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Polymorphism of Phosphatidylglycerol-Phosphatidylethanolamine Model Membrane Systems: a ^{31}P NMR Study

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SUMMARY

The structural preferences of saturated and unsaturated species of phosphatidylglycerol in isolation and in mixed systems with unsaturated (H_{II} phase) phosphatidylethanolamine have been investigated employing ^{31}P NMR techniques. It is shown that equimolar (with respect to change) Ca^{2+} induces a "rigid lattice" (no motion) situation in the phosphate region of the polar headgroup for a saturated species, but not for unsaturated species. Further, phosphatidylglycerol can stabilize a bilayer organization in the presence of phosphatidylethanolamine which would otherwise assume the hexagonal (H_{II}) phase, and structural bilayer-H_{II} transitions can then be triggered in these systems by the addition of Ca^{2+}.

Recent investigations (for review, see ref. 1) suggest that individual lipid species found in biological membranes belong to either of two major classes--those which assume a bilayer organisation on hydration, and those which prefer the hexagonal (H_{II}) phase. In the case of eukaryotic cell membranes it is remarkable that certain major lipid species, such as phosphatidylethanolamines prefer the hexagonal H_{II} arrangement at physiological temperatures, which has led to suggestions [1] that "non-bilayer" lipids and their associated structures may play functional roles which are not related to maintaining the membrane bilayer envelope.

For prokaryotic cell membranes, questions regarding the structural roles of lipids are equally intriguing. In the case of E. coli, for example, phosphatidylethanolamines commonly constitute 80% of the membrane phospholipid [2]. This does not necessarily indicate a predisposition of E. coli membranes for non-bilayer arrangements, however, as the relatively saturated nature of E. coli phosphatidylethanolamine can raise the temper-

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ature at which this lipid adopts the hexagonal H_{II} phase well above growth temperature [3].

The possibility that non-bilayer lipid configurations can occur in E. coli membranes is, however, supported by recent observations [4] that model systems composed of total lipid extracts of E. coli exhibit H_{II} phase components and "lipidic particle" [5] structure at 37°C. This would suggest that the presence of E. coli lipid species (other than phosphatidylethanolamine) in these mixed systems can induce non-bilayer alternatives. Given that phosphatidylglycerol is the next most abundant phospholipid, it is of obvious interest to investigate the polymorphic preferences of mixed phosphatidylethanolamine-phosphatidylglycerol model systems. The results of such studies are presented here. We show that saturated and unsaturated phosphatidylglycerols exhibit ^{31}P NMR spectra consistent with a bilayer organisation, and that phosphatidylglycerol stabilizes a bilayer organisation in the presence of non-bilayer (H_{II} phase) phosphatidylethanolamine. Further, in these latter systems Ca^{2+} can trigger isothermal bilayer to H_{II} polymorphic phase transitions.

MATERIALS AND METHODS

(Egg) phosphatidylglycerol, dimyristoyl phosphatidylglycerol and soya phosphatidylethanolamine were all prepared from the corresponding species of phosphatidylcholine employing the base exchange capacity of phospholipase D [6]. E. coli phosphatidylglycerol was isolated from a total lipid extract of freeze-dried E. coli (Sigma, St. Louis) employing carboxymethyl cellulose column chromatography. All lipids isolated were more than 99 pure as evidenced by thin-layer chromatography. The sodium salt of the phosphatidylglycerols was obtained as detailed elsewhere [7].

Samples for ^{31}P NMR studies were prepared from 50 μmol total phospholipid (unless indicated otherwise) in 1 ml chloroform, which was placed in a 10 mm NMR tube. The chloroform was evaporated under a stream of nitrogen, and the sample was subsequently placed under high vacuum for at least 1 hr. Subsequently the lipid was dispersed in 0.8 ml of a 10% D_2O buffer containing 100 mM NaCl, 10 mM Tris-HCl (pH = 7.4) and 2 mM EDTA employing a vortex mixer. Divalent cations were added in appropriate amounts from a 100 mM stock solution.

^{31}P NMR experiments were performed on a Bruker WP 200 Fourier transform NMR spectrometer operating at 81.0 MHz. Accumulated free induction decays were obtained from up to 1000 transients employing an 11 μsec 90° pulse, a 0.8 sec interpulse time, a 20 KHz sweep width and gated proton decoupling. An exponential multipication corresponding to a 50 Hz linebroadening was performed except where otherwise noted.

RESULTS AND DISCUSSION

In order to understand the behaviour of phosphatidylglycerol in mixed lipid systems, as well as the influence of Ca^{2+} on these systems, it is necessary to characterize the structural preferences of phosphatidylglycerol alone in the presence and absence of Ca^{2+}. Such information is given in Fig. 1 which presents the ^{31}P NMR spectra obtained from different species of phosphatidylglycerol under varying conditions of hydration and Ca^{2+} content. Three conclusions may be drawn. First, in the anhydrous sodium salt form, "rigid lattice" (no motion) ^{31}P NMR spectra are observed which are quantitatively similar to those observed for anhydrous phosphatidylcholine [8], sphingomyelin [9] and phosphatidylserine [7], consistent with a similar headgroup conformation in the phospate region for these different phospholipid species. Secondly, in the presence of excess aqueous buffer at 30°C, asymmetric ^{31}P NMR spectra with a low field shoulder and high field peak separated by approximately 40 ppm are observed, which are characteristic of liquid crystalline phospholipids in a lamellar organisation. This is with the exception that a narrow spectral component, superimposed on the bilayer lineshape, is observed for the dimyristoyl phosphatidylglycerol dispersion. Similar spectral components indicating that a portion of the phospholipids experience isotropic motional averaging are observed for saturated phosphatidylcholine [10] and sphingomyelin [9] and would be consistent with a population of small lamellar vesicles, which have been shown to occur in sphingomyelin dispersions [11]. A final point is that the addition of Ca^{2+} to obtain a Ca^{2+} to phosphatidylglycerol molar ratio of 0.5, (which results in immediate precipitation of the lipid dispersions) results in spectral changes which are sensitive to the fatty acid composition. In the case of the unsaturated (egg and E. coli phosphatidylglycerol) species, the spectra broaden somewhat but maintain a "bilayer" ^{31}P NMR lineshape. This contrasts with the behaviour of the Ca^{2+}-dimyristoyl phosphatidyl-

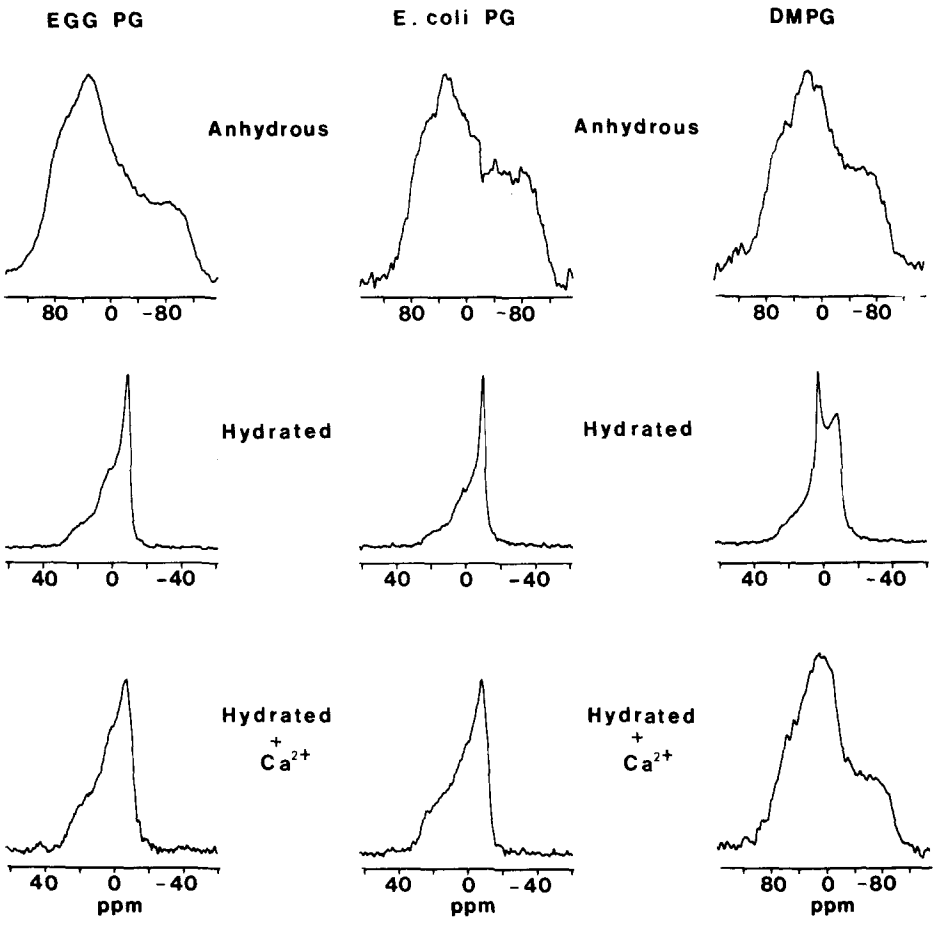

Figure 1. 81.0 MHz ^{31}P NMR spectra of egg, E. coli and dimyristoyl phosphatidylglycerol at 30°C in the anhydrous (sodium salt) form, in the presence of excess aqueous buffer and in the presence of excess aqueous buffer after addition of sufficient $CaCl_2$ to obtain a Ca^{2+}/phosphatidylglycerol ratio of 0.5 mol/mol. The spectra of the anhydrous phospholipids (and the hydrated DMPG in the presence of Ca^{2+}) were obtained from 200 μmol phospholipid employing a 50 KHz sweep width, a 20 sec interpulse time, reasonably high power (~5 G) proton decouplings and correspond to 200 transients. An exponential multiplication corresponding to 100 Hz linebroadening was also applied. In all other cases spectra were obtained from 1000 transients employing 50 μmol phospholipid, a 20 KHz sweep width, a 0.5 sec interpulse time and a 50 Hz linebroadening function. The aqueous buffer contained 100 mM NaCl, 10 mM Tris-HCl (pH = 7.0), 2 mM EDTA and 10% D_2O.

glycerol precipitate, which reverts to the rigid lattice lineshape characteristic of anhydrous phospholipids. A concomitant increase n the spin lattice relaxation time from 0.8 ± .2 sec to 15 ± 2 sec (as measured by a saturation-recovery technique) was also observed.

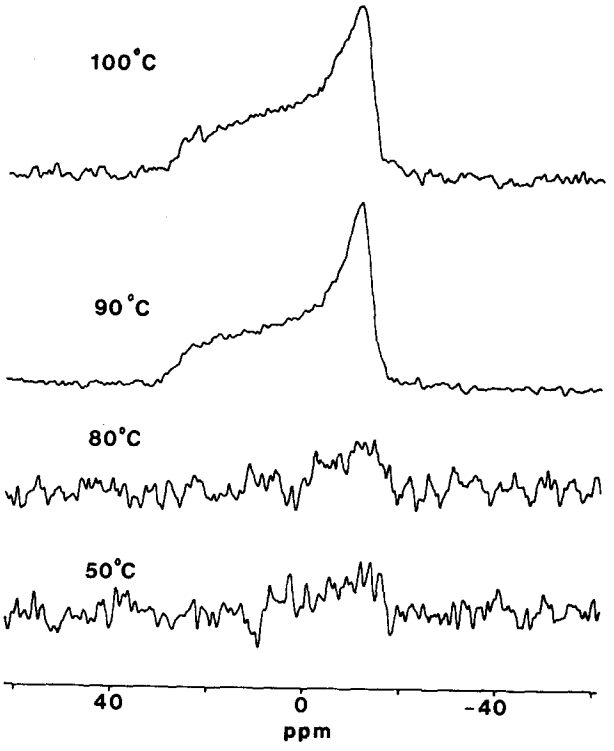

Figure 2. 81.0 MHz ^{31}P NMR spectra of 50 μmol hydrated dimyristoyl phosphatidylglycerol (DMPG) in the presence of 25 μmol Ca^{2+}, as a function of temperature. All spectra were obtained from 1000 transients employing identical conditions as those noted for the hydrated preparations of Fig. 1.

The ^{31}P NMR behaviour of dimyristoyl phosphatidylglycerol in the presence of Ca^{2+} is very similar to that oberved for (egg) phosphatidylserine [7]. Combined freeze-fracture and differential scanning calorimetry studies [12] reveal that similar condensed "cochleate" lipid structures are obtained, which undergo a melting transition to normal liposomes in the region of 80°C. This transition is also reflected in the ^{31}P NMR characteristics, as a liquid crystalline lamellar ^{31}P NMR spectrum is obtained above 80°C as shown in Fig. 2. It may be noted that this result contrasts with a recent report [13] suggesting the presence of H_{II} phase organisation for the ether analogue of dimyristoyl phosphatidylglycerol under similar experimental conditions.

Previous work has revealed, as may be logically expected, that phos-

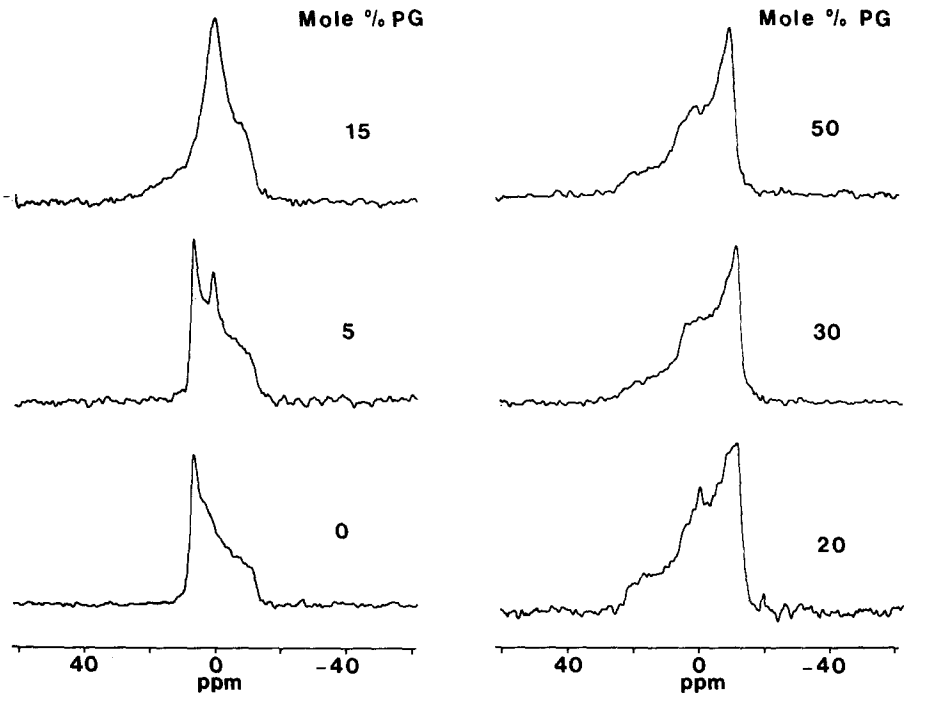

Figure 3. 81.0 MHz ^{31}P NMR spectra arising from aqueous dispersions of mixtures of (egg) phosphatidylglycerol and soya phosphatidylethanolamine at 30°C where the amounts of phosphatidylglycerol present correspond to 0, 5, 15, 20, 30 and 50 mol % of the total phospholipid. Sample preparation and spectral conditions as for Fig. 1 (hydrated samples).

pholipids such as phosphatidylcholine [14], sphingomyelin [9] and phosphatidylserine [15,16] which adopt a bilayer organisation in isolation are also able to stabilize a bilayer organisation in the presence of an H_{II} phase lipid such as unsaturated phosphatidylethanolamine. Phosphatidylglycerol displays a similar ability as indicated in Fig. 3 for mixtures of (egg) phosphatidylglycerol with soya phosphatidylethanolamine. Clearly, the presence of 20 mol % or more phosphatidylglycerol causes the vast majority of the phospholipid to assume bilayer structure. The narrow spectral components particularly visible at low phosphatidylglycerol contents could arise from a variety of sources, including small lamellar vesicles as indicated above. Given that increasing the amount of phosphatidylglycerol effects a hexagonal (H_{II}) to bilayer phase transition, however, we suggest that these components may likely arise from (intra-

bilayer) inverted micellar structures which can occur as intermediaries between lamellar and H_{II} phase organisation [17]. Such structures would also be expected to give rise to narrow ^{31}P NMR resonances characteristic of isotropic motional averaging [18].

A feature of mixed lipid systems where a bilayer organisation is stabilized by acidic (negatively charged) phospholipids such as phosphatidylserine [16] or cardiolipin [19] is that isothermal bilayer to hexagonal (H_{II}) polymorphic phase transitions may be triggered on addition of divalent cations such as Ca^{2+}. Such phenomena are of obvious interest with regard to the regulation and control of non-bilayer structure, which is of course vital if such structures are to be functionally useful. As shown in Fig. 4, Ca^{2+} also displays a similar ability to generate H_{II} phase structure in the mixed (egg) phosphatidylglycerol soya phosphatidylethanolamine systems. There are two aspects of these results that we wish to emphasize. First, in analogous systems where bilayer organisation is stabilied by phosphatidylserine or cardiolipin, equimolar (with respect to charge) amounts of Ca^{2+} are sufficient to trigger apparently complete H_{II} phase formation. In the case of the phosphatidylglycerolphosphatidylethanolamine mixture, however, this is clearly not the case, particularly for higher phosphatidylglycerol contents. This presumably reflects both a lower affinity of phosphatidylglycerol for Ca^{2+} as well as the fact that Ca^{2+}-(egg) phosphatidylglycerol complexes still prefer a bilayer organisation (c.f. Fig. 1). Secondly, consistent with results obtained for phosphatidylglycerolphosphatidylcholine mixtures [20] there is no evidence for a lateral segregation of phosphatidylglycerol by Ca^{2+}. This is supported by the spectra obtained for the 30 mol % phosphatidylglycerol system, where excess Ca^{2+} induces apparently complete H_{II} phase formation, with no evidence of 30% of the phospholipid remaining in the bilayer phase. This shows that the phosphatidylglycerol is directly incorporated into the H_{II} phase matrix in this system. This

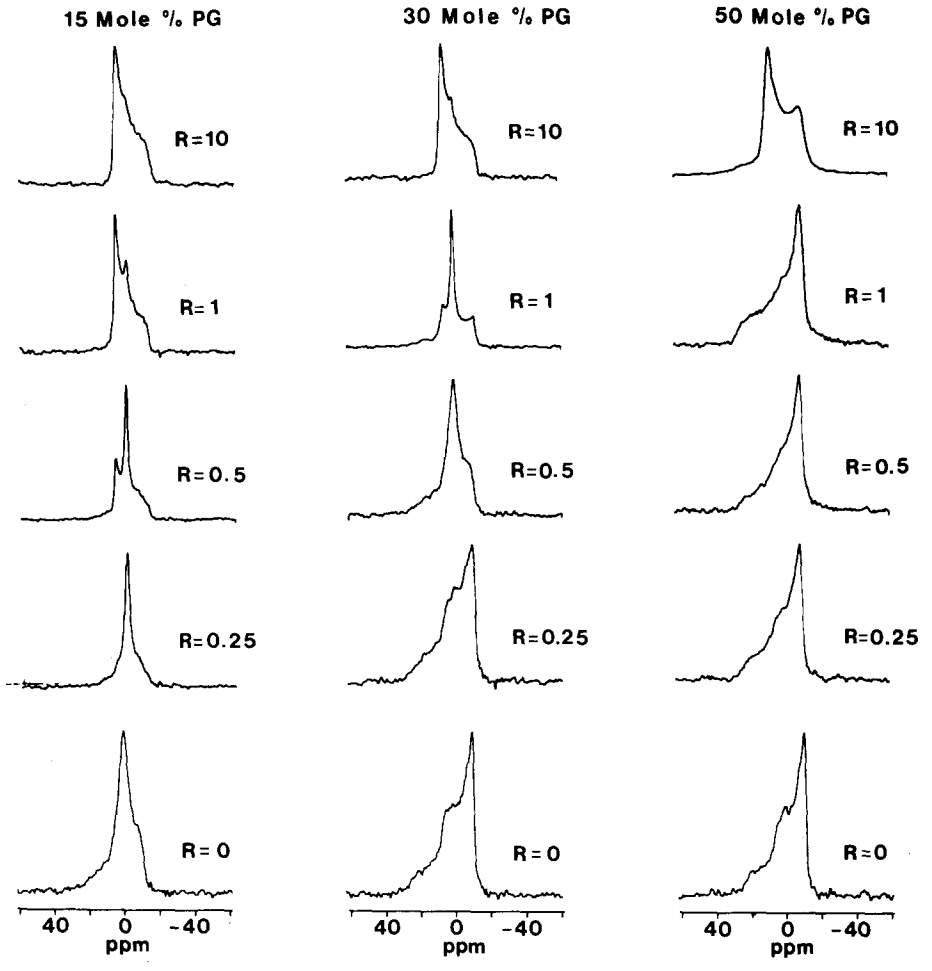

Figure 4. 81.0 MHz ^{31}P NMR spectra arising from aqueous dispersions of mixtures of soya phosphatidylethanolamine and 15, 30 and 50 mol % of egg phosphatidylglycerol in the presence of varying amounts of Ca^{2+}. The ratio R refers to the molar ratio of Ca^{2+} to egg phosphatidylglycerol. All other experimental conditions are identical to those employed for the hydrated preparations of Fig. 1.

behaviour contrasts with phosphatidylserine containing systems, where Ca^{2+} induces lateral segregation of the phosphatidylserine, allowing the phosphatidylethanolamine to revert to the hexagonal H_{II} phase structure it prefers in isolation [15].

In summary, phosphatidylglycerol assumes a bilayer organisation on hydration, and is able to stabilize bilayer structure in the presence of unsaturated (H_{II} phase) phosphatidylethanolamine. The influence of

Ca^{2+} on pure phosphatidylglycerol systems is sensitive to the fatty acid composition, and ^{31}P NMR spectra indicative of condensed crystalline "cochleate" [21] structure are only observed for saturated species in the presence of Ca^{2+}. Finally, in mixed phosphatidylglycerolphosphatidylethanolamine systems Ca^{2+} can trigger bilayer-H_{II} transitions. In such situations, the phosphatidylglycerol actually enters the H_{II} phase matrix.

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