Structure and Function Relationship of Phosphatidylglycerol in the Stabilization of the Phosphatidylethanolamine Bilayer†

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ABSTRACT: Differential scanning calorimetry was used to examine the structure–function relationship of the phospholipids on the Lα-phase stabilization of phosphatidylethanolamine (PE). Phosphatidylglycerol (PG) was chosen as a model stabilizer. Dielaidoylphosphatidylethanolamine (DEPE) was mixed with various PGs to study the effects of (i) chain length, (ii) chain unsaturation, and (iii) chain number of the stabilizer on the Lα-phase stabilization. At low concentrations of stabilizer, both bilayer stabilization and destabilization were observed. Phase separations also were seen, as revealed by split peaks of the Lβ → Lα transition; these were particularly prone to occur in the destabilization cases. When saturated PGs were compared, shorter chains (C12:0 and C14:0) promoted bilayer stabilization whereas longer chains (C16:0 and C18:0) promoted bilayer destabilization. Unsaturated PG with larger hydrophobic volumes (C18:2) favored bilayer destabilization, relative to unsaturated PG with smaller hydrophobic volumes (C18:1). Lyso-PG (C14:0) showed higher bilayer stabilization activity than their double-chain counterparts. Thus, at low concentrations of stabilizer, the acyl chain composition plays a vital role in bilayer-phase stabilization. However, at higher concentrations (≥8 mol %), all PGs become active bilayer stabilizers. This is probably because the increased head-group hydration becomes the dominant factor in the stabilization. The effect of acyl chain composition of the stabilizer was also studied by using small unilamellar vesicles composed of dioleoylphosphatidylethanolamine (DOPE). Fluorescence quenching of calcein entrapped in liposomes was used to monitor the stability of the liposomes. Similar acyl chain effects on liposomal stabilization were obtained. However, a higher mole percent stabilizer is required to stabilize DOPE liposomes than to stabilize the Lα phase of DEPE. Furthermore, lyso-PG, which was an active DEPE bilayer stabilizer, was a poor DOPE liposomal stabilizer. This is probably due to the coexistence of Lα and isotropic, or micellar, phases in the binary mixtures of DOPE and lyso-PG, or to a phase separation in the fluid liposome membrane.

Liposomes have long been envisioned as tools for cellular delivery due to their ability to encapsulate a variety of drugs, antibodies, enzymes, and nucleic acids [for a recent review, see Gregoriadis (1988)]. To improve the efficiency of the liposomal delivery of the macromolecules to target cells, immunoliposomes were developed [for a recent review, see Connor and Huang (1987)]. These vesicles bear the antibody specific for tumor or viral antigens on target cells and are taken up by the cells through receptor-mediated endocytosis (Straubinger et al., 1983; Huang et al., 1983). However, the eventual fate of the immunoliposome is delivery to the lysosomes and degradation of the liposome and its contents (Huang et al., 1983). In order to avoid lysosomal degradation, pH-sensitive immunoliposomes (Huang et al., 1984; Connor et al., 1984; Düzgünes et al., 1984; Ellens et al., 1984) and target-sensitive immunoliposomes (Ho et al., 1986a,b) were developed. The basic strategy of the pH-sensitive immunoliposome is to stabilize dioleoylphosphatidylethanolamine (DOPE)[1] into bilayers by addition of weakly acidic amphiphiles. At neutral pH, the liposomes are stable; however, when these liposomes enter into the acidic endosomal compartments, protonation of the amphiphilic lipid leads to liposome destabilization, thus releasing the encapsulated contents into the cytoplasm. Target-sensitive immunoliposomes are also made of DOPE but are stabilized by a fatty acid derivatized antibody (Ho et al., 1986a,b). Upon binding to the target, the liposomes are rapidly destabilized, leading to the release of the entrapped contents at the cell surface [for a review, see Collins and Huang (1988)]. Stabilization of the DOPE bilayer phase in the formation of these liposomes is crucial because pure DOPE tends to aggregate into a hexagonal phase (H_{II}) at room temperature and physiological pH (Cullis & de Kruijff, 1979). It is known that certain amphiphiles can stabilize the Lα phase of DOPE and make it possible to form stable bilayer liposomes (Cullis & de Kruijff, 1979). A main driving force of the bilayer stabilization depends on the ability of the stabilizer to increase the hydration of PE molecules. It has also been shown that the acyl chain composition of the stabilizer molecule, in particular, chain length (Tate & Gruner, 1987) and chain number (Epand, 1985), plays an important role in PE Lα-phase stabilization. Nonetheless, it is not clearly understood how the hydrophobic portion of the stabilizer can affect its Lα-phase stabilization activity. Thus, we have undertaken a systematic investigation, using PG as a model stabilizer, to study the effects of (i) chain length, (ii) chain unsaturation, and (iii) chain number of the stabilizer on the Lα-phase stabilization activity. We have used high-sensitivity differential scanning calorimetry to study the Lα-phase stabilization of DEPE by various PGs. Also, the stability of DOPE liposomes was studied by the fluorescence quenching of an entrapped dye, calcein.

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[1] Abbreviations: PE, phosphatidylethanolamine; DOPE, dioleoyl-PE; DEPE, dielaidoyl-PE; PG, phosphatidylglycerol; DLPG, dilauroyl-PG; DMPG, dimyristoyl-PG; DPPG, dipalmitoyl-PG; DSPG, distearoyl-PG; DOPG, dioleoyl-PG; DLOPG, dilinoleoyl-PG; MMPG, 2-myristoyllyso-PG; MPPG, 2-palmitoyllyso-PG; PC, phosphatidylcholine; DMPC, dimyristoyl-PC; DPPC, dipalmitoyl-PC; MMPC, 2-myristoyllyso-PC; MPPC, 2-palmitoyllyso-PC; H_{II}, hexagonal phase; PBS, phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, and 0.1 mM Na_2HPO_4); DSC, differential scanning calorimetry.

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Materials and Methods

Materials. The phospholipids were purchased from Avanti Polar Lipids (Birmingham, AL). Their purity was verified by thin-layer chromatography, and their concentration was measured by a phosphorus assay (Bartlett, 1959). Calcein and deoxycholate were obtained from Sigma (St. Louis, MO) and used without further purification.

Differential Scanning Calorimetry. An MC-2 high-sensitivity differential scanning calorimeter (Microcal Co., Amherst, MA) was used. Heating scans were done between 25 and 80 °C with a scan rate of 20 °C/h. The chain melting (T_m) and the $L\alpha \rightarrow H_{II}$ transition (T_h) temperatures for pure DEPE were 37.9 and 65 °C, respectively, when the lipid was dispersed in PBS, pH 7.4. Various PGs, ranging from 0 to 12 mol %, were mixed with DEPE. After the solvent was removed, the lipid mixture (10 mM) was hydrated in PBS at 50 °C for 3.5 h and then left overnight at room temperature for equilibration. The lipid suspensions were degassed under vacuum before being loaded into the calorimeter. T_h was the peak temperature of the transition determined by computer. It was measured with an accuracy of ±1 °C standard deviation.

Liposome Preparation for Spectrophotometry. Various PGs, ranging from 0 to 20 mol %, were mixed with DOPE in chloroform. A trace amount of hexadecyl [^{3}H]cholestanyl ether was added as a lipid marker. Chloroform was evaporated from the lipid mixture by a stream of nitrogen gas. The last traces of chloroform were removed by vacuum desiccation for at least 1 h. The dry lipid mixture was hydrated at 5 μmol/mL at room temperature for 1 h with PBS containing 50 mM calcein (390 mOsm/kg). The samples were sonicated at room temperature using a bath-type sonicator (Laboratory Supplies, Hicksville, NY) to form unilamellar vesicles. The pH of the sonicate was adjusted to 7.4 with NaOH. The liposomes were stored at 4 °C overnight before the untrapped calcein was removed by passage over a Bio-Gel A0.5M column. Liposome-containing fractions as determined by ^{3}H counting were pooled and immediately measured for calcein fluorescence.

Fluorescence Measurements. A Perkin-Elmer LS5 fluorescence spectrophotometer was used to measure the self-quenching of entrapped calcein. Lipid concentration was 50 μM. The excitation and emission slit widths were 5 and 3 nm, respectively, with λ_{ex} = 490 nm and λ_{em} = 520 nm. Percent fluorescence quenching was calculated by using the equation:

$$\% Q = (1 - F_0/F_t) \times 100$$

where F_t and F_0 are the fluorescence intensity in the presence and absence of 2% deoxycholate, respectively.

Results

High-sensitivity DSC was used to study the acyl chain effects of various PGs on the Lα-phase stabilization of DEPE. A stabilizer increases the $L\alpha \rightarrow H_{II}$ phase transition temperature (T_h) of DEPE to above 65 °C, and a destabilizer decreases the T_h (Epand, 1985; Epand & Bottega, 1987; Epand et al., 1988).

A series of saturated PGs, ranging from C12:0 to C18:0, was used to study the effect of acyl chain length on the activity of stabilizer. As shown in Figure 1, short-chain PGs (C12:0 and C14:0) were active stabilizers; there was a continuous increase in T_h when the concentration of PG was increased from 0 to 6 mol %. However, longer chain PGs (C16:0 and C18:0) showed a biphasic effect on T_h. Destabilization (decrease of T_h) was seen at low concentrations, whereas bilayer stabilization (increase of T_h) was observed at higher concentrations. The mole percent of PG at which bilayer destabilization ceased and stabilization began depended on the chain length of saturated PG. DPPG at 4 mol % had Lα-phase stabilization activity equal to that of DMPG whereas DSPG at the same concentration still promoted bilayer destabilization. The T_h values were approximately 13 °C apart from each other. Bilayer stabilization by DSPG was not observed until at least 8 mol % was included in DEPE. The relative order of Lα-phase stabilization activity of the saturated PG was therefore C12:0 > C14:0 > C16:0 > C18:0. Also, phase separations, observed as either split peaks or peak shoulders, were seen in certain $L\beta \rightarrow L\alpha$ transitions. They were found in all DLPG and DSPG scans and also in the scan of 2 mol % DPPG.

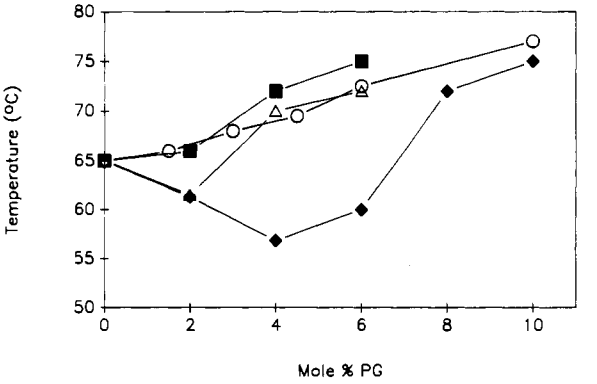

FIGURE 1: Effect of saturated PG on the $L\alpha \rightarrow H_{II}$-phase transition temperature (T_h) of DEPE. T_h values were determined by DSC and plotted vs mole percent PG. (□) DLPG; (○) DMPG; (△) DPPG; (◇) DSPG. Closed symbols indicate the existence of phase separation from the $L\beta \rightarrow L\alpha$ transition in the corresponding scans. Also, the T_h of 12 mol % DMPG in DEPE was not detected up to 85 °C.

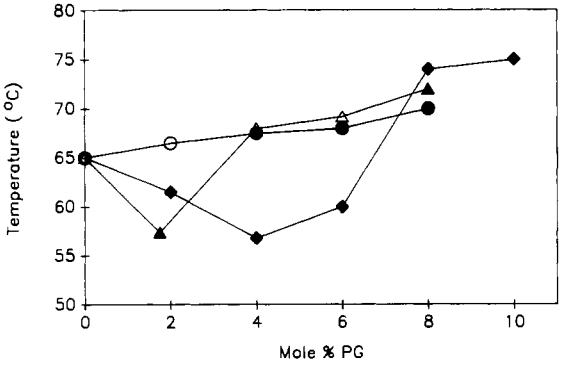

FIGURE 2: cis unsaturation effect of C18 PG on the $L\alpha \rightarrow H_{II}$-phase transition temperature (T_h) of DEPE. (○) DOPG; (△) DLOPG. Closed symbols indicate the existence of phase separation from the $L\beta \rightarrow L\alpha$ transition in the corresponding scans. Data for (◇) DSPG are also represented here for comparison.

C18:1 PG (DOPG) and C18:2 PG (DLOPG) were used to examine the role of cis unsaturation on the DEPE Lα phase. DOPG showed a continuous stabilization effect up to 8 mol %, whereas DLOPG showed a biphasic effect (Figure 2). At 1.7 mol % DLOPG, an 8 °C decrease in the T_h was obtained. However, at higher concentrations, the stabilization activity of DLOPG was indistinguishable from that of DOPG. Thus, we conclude that the degree of acyl chain unsaturation is an important factor in determining bilayer stabilization activity only at low concentrations of PG and a high level of unsaturation causes bilayer destabilization. Phase separation was observed with DLOPG at all concentrations. The effect of DSPG (C18:0) on the T_h is also shown in Figure 2 as a comparison.

We have also studied the effect of acyl chain number on Lα-phase stabilization by comparing DMPG and MMPG

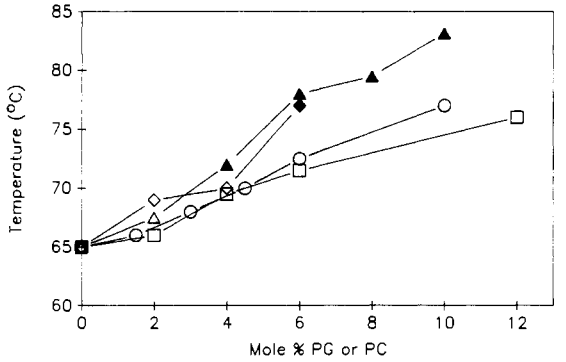

FIGURE 3: Chain number effect of PG or PC on the $L\alpha \rightarrow H_{II}$ transition temperature (T_h) of DEPE. (○) DMPG; (△) MMPG; (□) DMPC; (◇) MMPC. Closed symbols indicate the existence of phase separation from $L\beta \rightarrow L\alpha$ in the corresponding scans.

(Figure 3). Only stabilization of the DEPE $L\alpha$ phase was observed at all concentrations of both PG and lyso-PG, with lyso-PG showing a stronger activity than double-chain PG. Thus, the single-chain stabilizer has higher $L\alpha$-phase stabilization activity than the corresponding double-chain stabilizer. To confirm this observation, we have also compared the stabilization effects of DMPC and MMPC (Figure 3). Again, the lyso-PC showed a higher activity than the corresponding PC. Also apparent from the data in Figure 3 is that DMPG and DMPC showed similar stabilization effect up to 6 mol %. At higher concentrations, DMPG's stabilization activity was higher than DMPC's. For example, the $L\alpha \rightarrow H_{II}$ transition was not detectable up to 85 °C for DEPE containing 12 mol % DMPG, whereas the T_h for the corresponding sample containing DMPC at the same concentration was 76 °C. These results indicate that the acyl chain composition of the stabilizer dictates the stabilization effect at low concentrations of the stabilizer, whereas at high concentrations the head-group effect dominates the activity. Phase separations were found in both lyso-PG and lyso-PC scans.

In all cases except DSPG, the T_m of DEPE decreased with increasing concentrations of stabilizer. For instance, when 10 mol % DSPG was used, the T_m of DEPE was driven up to 40.6 °C. All PG stabilizers used have a chain melting temperature close to or lower than that of DEPE, except for DSPG whose chain melting temperature is 54.8 °C (McElhaney, 1982). Thus, the melting temperature of the stabilizer can alter the T_m of the host lipid. This means that the stabilizers used here mix well with DEPE in the majority of cases. However, broad peaks, peaks with shoulders, or even split peaks were seen in some cases (closed symbols in Figures 1–3), particularly the cases in which PG had destabilized the $L\alpha$ phase of DEPE. Nonideal mixing or phase separation was evident.

Figure 4 shows representative scans for pure DEPE, 6 mol % DLPG, DMPG, and DSPG in DEPE. DSPG showed bilayer destabilization, but DLPG and DMPG showed bilayer stabilization at this concentration. Phase separations were shown in both DLPG and DSPG scans.

The stability of DOPE-containing liposomes was also evaluated by the entrapment of calcein. Stable liposomes should have a higher degree of fluorescence quenching than unstable (or leaky) liposomes. If calcein is stably entrapped in the liposomes, its concentration inside the liposomes should be 50 mM, which yields approximately 70–80% fluorescence quenching (Ho et al., 1986a). A more active stabilizer should allow calcein trapping in the DOPE liposomes at a lower mole percent than a weaker bilayer stabilizer.

Similar to our DSC studies, acyl chain effects on liposomal stabilization were observed. As can be seen in Figure 5, all

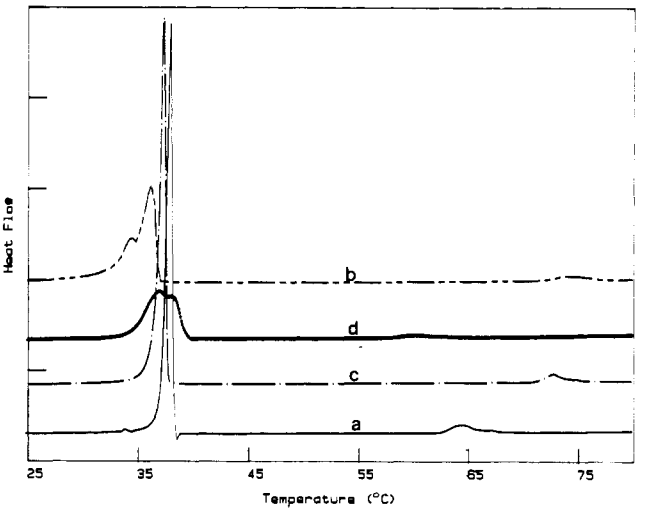

FIGURE 4: Representative DSC scans of (a) pure DEPE, (b) 6 mol % DLPG, (c) 6 mol % DMPG, or (d) 6 mol % DSPG in DEPE.

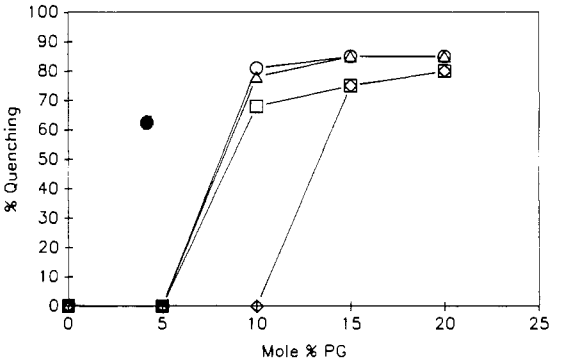

FIGURE 5: Effect of saturated PG on DOPE liposome stabilization. Percent quenching was plotted vs mole percent PG. (□) DLPG; (○) DMPG; (△) DPPG; (◇) DSPG.

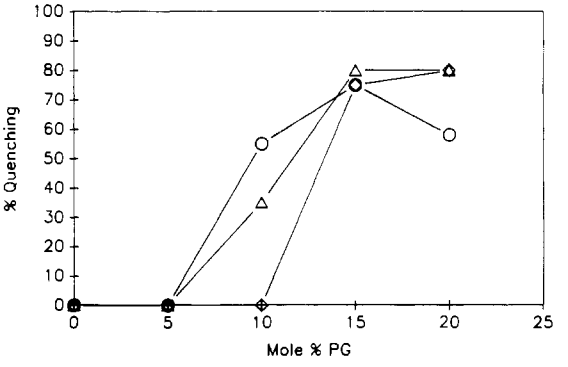

FIGURE 6: cis unsaturation effect of C18 PG on DOPE liposome stabilization. Percent quenching was plotted vs mole percent PG. (○) DOPG; (△) DLOPG. Data for (◇) DSPG are also shown here for comparison.

PGs at greater than 10 mol % stabilized the DOPE bilayers. However, different degrees of stabilization activity were observed, especially at 10 mol % PG. It was clear that DSPG did not stabilize liposomes at this concentration whereas other saturated PGs did. The relative stabilization activity was as follows: C14:0 ≥ C16:0 > C12:0 ≫ C18:0.

At 10 mol % PG, liposomes containing unsaturated PG exhibited more fluorescence quenching than those containing saturated PG (Figure 6). The stabilization activity of DOPG at 10 mol % was slightly higher than that of DLOPG.

At 10 or 15 mol % of either DMPG or DPPG, double-chain PG fully stabilized liposomes, but the lyso-PG counterparts did not (Figure 7). The sonicated mixtures of DOPE and lyso-PG at these concentrations were of low turbidity, indi-

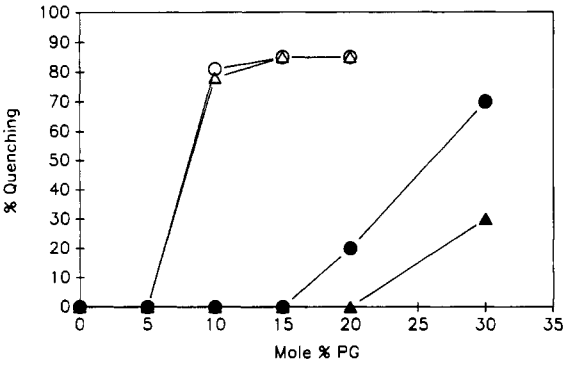

FIGURE 7: Chain number effect of PG on DOPE liposome stabilization. Percent quenching was plotted vs mole percent PG. (○) DMPG; (●) MMPG; (△) DPPG; (▲) MPPG.

cating the absence of extensive H_{II} phase. However, the mixtures did not entrap calcein. Lyso-PG began to show some stabilization activity at higher mole percent; however, the liposomes were quite leaky. The fluorescence quenching decayed to nearly 0% after 5 h at room temperature. Furthermore, the liposome suspensions were slightly foamy. This indicates the coexistence of micelles and bilayers under these conditions. This result indicates that double-chain amphiphiles are more potent liposomal stabilizers than their single-chain counterparts. To confirm this observation, DMPC, DPPC, and their corresponding single-chain counterparts were used to stabilize the DOPE liposomes (Figure 8). Again, the lyso-PC showed weaker stabilization activity than the double-chain PC. The lyso-PC-stabilized liposomes were also leaky, losing quenched calcein fluorescence in approximately 4 h. Comparing the two lyso-PCs and the two lyso-PGs, shorter chain amphiphiles exhibit a higher stabilization activity than those with longer acyl chains (Figures 7 and 8).

It is also evident that PG is a stronger stabilizer than the corresponding PC. Both DMPG and DPPG stabilized the DOPE liposomes at 10 mol %, whereas DMPC and DPPC required at least 20 mol % to show the same stabilization effect (Figures 7 and 8).

Discussion

Liposomes composed of unsaturated PE, such as DOPE, have become popular as drug delivery [for a review, see Collins and Huang (1988)] and diagnostic reagents [for a review, see Ho and Huang (1988)]. This is because of the tendency of unsaturated PE to aggregate into nonbilayer phases (Cullis & de Kruijff, 1979; Gruner et al., 1985). Nonbilayer structures such as the isotropic phase or the lipidic particles have been inferred to as intermediate structures of membrane fusion (Siegel, 1986) and seem to be important for liposomal drug delivery. Thus, it is important to understand the stabilization and destabilization of the bilayer phase of unsaturated PE. In the present study, we have examined the structural requirements of stabilizer molecules which can be combined with unsaturated PE to form stable liposomes. We have chosen PG as a model stabilizer because many structural analogues are available. High-sensitivity DSC is used to study the stabilization of the $L\alpha$ phase of DEPE. Liposomes composed of DOPE and a variety of different PGs have also been studied with calcein entrapment.

By using PG with various acyl chain structures, it is clear from both types of study that the acyl chain structure of a charged stabilizer is only important for the stabilization activity when the concentration of the stabilizer is low. At higher concentrations, all PGs become active stabilizers. This is because the H_{II}-phase formation requires close bilayer contact (Ellens et al., 1986). PE bilayers containing a relatively high concentration of a charged stabilizer cannot be closely apposed due to charge repulsion. The observation that the stabilization activities of PG and lyso-PG are higher than the corresponding PC and lyso-PC at high concentrations (Figure 3) is due to the same charge repulsion effect, since the head groups of PC and lyso-PC are zwitterionic. At low stabilizer concentrations, the acyl chain structure plays an important role in the activity of the stabilizer. Physical parameters other than the charge of the head group have to be considered.

An interesting observation is that at low mole percent, long-chain PGs such as DPPG and DSPG are actually destabilizers of the $L\alpha$ phase whereas shorter chain PGs such as DLPG and DMPG are active stabilizers at the same concentrations (Figure 1). The destabilization effect is apparently proportional to the acyl chain length since DSPG is a stronger destabilizer than DPPG. Long-chain lipids such as fatty acids and alkanes are known to stabilize the H_{II} phase of DOPE (Tate & Gruner, 1987). This is due to the fact that long-chain hydrocarbons can fill in the hydrophobic void space between the H_{II} tubes, i.e., the space near the hexagonal lattice, hence minimizing the free energy of the H_{II} phase (Tate & Gruner, 1987). The longer chain amphiphiles may also reduce the "intrinsic radius of curvature", R_0, of the H_{II} tubes (Tate & Gruner, 1987). The destabilization effect noted for DPPG and DSPG is related to this chain length effect. For the stabilization of DOPE liposomes, DSPG is also a weaker stabilizer than shorter chain PGs (Figure 5). The same chain length effect also controls the stability of the liposomes. At higher concentrations of DPPG and DSPG, the head-group hydration and/or charge overcomes the effect of acyl chain length, and both PG become active stabilizers. Such biphasic behavior of an amphiphile in stabilizing the DEPE $L\alpha$ phase has been reported for cholesterol (Epand & Bottega, 1987).

Comparing DOPG and DLOPG, it is clear that lipids with bulky, unsaturated acyl chains do not show a strong stabilization activity for the $L\alpha$ phase of DEPE (Figure 2), nor for the stability of the DOPE liposomes (Figure 6). The introduction of a cis double bond in the hydrocarbon chains not only shortens the effective acyl chain length but also increases the hydrophobic volume of the molecule. Israelachvili et al. (1980) have proposed a useful molecular shape model to determine the relative tendency of a lipid to assume the H_{II} phase. The critical parameters for the molecular shape are v/al, where v is the volume of the hydrophobic moiety of the molecule, a is the surface area of the hydrophilic head group, and l is the effective length of hydrocarbon chains. Molecules having a large value of v/al show a high tendency to aggregate into the H_{II} phase and vice versa. Comparing DSPG, DOPG, and DLOPG, the relative order for l is DSPG > DOPG > DLOPG, and the relative order for v is DLOPG > DOPG > DSPG. While a is the same for all three PGs, the relative order for the v/al value is DLOPG > DOPG > DSPG. While the theory successfully predicts that DLOPG is not as strong a stabilizer as DOPG, it fails to predict the poor stabilization activity of DSPG. Therefore, the chain length effect discussed above seems to be a more dominating consideration than the overall molecular shape effect. For the stabilization of DOPE liposomes, the relative order of DOPG > DLOPG > DSPG is again observed (Figure 6), indicating the same physical parameters also control liposome stability.

Madden and Cullis (1982) have reported that lyso-PC is a stabilizer for the $L\alpha$ phase of unsaturated PE. This is in line with the molecular shape theory, since the volume of the hydrophobic moiety of a single-chain lipid is considerably

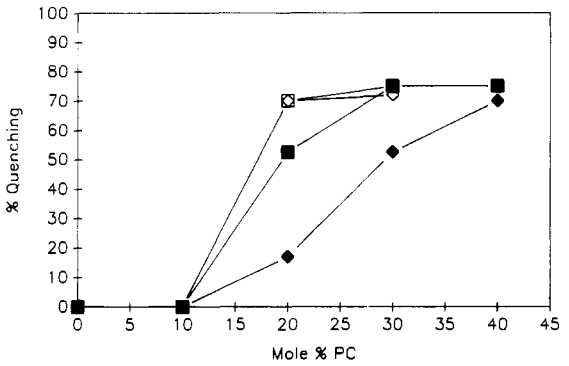

FIGURE 8: Chain number effect of PC on DOPE liposome stabilization. Percent quenching was plotted vs mole percent PC. (□) DMPC; (■) MMPC; (◇) DPPC; (◆) MPPC.

smaller than the corresponding double-chain counterpart. In fact, this observation, together with others, becomes the basis of the "shape concept" which is quite similar to the molecular shape theory of Israelachvili et al. (1980). Unsaturated PEs having a cone-shaped configuration are prone to form the H_{II} phase, whereas lysophospholipids having an inverted cone shape favor the micellar phase [for a review, see Cullis and de Kruijff (1979)]. The concept also predicts that lysophospholipid should stabilize the bilayer phase of the unsaturated PE due to a complementary effect in their shapes, because the bilayer-forming lipids are those having a cylindrical shape (Cullis & de Kruijff, 1979). Although the "shape concept" has been criticized for the lack of accuracy (Gruner et al., 1985), it adequately supports the data in Figure 3 which show a stronger $L\alpha$-phase stabilization effect for both lyso-PG and lyso-PC than for the corresponding PG and PC. What is surprising is that the opposite effect was observed for the DOPE liposome stabilization effect (Figures 7 and 8). Both lyso-PG and lyso-PC are much weaker liposome stabilizers than their double-chain counterparts over a wide range of concentrations. Even those concentrations at which liposomes could be prepared by our protocol, the liposomes were not stable because the entrapped calcein leaked out within 4–5 h at room temperature. The reason for the discrepency is not clear. One possibility is that the $L\alpha$ phase for the binary mixture of DOPE and lyso-PG or lyso-PC appears only within a narrow range of the lysophospholipid mole percent at 25 °C. Even at this $L\alpha$-phase range, isotropic, or micellar phases could be coexisting; thus, no stable liposomes entrapping calcein could be prepared. Another possibility is a phase separation in the $L\alpha$ phase of the fluid membrane of liposome; i.e., the lysophospholipids and DOPE are not mixed uniformly in the $L\alpha$ phase. Fluid-phase domains have been reported for DMPC and cholesterol binary mixtures (Recktenwald & McConnell, 1981) which could give rise to the instability of the liposomes. We have observed a phase separation in the $L\beta$ phase when lyso-PG is mixed with DEPE as indicated by a peak with a shoulder or split peaks of the $L\beta \rightarrow L\alpha$ transition. The $L\alpha \rightarrow H_{II}$ transition is a small broad peak even in pure DEPE. It would be difficult to detect any phase separation in the $L\alpha$ phase by DSC. Other methods will have to be used to check this possibility.

Another discrepancy between the data of DSC and liposome stabilization is that the liposome stabilization generally requires much higher concentrations of the stabilizer than those required by the DEPE $L\alpha$-phase stabilization. The concentrations of the stabilizer used to stabilize liposomes would generally abolish the $L\alpha \rightarrow H_{II}$ transition from the DSC scans, e.g., 12 mol % DMPG (Figures 3 and 5). This could be due to the fact that DOPE has a much higher tendency to form the H_{II} phase than DEPE (T_h for DOPE = 10 °C, T_h for DEPE = 65 °C). Thus, larger amounts of stabilizer are required to stabilize the $L\alpha$ phase of DOPE than the same phase of DEPE.

In conclusion, we have completed a systematic structure–function study on the stabilization activity of the bilayer-phase stabilizer for unsaturated PE. To have an active bilayer stabilizer, one should use a fully saturated, medium-chain (C14) lipid with a charged head group. Depending on the purpose of the investigation, a single-chain lipid may be more useful in the $L\alpha$-phase stabilization, but a double-chain lipid is more applicable in preparing stable liposomes. This information should be useful in the future designs of PE-based liposomes for drug delivery and diagnostic purposes.

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