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### STABILIZATION OF BILAYER STRUCTURE FOR UNSATURATED PHOSPHATIDYLETHANOLAMINES BY DETERGENTS

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The structural preferences of mixed lipid systems containing egg yolk or 18:1<sub>c</sub>/18:1<sub>c</sub> phosphatidylethanolamine and representative detergents (Triton X-100, deoxycholate, octylglucoside and lyso-phosphatidylcholine) have been examined. It is shown that all these detergents exhibit an ability to stabilize a bilayer organization for the phosphatidylethanolamine at detergent to phosphatidylethanolamine molar ratios of 0.05 to 0.5, depending on the detergent and/or phosphatidylethanolamine species. These results are interpreted in terms of molecular shape, where the 'inverted cone' shape detergents combine in a complementary fashion with 'cone shaped' phosphatidylethanolamine to result in net bilayer structure.

Israelachvili and coworkers [1–3] have shown that the macromolecular aggregates formed by various lipids on hydration are sensitive to geometric considerations (i.e. the molecular 'shape' of the lipid) as well as thermodynamic factors. This proposal is of considerable interest, as it leads to a possible rationale for lipid diversity in membranes in terms of a requirement for lipids with a variety of shapes. In particular, other authors [4] have suggested that the presence of lipids with particular shapes is a conserved quantity in membranes. Results presented by McElhaney and Silvius [5] could also be employed to support such a conjecture. Further, as we have noted elsewhere [6], the abundant evidence that lipid composition in bacteria is regulated by factors such as growth temperature, which has previously been interpreted in terms of a requirement to maintain a certain membrane 'fluidity', can equally well support the notion that membrane lipid shape is the conserved quantity.

Within the shape hypothesis there are three broad categories of lipids [7], those preferring a micellar organization on hydration (inverted cone

shape), a bilayer phase (cylindrical shape) or the hexagonal ( $\text{H}_{\text{II}}$ ) phase (cone shape). In this communication we examine a simple prediction of the shape hypothesis which suggests that appropriate mixtures of cone ( $\text{H}_{\text{II}}$  phase) and inverted cone (micellar) lipids should give rise to a net bilayer organization due to shape complementarity. This possibility is tested employing two species of  $\text{H}_{\text{II}}$  phase lipids (egg phosphatidylethanolamine and synthetic 18:1<sub>c</sub>/18:1<sub>c</sub> phosphatidylethanolamine) in the presence of several common detergents (sodium deoxycholate, Triton X-100, octylglucopyranoside and egg lysophosphatidylcholine). We show that all these detergents are able to stabilize a net bilayer organization for the phosphatidylethanolamines under conditions where this structure is not available to either lipid species in isolation.

**Materials and Methods.** Phosphatidylethanolamine was isolated from a total lipid extract of egg yolk employing a Waters Prep 500 LC apparatus according to established procedures [8]. The final product, which was at least 99% pure as determined by thin-layer chromatography, was

freeze-dried (from benzene/methanol, 70:30, v/v) and stored in 100-mg ampoules under liquid nitrogen. 1,2-Oleoyl-*sn*-3-glycerophosphoethanolamine (18:1<sub>c</sub>/18:1<sub>c</sub> phosphatidylethanolamine) was synthesized as indicated elsewhere [9]. Oleoyllysophosphatidylcholine and egg lysophosphatidylcholine (containing primarily palmitic and stearic acid acyl chain substituents) were obtained from Sigma (St. Louis). Sodium deoxycholate, octyl- $\beta$ -D-glucopyranoside (grade A; octylglucoside) and Triton X-100 (scintillation grade) were obtained from MCB (Norwood, U.S.A.), Calbiochem (La Jolla) and BDH (Poole, U.K.), respectively, and were used without further purification.

Phosphatidylethanolamine and phosphatidylethanolamine-detergent dispersions were prepared from 74 mg of phospholipid and appropriate amounts of detergent in benzene/methanol (70:30, v/v) solutions which were subsequently freeze-dried. The lipid was hydrated in 1.0 ml of a 100 mM NaCl, 50 mM Hepes (pH 7.4), 0.2 mM EDTA buffer containing 10%  $^2\text{H}_2\text{O}$ .

$^{31}\text{P}$ -NMR spectra were recorded employing a Bruker WP 200 Fourier Transform NMR spectrometer operating at 81.0 MHz. Free induction decays were obtained from up to 2000 transients employing an 11  $\mu\text{s}$  90° pulse, an interpulse time of 0.8 s and a sweepwidth of 20 kHz. An exponential filter corresponding to a 50 Hz line broadening was applied to the free induction decay prior to Fourier transformation. Spectra were accumulated in the presence of broadband proton decoupling.

Samples for freeze-fracture contained 30% glycerol and were quenched from 37°C, and were fractured and visualized employing a Balzers freeze-fracture apparatus and a Philips 400 electron microscope.

**Results and Discussion.** As shown elsewhere [10], fully hydrated egg phosphatidylethanolamine undergoes a bilayer to hexagonal ( $\text{H}_{\text{II}}$ ) phase transition as the temperature is increased through 30°C. Thus at 37°C this lipid prefers the  $\text{H}_{\text{II}}$  organization as indicated by the characteristic  $^{31}\text{P}$ -NMR spectra obtained in the absence of detergent (see Fig. 1). This asymmetric spectra, which has a low-field peak and high-field shoulder has reversed asymmetry and is approximately half as broad as the spectra obtained from phospholipids in large (> 2000 Å diameter) bilayer systems [7]. Phos-

pholipid in smaller lamellar systems (e.g. sonicated vesicles) or in non-lamellar structures such as inverted micelles (lipidic particles [11,12]) or cubic phases [13,14] exhibit narrow, symmetric spectra.

As shown in Fig. 1, all the detergents investigated demonstrated an ability to stabilize a bilayer organization for egg phosphatidylethanolamine at 37°C. In the case of the ionic detergents deoxycholate and lysophosphatidylcholine, relatively complete stabilization occurs at detergent-phospholipid molar ratios  $R = 0.05$  whereas higher amounts ( $R = 0.2$ ) of the non-ionic detergents octylglucoside and Triton X-100 are required to produce equivalent effects. It may be noted that octylglucoside stabilizes the bilayer particularly effectively for  $R = 0.2$ , with very little evidence of a narrow spectral component indicating phospholipid in structures allowing isotropic motional averaging. Deoxycholate has similar effects at molar ratios  $R = 0.05$ . In contrast, whereas both lysophosphatidylcholine and Triton X-100 can induce the majority of the phospholipid to adopt the bilayer organization at appropriate concentrations, a strong 'isotropic' component is also present. In the case of lysophosphatidylcholine this component may arise, at least in part, from the fraction of lipid in micellar form as dictated by the membrane-buffer partition coefficient. This is not the case for Triton X-100 — egg phosphatidylethanolamine dispersions, which appear to adopt a structure compatible with isotropic motional averaging at intermediate detergent concentrations below those required for relatively complete bilayer stabilization (see spectrum for  $R = 0.1$ ). In order to ascertain the nature of the structures present, freeze fracture studies were performed for the  $R = 0.1$  sample and the micrographs obtained indicated the presence of apparently multilamellar systems with an average diameter of approx. 1000 Å. Phospholipids in such structures can experience isotropic motional averaging on the NMR time scale, primarily due to lateral diffusion processes [15] for lateral diffusion rates of  $10^{-8}$  cm<sup>2</sup>/s or faster. Concentrations of detergent appreciably higher than those required for bilayer stabilization also exhibit narrow spectral components, presumably due to the formation of mixed detergent-phospholipid micelles.

The ability of those detergents to stabilize a

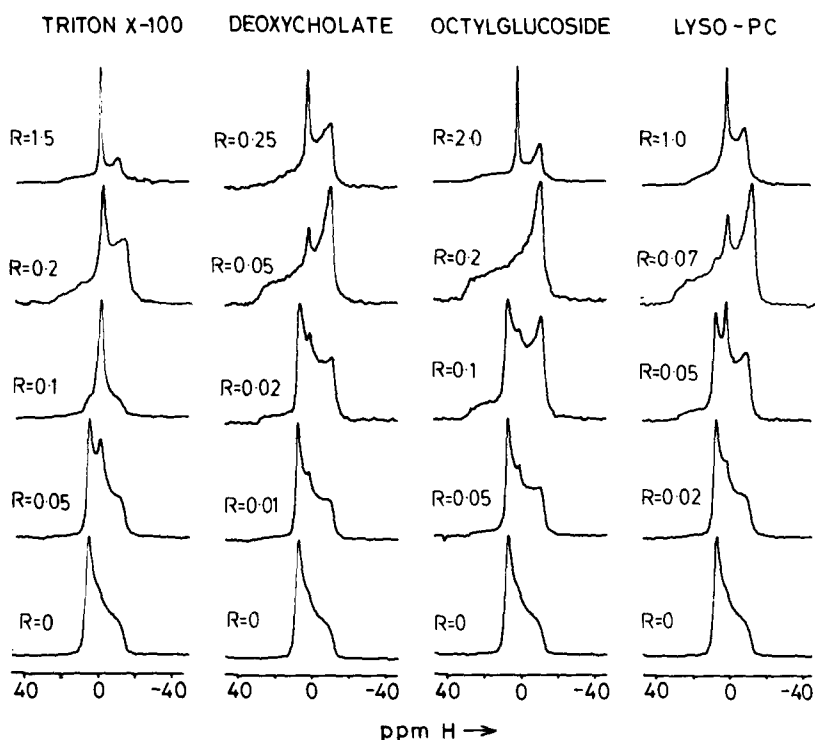


Fig. 1. 81.0 MHz  $^{31}\text{P}$ -NMR spectra at  $37^\circ\text{C}$  of aqueous dispersions of egg yolk phosphatidylethanolamine in the presence of increasing amounts of various common detergents. 0 ppm corresponds to the resonance position of sonicated phosphatidylcholine vesicles in this and subsequent figures. For details of sample preparation and signal accumulation see Methods.

bilayer organization for egg phosphatidylethanolamine at  $37^\circ\text{C}$  corresponds to an increase in the bilayer to hexagonal ( $\text{H}_{\text{II}}$ ) transition temperature ( $T_{\text{BH}}$ ). As indicated elsewhere [10], in the absence of detergent  $T_{\text{BH}} = 28^\circ\text{C}$ , whereas the presence of deoxycholate to obtain a detergent to phospholipid ratio of  $R = 0.05$  increases  $T_{\text{BH}}$  to approx.  $40^\circ\text{C}$  (see Fig. 2). The presence of the other detergents at the same detergent to phospholipid ratios resulted in  $T_{\text{BH}} = 34^\circ\text{C}$  for octylglucoside and  $40^\circ\text{C}$  for egg lysophosphatidylcholine.

These results can clearly be taken to support the shape concept, in that they are consistent with the proposal that the cone shaped phosphatidylethanolamine combines with the inverted cone detergent in a complementary fashion to arrive at a net bilayer structure. In this regard, it would be expected that the amount of detergent required to stabilize bilayer organization for a phosphatidylethanolamine with a more pronounced cone shape

(e.g. a more unsaturated variety with a lower  $T_{\text{BH}}$ ) would be somewhat higher. We therefore investigated the influence of octylglucoside on the phase characteristics of  $18:1_c/18:1_c$  phosphatidylethanolamine which has a  $T_{\text{BH}}$  in the region of  $10^\circ\text{C}$  [9] as indicated in Fig. 3. In this case, detergent to phospholipid ratios of  $R = 0.4$  are required to achieve bilayer stabilization at  $37^\circ\text{C}$ , as compared to  $R = 0.2$  for egg phosphatidylethanolamine.

The results of this investigation are therefore consistent with the proposal that lipids have characteristic geometry and that the macromolecular structures adopted by pure and mixed lipid systems on hydration are dependent on these shape factors. It may be noted that the effects of the detergent appear to be distributed and do not involve the formation of detergent-phosphatidylethanolamine complexes of fixed stoichiometry. This is indicated by an increase in  $T_{\text{BH}}$  for the entire phosphatidylethanolamine dispersion in the

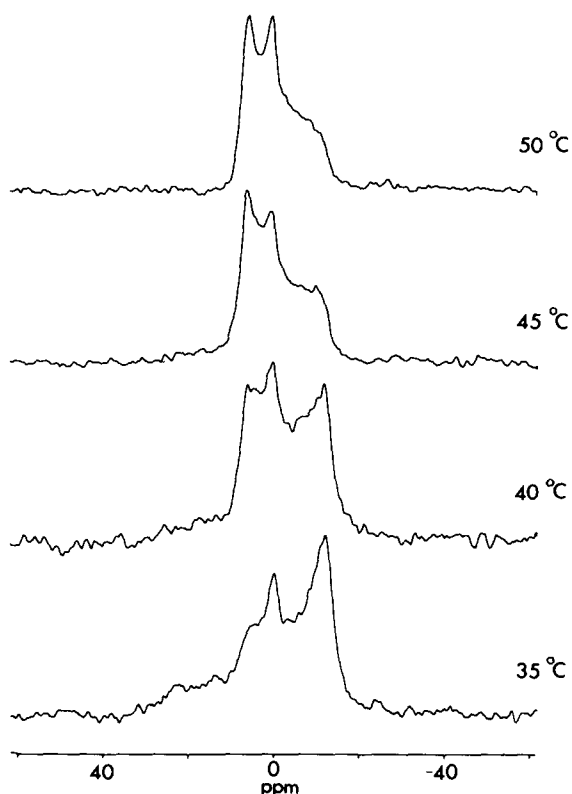


Fig. 2. 81.0 MHz  $^{31}\text{P}$ -NMR spectra of an aqueous dispersion of egg yolk phosphatidylethanolamine in the presence of sodium deoxycholate ( $R=0.05$ ) as a function of temperature. For details see Methods.

presence of detergent, as opposed to observation of a detergent dependent proportion of the phosphatidylethanolamine adopting the lamellar phase. In addition, it should be noted that while Triton X-100, octylglucoside and lysophosphatidylcholine form micelles in solution above their critical micelle concentrations sodium deoxycholate adopts a more complex structure [16]. The stabilization of phosphatidylethanolamine in a lamellar phase by this detergent may reflect its ability to intercalate between the phospholipid headgroups at the interfacial region of the bilayer thus providing an effective inverted cone shape.

Other workers have reported that lamellar structures can be obtained from non-bilayer components. These involve mixtures of lysophosphatidylcholine with cholesterol [17] and fatty acids [18]. While such behaviour is consistent with the shape hypothesis (both cholesterol [19,20] and fatty

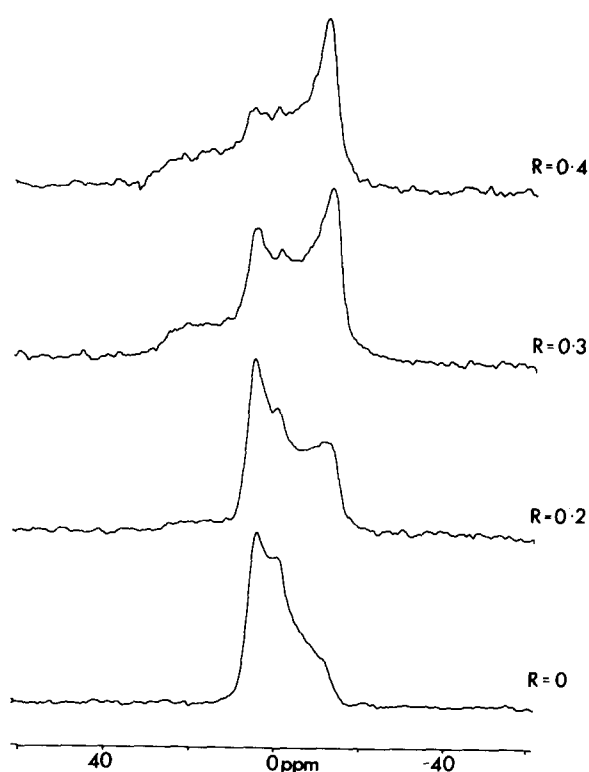


Fig. 3. 81.0 MHz  $^{31}\text{P}$ -NMR spectra at 37 °C of aqueous dispersions of 18:1<sub>c</sub>/18:1<sub>c</sub> phosphatidylethanolamine in the presence of various amounts of octylglucoside. For details see Methods.

acids [21] can induce  $H_{II}$  phase structure in previously bilayer systems, suggesting they are cone-shaped molecules) it may be more appropriate to consider that a cholesterol or a fatty acid can substitute for the missing acyl chain of lysophosphatidylcholine as suggested by the 1:1 stoichiometry involved. A further example concerns representative anaesthetics which can also stabilize the bilayer for unsaturated (egg) phosphatidylethanolamine [22].

The ability of non-bilayer lipid components to combine to form a bilayer structure suggests that the net bilayer structure of biomembranes may result from a complex interplay between protein and lipid components which may not support bilayer structure of themselves. Further, in reconstituted lipid-protein systems, particularly with  $H_{II}$  phase lipid components, residual detergent may have rather unexpected effects, serving to stabilize rather than destabilize bilayer organization.

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