ^{31}P NMR spectra of aqueous dispersions of soya phosphatidylethanolamine/phosphatidylcholine mixtures in the presence and absence of equimolar cholesterol: (a) soya phosphatidylethanolamine/35 mol% egg yolk phosphatidylcholine (T = 30°C), (b) as (a) plus equimolar cholesterol; (c) soya phosphatidylethanolamine/30 mol% 16 : 0/16 : 0 phosphatidylcholine (T = 40°C), (d) as (c) plus equimolar cholesterol. All dispersions contained 25 mM Tris/acetic acid (p^2H 7.0) and 2 mM EDTA.

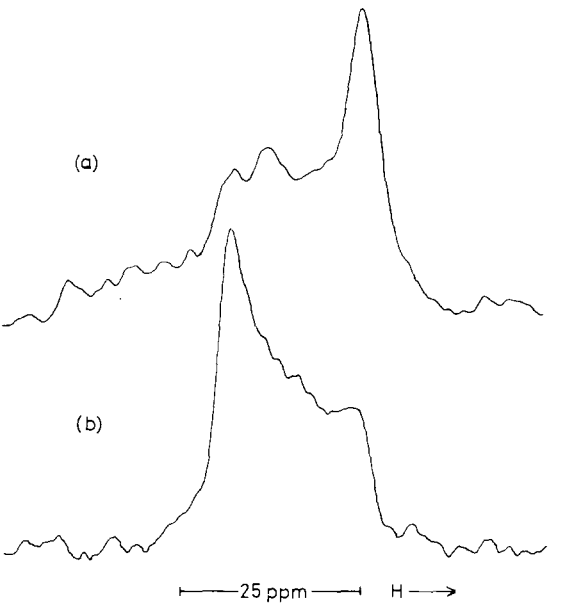


Fig. 5. 36.4 MHz ^{31}P NMR spectra of aqueous dispersions of 18 : 1_c/18 : 1_c phosphatidylethanolamine (a) in the absence and (b) in the presence of equimolar cholesterol (T = 3°C). All dispersions contained 25 mM Tris/acetic acid (pH 7.0) and 2 mM EDTA.

shown)) equimolar cholesterol stabilizes the bilayer phase) (Fig. 4(d)).

In order to demonstrate more definitively the ability of cholesterol to destabilize bilayer structure in the presence of unsaturated phosphatidylethanolamines the ^{31}P NMR behaviour of 18 : 1_c/18 : 1_c phosphatidylethanolamine in the presence and absence of equimolar cholesterol at 5°C was examined. As shown in Fig. 5 equimolar cholesterol induces the hexagonal (H_{II}) phase in such a system. It also was noted that the bilayer-hexagonal (H_{II}) transition temperature for 18 : 1_c/18 : 1_c phosphatidylethanolamine was decreased from 10—15°C [6] to —5—0°C in the presence of equimolar cholesterol.

Discussion

The results obtained here illustrate both the potential of the ^{31}P NMR technique to elucidate the polymorphic phase behaviour of hydrated lipid systems and provide new information on factors which may affect this polymorphism.

As noted in detail in Appendix and more briefly elsewhere [6] the ^{31}P NMR spectra obtained from lipids in the bilayer and hexagonal (H_{II}) phases are fully consistent with the ability of (liquid-crystalline) phospholipids to rotate rapidly about their long axis in the bilayer configuration, whereas in the hexagonal (H_{II}) phase they also diffuse laterally around the aqueous channels. Alternatively, phospholipids in other phases such as the inverted micellar, cubic or rhombic [18] are able to undergo effectively isotropic motion (on the time scale of 10^{-4} s) due to lateral diffusion, which gives rise to much narrower symmetric ^{31}P NMR spectra. It should be noted that these identifications assume

rapid lateral diffusion rates ($D_t \geqslant 10^{-8}$ cm^2/s) and thus may not be correct for systems containing gel state phospholipids, which have been shown to experience much slower lateral diffusion rates [19]. However, as pointed out by Luzzati et al. [18] no gel state phospholipids have been found to exist in phases other than bilayer, presumably due to packing constraints in the hydrocarbon region.

It may be concluded that ^{31}P NMR is a convenient diagnostic tool for determining the polymorphism of hydrated phospholipids. It could be argued that some of the observed effects may be caused by changes in the orientation of the phosphate segment for phospholipids which remain in the bilayer configuration. This must be considered as rather unlikely, however, as previous results indicate relatively invariant orientation and local motion in this region, regardless of the fatty acid composition or phospholipid head group [11]. Further, the agreement with theoretical considerations, particularly for the hexagonal (H_{II}) phase, is excellent. It may be noted that this close agreement indicates that the local motion and conformation in the phosphate region is very similar in the bilayer phase and the "inverted" hexagonal phase. Finally, the occurrence of ^{31}P NMR spectra identified with the hexagonal (H_{II}) phase coincides with the observation of structure consistent with the hexagonal phase in freeze fracture studies [16]. One drawback to the technique which should be mentioned, however, is that accurate determinations of the characteristic dimensions of the non-bilayer phases are not available. This applies particularly to phospholipids in the inverted micellar, cubic or rhombic configurations.

The observation that soya phosphatidylethanolamine undergoes a (partial) bilayer-hexagonal (H_{II}) transition below -10°C corresponds with previous results from X-ray studies on phosphatidylethanolamines from natural sources [4] where a similar transition was observed at somewhat higher temperatures. The different transition temperatures likely reflect the different fatty acid compositions. In this regard the very unsaturated nature of soya phosphatidylethanolamine (see p. 211) and low bilayer-hexagonal transition temperature may be compared to the higher bilayer-hexagonal transition temperature of 18 : 1_c/18 : 1_c phosphatidylethanolamine [6]. This is consistent with the notion that the preference of phospholipids for the bilayer or hexagonal (H_{II}) phase is dictated by the (dynamic) volume assumed by the fatty acid chains. An increased unsaturation may therefore be considered to endow phosphatidylethanolamine with a more pronounced "wedge" shape, with the polar headgroup at the thinner end of the wedge, encouraging formation of inverted phases such as the hexagonal (H_{II}). This would also be consistent with the observation of ^{31}P NMR spectra consistent with bilayer structure for 12 : 0/12 : 0, 14 : 0/14 : 0 [6] and 16 : 0/16 : 0 [20] phosphatidylethanolamine at temperatures well above their respective hydrocarbon transition temperatures.

The ability of phosphatidylcholines to stabilize bilayer structure in the presence of unsaturated phosphatidylethanolamines is of obvious interest with regard to the stability of bilayer structure in biological membranes. The results obtained here indicate that 50 mol% egg yolk phosphatidylcholine performs such a stabilizing function. The fact that similar amounts of 16 : /16 : 0 phosphatidylcholine were required to induce comparable bilayer stabilization (at temperatures above the hydrocarbon transition temperature of the phosphati-

dylcholine component) suggests that the ability of phosphatidylcholine to stabilize the bilayer phase is not particularly sensitive to the saturated or unsaturated nature of the acyl chains. However, the observation that only 15 mol% of 16 : 0/16 : 0 phosphatidylcholine induces appreciable bilayer phase at temperatures below the phase transition of this component imply that gel state phosphatidylcholines are very effective in promoting bilayer structure, even under conditions of lateral phase separation.

An interesting feature of the bilayer stabilization process concerns the observation of an intermediate phase between the hexagonal (H_{11}) phase of pure soya phosphatidylethanolamine and the bilayer phase obtained in the presence of 50 mol% egg yolk phosphatidylcholine. The structure of this intermediate phase which is characterized by the possibility of isotropic motional averaging and corresponding narrow ^{31}P NMR lineshape is unknown. It may reflect the presence of short (length $\leqslant$ 500 Å) regions of H_{11} phase (inverted micelles) or regions of cubic or rhombic [18] phase. Whatever the structure, it is clear that the constituent lipids enjoy considerable motional freedom arising from lateral diffusion processes.

The influence of cholesterol on the polymorphic phase behaviour of phosphatidylethanolamine is of particular interest. The results obtained here provide clear evidence that cholesterol encourages formation of the hexagonal (H_{11}) phase over the bilayer phase for soya phosphatidylethanolamine and 18 : 1_c/18 : 1_c phosphatidylethanolamine (see Fig. 5) and when unsaturated phosphatidylcholine is present encourages formation of the intermediary phase over bilayer structure (Fig. 4(b)). It may therefore be concluded that cholesterol destabilizes bilayer structure in (unsaturated) lipid systems containing phosphatidylethanolamine. This result is consistent with the previous suggestion [21] that cholesterol has a dynamic wedge shape (where the hydroxyl group is at the thinner end of the wedge) which may be conjectured to be more easily accomodated in an "inverted" phase such as the hexagonal (H_{11}). This effect could provide a rationale for certain aspects of the widely varying lipid compositions of various biological membranes. In particular, biomembranes containing high amounts of phosphatidylethanolamine invariably contain little or no cholesterol [22]. It may be speculated that cholesterol is not incorporated because its presence would tend to destabilize bilayer structure with resultant loss of cellular integrity. In this regard it is relevant to note that differential scanning calorimetry studies [16] indicate that phosphatidylethanolamines have a relatively low affinity for cholesterol as compared to (saturated) phosphatidylcholines or sphingomyelin.

The bilayer destabilizing capacity of cholesterol in phosphatidylethanolamine/phosphatidylcholine mixtures is only manifest for unsaturated phosphatidylcholines, however. When 16 : 0/16 : 0 phosphatidylcholine is present the introduction of equimolar cholesterol positively stabilizes bilayer structure even above the hydrocarbon transition temperature of the phosphatidylcholine component. We suggest that such effects could arise from a larger reduction in the dynamic volume occupied by the acyl chains of 16 : 0/16 : 0 phosphatidylcholine due to the presence of cholesterol than is possible for unsaturated phosphatidylcholines. This would be consistent with the observation for 16 : 0/16 : 0 phosphatidylcholine that high concentrations of cholesterol induce an

essentially all trans-conformation in that part of the hydrocarbon chain in contact with the steroid nucleus [23]. This would be expected to result in a very large reduction of the dynamic volume occupied by the acyl chains as compared to the liquid-crystalline state. Alternatively, for unsaturated phosphatidylcholines, the presence of cis-unsaturated bond at the C(9) position would place a lower limit on such effects. Thus it may be speculated that the (hypothetical) cholesterol · saturated phosphatidylcholine complex does not have a net wedge shape, and therefore acts to stabilize bilayer structure, whereas the (hypothetical)cholesterol · unsaturated phosphatidylcholine complex does have a net wedge shape (with the polar region at the smaller end of the wedge) which is more easily accommodated in "inverted" phases such as the hexagonal (H_{11}). These results dramatically illustrate the diverse effects which may occur as a result of relatively subtle changes in fatty acid composition.

The preference of unsaturated phosphatidylethanolamine and other lipid species for hexagonal H_{11} phases or other inverted phases in an aqueous environment must be expected to have profound consequences for the struc-

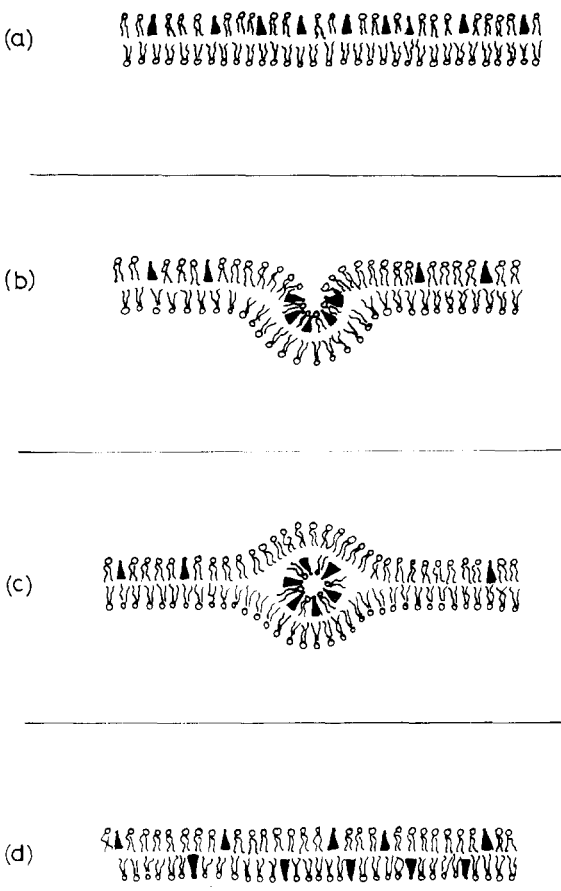


Fig. 6. A possible mechanism of "flip-flop" phenomena in biomembranes. In (a) "non-bilayer" lipid is introduced into one monolayer, and is subsequently redistributed across the membrane via formation of an intermediary "inverted" phase as indicated in (b) and (c).

ture and function of biological membranes. This is particularly true if there are possibilities of segregation of particular species of phospholipid either in the plane of the membrane, or across the membrane. In such situations it is suggested here that the bilayer structure itself may be in dynamic equilibrium with other available phases. As indicated in Fig. 6 such a possibility could be envisaged to allow rapid "flip-flop" of particular species of lipid across the membrane, as local high concentrations of "non-bilayer" lipid in one monolayer result in destabilization of that monolayer, intermediate formation of the H_{II} or other inverted phase such as inverted micellar, and subsequent redistribution of the "non-bilayer" lipid on the other monolayer of the membrane. Other species of lipid also induce the H_{II} phase in phospholipid-containing systems, including fatty acids (Cullis, P.R. and Hope, M., unpublished) and thus could be transported by a similar mechanism. Such possibilities also cast some doubts on the use of phospholipases to determine asymmetric distributions of phospholipids across biological membranes [24] as the products may result in local destabilization of bilayer structure and a redistribution of membrane lipids across the bilayer.

Appendix

The contribution of chemical shift anisotropy to the phospholipid phosphorus spin Hamiltonian may be written

$$\mathcal{H}_{CSA} = \gamma_p H(1 - \sigma_a)S_z + \Delta\sigma_{CSA}^{EFF}\gamma_p \underline{H} \cdot \begin{bmatrix} 1 & & \\ & 1 & \\ & & -2 \end{bmatrix} \cdot \underline{S} \tag{1}$$

where γ_p is the phosphorus gyromagnetic ratio, $\underline{H}$ is the magnetic field, S_z is the z component of the spin $\underline{S}$ and $\Delta\sigma_{CSA}^{EFF}$ is the effective chemical shift anisotropy (measured in parts per million). Eqn. 1 includes the effects of rapid axial rotation about an axis perpendicular to the bilayer common to liquid-crystalline phospholipids [11] and the corresponding lineshape [9] is illustrated in Fig. 1. Lipids in the hexagonal (H_{II}) phase may experience additional motional averaging due to lateral diffusion around the aqueous channels. If it is assumed (without loss of generality) that the axis of the aqueous channel is in the X direction, this may be mathematically expressed as an averaging of the Y and Z components of the tensor of Eqn. 1, resulting in

$$\mathcal{H}_{CSA} = \gamma_p H(1 - \sigma_a)S_z - \frac{\Delta\sigma_{CSA}^{EFF}}{2}\gamma_p \underline{H} \cdot \begin{bmatrix} -2 & & \\ & 1 & \\ & & 1 \end{bmatrix} \cdot S \tag{2}$$

It is then straightforward to show that if the X axis subtends an angle θ with respect to $\underline{H}$, the chemical shift of the ^{31}P NMR signal obtained from a particular lipid cylinder is

$$\Delta\nu(\theta) = -\gamma_p H \frac{\Delta\sigma_{EFF}^{CSA}(3\cos^2\theta - 1)}{6} \tag{3}$$

The lineshapes expected from unoriented hexagonal (H_{II}) phase systems may be simulated employing Eqn. 3, arriving at a lineshape with reversed asymmetry and half the chemical shift anisotropy as compared to lineshapes expected for bilayer structure (see Fig. 1).

The correlation time τ_c of the motion around the channel for lateral diffu-

sion rates $D_t \geqslant 10^{-8}$ cm^2/s, assuming a channel diameter of 20 Å may be calculated to be $\tau_c \lesssim 10^{-6}$ s. For significant motional averaging to occur the relation $\omega_0^2 \tau_c^2 << 1$ must be observed where $\omega_0^2 \approx (\gamma_p H \Delta\sigma_{CSA}^{EFF})^2$. For $\Delta\sigma_{CSA}^{EFF} = 40$ ppm at an observation frequency of 36.4 MHz it is therefore required that $\tau_c << 10^{-4}$ s, which condition would be well satisfied for $D_t \gtrsim 10^{-8}$ cm^2/s.

If the cylinders of the hexagonal (H_{II}) phase become shorter an increasing amount of the lipid will be able to diffuse around the end of the cylinders in a time $\tau_c << 10^{-4}$ s until the limiting case of an inverted micelle is reached, in which case isotropic averaging will occur. This would result in a narrow, symmetric lineshape. However, this narrow line may also result from cubic or rhombic [18] phases which have been observed for hydrated phospholipids. Both these phases consist of short rods joined three by three, and as the length of these rods is not much longer than their diameter (≈20 Å) the possibility exists that lipids can diffuse from one rod to another (which has a different orientation) in times much less than 10^{-4} s, which would also result in isotropic averaging. The ^{31}P NMR spectra expected from the various available phospholipid phases are summarized in Fig. 1.

Acknowledgements

We would like to thank Professor L.L.M. van Deenen for his support and Dr. D.W.M. van Dijck for performing the differential scanning calorimetry experiments. P.R.C. is a Post-Doctoral Fellow of the European Molecular Biology Organization (1977) and acknowledges their support.

References

1 Singer, S.J. and Nicolson, G.L. (1972) Science 145, 720—731
2 Cullis, P.R. and Grathwohl, Ch. (1977) Biochim. Biophys. Acta 471, 213—226
3 Rand, R.P. and Sengupta, S. (1972) Biochim. Biophys. Acta 255, 484—492
4 Rand, R.P., Tinker, D.O. and Fast, P.G. (1971) Chem. Phys. Lipids 6, 333—342
5 Junger, E. and Reinauer, H. (1969) Biochim. Biophys. Acta 183, 304—308
6 Cullis, P.R. and de Kruijff, B. (1976) Biochim. Biophys. Acta 436, 523—540
7 Träuble, H. and Overath, P. (1973) Biochim. Biophys. Acta 307, 491—512
8 McLaughlin, A.C., Cullis, P.R., Berden, J.A. and Richards, R.E. (1975) J. Mag. Resonance 20, 146—165
9 Cullis, P.R., de Kruijff, B. and Richards, R.E. (1976) Biochim. Biophys. Acta 426, 433—446
10 McLaughlin, A.C., Cullis, P.R., Hemminga, M.A., Hoult, D.I., Radda, G.K. and Richards, R.E. (1975) FEBS Lett. 57, 213—218
11 Cullis, P.R. and McLaughlin, A.C. (1977) Trends Biochem. Sci. 2, 196—199
12 Cullis, P.R. (1976) FEBS Lett. 68, 183—176
13 de Kruijff, B., Cullis, P.R., Radda, G.K. and Richards, R.E. (1976) Biochim. Biophys. Acta 419, 411—424
14 Comfurius, P. and Zwaal, R.F.A. (1977) Biochim. Biophys. Acta 488, 36—42
15 van Deenen, L.L.M. and de Haas, G.H. (1964) Adv. Lipid Res. 2, 168—229
16 van Dijck, P.W.M., de Kruijff, B., van Deenen, L.L.M., de Gier, J. and Demel, R.A. (1976) Biochim. Biophys. Acta 455, 576—587
17 de Kruijff, B., Cullis, P.R. and Radda, G.K. (1976) Biochim. Biophys. Acta 406, 6—20
18 Luzzati, V., Gulik-Krzywicki, T. and Tardieu, A. (1969) Nature 218, 1031—1034
19 Cullis, P.R. (1976) FEBS Lett. 70, 223—228
20 Seelig, J. and Gally, H. (1976) Biochemistry 15, 5199—5204
21 de Kruijff, B., Cullis, P.R. and Radda, G.K. (1976) Biochim. Biophys. Acta 436, 729—740
22 Demel, R.A., Jansen, J.W.C.M., van Dijck, P.W.M. and van Deenen, L.L.M. (1976) Biochim. Biophys. Acta 465, 1—10
23 Gally, H.U., Seelig, A. and Seelig, J. (1976) Hoppe-Seyler's Z. Physiol. Chem. 357, 1447—1450
24 Zwaal, R.F.A., Roelofsen, B. and Colley, C.M. (1973) Biochim. Biophys. Acta 300, 159—170