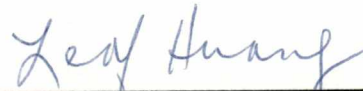


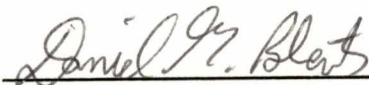
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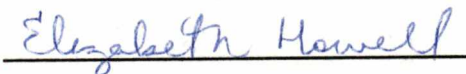
I am submitting herewith a thesis written by Ana Maria Tari entitled "Structure and function relationship of phosphatidylglycerol in the stabilization of phosphatidylethanolamine bilayer." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Biochemistry.



Leaf Huang, Major Professor

41
We have read this thesis and
recommend its acceptance:





Accepted for the Council:



Vice Provost
and Dean of The Graduate School

STRUCTURE AND FUNCTION RELATIONSHIP OF PHOSPHATIDYLGLYCEROL
IN THE STABILIZATION OF PHOSPHATIDYLETHANOLAMINE
BILAYER

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Ana Maria Tari

May 1989

DEDICATION

I would like to dedicate this thesis to my beloved family for all their support they have given me.

ACKNOWLEDGMENTS

First, I would like to express my gratitude to Dr. Leaf Huang for his patience while I was studying in his laboratory. Second, I would like to express my appreciation and thanks to my lab peers, especially David Collins for his helpful guidance. Third, I would like to thank Carolyn Drake for her word processing expertise.

Lastly, I would like to thank David Reed for his encouragement and faith in me.

ABSTRACT

Differential scanning calorimetry was used to examine the structure-function relationship of the stabilizer on the $L\alpha$ phase stabilization of phosphatidylethanolamine (PE). Phosphatidylglycerol (PG) was chosen as a model stabilizer. Dielaidoylphosphatidylethanolamine (DEPE) was mixed with various PG to study the effects of: (i) chain length, (ii) chain unsaturation and (iii) chain number of the stabilizer on the $L\alpha$ phase stabilization. At low concentration of stabilizer, both bilayer stabilization and destabilization were observed. Phase separation revealed as split peaks of the $L\beta \rightarrow L\alpha$ transition, which was particularly prone in the destabilization cases, were also seen. When saturated PG were compared, shorter chains (C12:0 & C14:0) promoted bilayer stabilization whereas longer chains (C16:0 & C18:0) promoted bilayer destabilization. Unsaturated PG with larger volume of the hydrophobic moiety (C18:2) was found to more favor bilayer destabilization than the PG with smaller volume (C18:1). Lyso PG (C14:0) showed higher bilayer stabilization activity than their double-chain counterparts. Thus at low concentrations of the stabilizer, the acyl chain composition played a vital role in PE bilayer phase stabilization. However, at higher concentration (≥ 8 mole %), all PG became active bilayer stabilizers, which was probably due to the head group hydration effect being the dominant factor in the bilayer stabilization activity. The effect of acyl chain composition of the stabilizer was also used studied by using dioleoylphosphatidylethanolamine (DOPE) liposomes prepared by sonication. 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 % of the stabilizer is required to stabilize DOPE liposomes than that required to stabilize the $L\alpha$ phase of DEPE. Furthermore, lyso PG which was active DEPE bilayer stabilizers were found to be poor DOPE liposomal stabilizers. This is probably due to the coexistence of $L\alpha$ and isotropic, or micellar, phase in the binary mixtures of DOPE and lyso PG, or due to a phase separation in the fluid liposome membrane.

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ABBREVIATIONS

PE,	phosphatidylethanolamine
DOPE,	dioleoyl PE
DEPE,	dielaidoyl PE
PG,	phosphatidylglycerol
DLPG,	dilauroyl PG
DMPG,	dimyristoyl PG
DPPG,	dipalmitoyl PG
DSPG,	disteroyl PG
DOPG,	dioleoyl PG
DLOPG,	dilinoleoyl PG
MMPG,	2-myristoyl lyso PG
MPPG,	2-palmitoyl lyso PG
PC,	phosphatidylcholine
DMPC,	dimyristoyl PC
DPPC,	dipalmitoyl PC
MMPC,	2-myristoyl lyso PC
MPPC,	2-palmitoyl lyso PC
H _{II} ,	hexagonal phase
PBS,	phosphate buffered saline (137mM NaCl, 2.7mM KCl, 1.5mM KH ₂ PO ₄ , 0.1mM Na ₂ HPO ₄)
DSC,	differential scanning calorimetry

CHAPTER I

INTRODUCTION

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 recent reviews, see Gregoriadis (1988)]. To improve the efficiency of the liposomal delivery of the macromolecules to target cells, immunoliposomes have been developed [for a recent review, see Connor & Huang, (1987)]. These vesicles bear the antibody specific for tumor or viral antigens on target cells and are then taken up by the cells through a receptor-mediated endocytosis (Straubinger et al., 1983; Huang et al., 1983). However, the eventual fate of the immunoliposomes is delivery to the lysosomes and degradation of liposome and its contents occurs (Huang et al., 1983). In order to overcome 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., 1986 a, b) have been developed. The basic components of the pH-sensitive immunoliposomes are DOPE stabilized by a weakly acidic amphiphile at neutral or basic pH. However, when these liposomes enter into the acidic endosomal compartments, protonation of the amphiphile leads to liposomal destabilization and fusion with the endosomal membrane, thus releasing the encapsulated contents into the cytoplasm. Target-sensitive immunoliposomes are also made of DOPE but stabilized by a fatty-acid derivatized antibody molecule (Ho et al., 1986 a, b). Upon

binding to the target, the liposomes are rapidly destabilized leading to the release of its contents at the cell surface [for review, see Collins & Huang (1988)]. The stabilization of DOPE bilayer phase in the formation of these liposomes is crucial because pure DOPE tends to aggregate into hexagonal phase (H_{II}) at room temperature and physiological pH (Cullis & de Kruijff, 1979). However, it is known that certain amphiphiles can stabilize the $L\alpha$ phase of DOPE and make it possible to form stable bilayer liposomes (Cullis & de Kruijff, 1979). The main driving force of the bilayer stabilization depends on the ability of the stabilizer to increase the hydration of PE molecules. However, it has also been shown that the acyl chain composition of the stabilizer molecule, such as chain length (Tate & Gruner, 1987) and chain number (Epand, 1985), may play an important role in the $L\alpha$ phase stabilization. Nonetheless it is not clearly understood how the hydrophobic portion of the stabilizer can affect its $L\alpha$ 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 $L\alpha$ phase stabilization activity. We have used high-sensitivity differential scanning calorimetry to study the $L\alpha$ phase stabilization of DEPE by various PG. Also the stability of DOPE liposomes was studied by the fluorescence quenching of an entrapped dye, calcein.

CHAPTER II

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 phosphorous assay (Bartlett, 1959). Calcein and deoxycholate were obtained from Sigma (St. Louis, MO).

Differential Scanning Calorimetry

A MC-2 high-sensitivity differential scanning calorimeter (Microcal Co., Amherst, MA) was used. Heating scans were done between 25°C to 80°C with a scan rate of 20°C/hr. The chain melting (T_m) and the $L\alpha \rightarrow H_{II}$ transition (T_h) temperatures for pure DEPE were 37.9°C and 65°C, respectively, when DEPE was dispersed in PBS, pH 7.4. Various PG, ranging from 0 to 12 mole %, were mixed with DEPE. After the solvent was removed, the lipids (10 mM) were hydrated in PBS at 50°C for 3.5 hours and then left overnight at room temperature for equilibration. The lipid suspensions were degassed under vacuum before loaded into the calorimeter.

Liposome Preparation for Spectrophotometry

Various PG, ranging from 0 to 20 mole %, were mixed with DOPE. A trace of hexadecyl [^3H]cholestanyl ether was used as a lipid marker. Chloroform was evaporated from the lipids by a stream of nitrogen gas.

Last traces of chloroform were removed by vacuum desiccation for at least one hour. Lipids were hydrated at 5 μ mole/ml at room temperature for 1 hour with PBS containing 50 mM calcein (390 mOsm/kg). They were then sonicated to form unilamellar vesicles at room temperature with a bath type sonicator (Laboratory Supplies, Hicksville, NY). The pH of the sonicate was adjusted to 7.4 with NaOH and was stored at 4°C overnight. Untrapped calcein was removed by passing through a BioGel A0.5M column and liposome fractions were pooled and immediately measured for calcein fluorescence.

Fluorescence Measurements

A Perkin-Elmer LS5 fluorescence spectrophotometer was used to measure the self-quenching of calcein entrapped in the liposomes. The excitation and emission slit widths were 5 nm and 3 nm, respectively. λ_{ex} = 490 nm and λ_{em} = 520 nm. Percent of quenching was calculated with the following equation:

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

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

CHAPTER III

RESULTS

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

A series of saturated PG, 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 Fig. 1*, short-chain PG (C12:0 & C14:0) were active stabilizers; there was a continuous increase in T_h when the concentration of PG was increased from 0 to 6 mole %. However, longer-chain PG (C16:0 & C18:0) showed a biphasic effect on T_h . A destabilization effect (decrease of T_h) was seen at low concentrations, whereas a stabilization effect (increase of T_h) was observed at higher concentrations. The mole % of PG at which the destabilization effect had turned to the stabilization effect depended on the chain length of saturated PG. DPPG at 4 mole % had stabilized the $L\alpha$ phase of DEPE as strongly as DMPG whereas DSPG at the same concentration still caused a strong destabilization effect. T_h were approximately 13°C apart from each other. The stabilization effect of DSPG was not observed until at least 8 mole % was included in DEPE. The relative order of stabilization activity of the saturated PG was therefore: C12:0 > C14:0 > C16:0 > C18:0. Also, phase separations marked as either split peaks or

* All figures may be found in the Appendix.

shoulder, 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 mole % DPPG.

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

We have also studied the chain number effect by comparing C14:0 PG and 2-C14:0 lyso PG (Fig. 3). Only stabilization of DEPE $L\alpha$ phase was observed at all concentrations of both PG and lyso PG, with lyso PG showing a stronger activity than the PG. Thus, single-chain stabilizer has a higher $L\alpha$ phase stabilization activity than the corresponding double-chain stabilizer. To confirm this observation, we have also compared the stabilization effects of C14:0 PC and 2-C14:0 lyso PC (Fig. 3). Again, the lyso PC showed a higher activity than the corresponding PC. Also apparent from the data in Fig. 3 is that DMPG and DMPC showed similar stabilization effect up to 6 mole %. At higher concentrations, DMPG's stabilization activity was higher than that of the DMPC. For example, the $L\alpha \rightarrow H_{II}$ transition was not detectable up to

85°C for DEPE containing 12 mole % 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.

Generally in all cases, the T_m of DEPE decreased with increasing concentration of stabilizer except in the case of DSPG. For instance, when 10 mole % 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 host lipid is affected by the stabilizer according to the melting temperature of the stabilizer. This means that the stabilizer mixes well with DEPE in the majority of cases. However, broad peak, peak with a shoulder or even split peaks were seen in some cases (filled symbols in Fig. 1, 2 & 3), particularly in the cases which the PG had destabilized the $L\alpha$ phase of DEPE. Non-ideal mixing or phase separation was evident.

Fig. 4 shows representative scans for pure DEPE, 6 mole % 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 liposomes was also evaluated by the entrapment of calcein in DOPE. Stable liposomes would have a higher degree of fluorescence quenching than unstable (or leaky) liposomes. If calcein is stably entrapped in the liposomes, its concentrations inside the liposomes should be 50 mM which gives approximately 70-80% fluorescence quenching (Ho et al., 1986a). An active stabilizer would stabilize the DOPE liposomes at a low mole %.

Similar acyl chain effects on liposomal stabilization were found. As can be seen in Fig. 5, all PG at greater than 10 mole % showed stabilization activities. However, different degrees of stabilization activity were observed, especially when 10 mole % PG were compared. It was clear that DSPG did not stabilize liposomes at this concentration whereas other saturated PG did. The relative stabilization activity was as follows: C14:0 $\geq$ C16:0 > C12:0 >> C18:0.

When 10 mole % PG were used, both unsaturated PG gave a higher degree of fluorescence quenching than the saturated one (Fig. 6). The stabilization activity of DOPG at 10 mole % was slightly higher than that of the DLOPG.

At 10 or 15 mole % of either C14:0 or C16:0 PG, double-chain PG fully stabilized liposomes but the lyso PG counterparts did not (Fig. 7). The sonicated mixtures of DOPE and lysoPG at these concentrations were of low turbidity, indicating 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 %; however, the liposomes were quite leaky. The fluorescence quenching decayed to nearly 0%

after 5 hours at room temperature. Furthermore, the liposome suspension was a little sudsy, indicating the coexistence of micelles under this condition. This result indicates that a double-chain amphiphile is a stronger liposomal stabilizer than the single-chain counterpart. To confirm this observation, double-chain and single-chain C14:0 and C16:0 PC were used to stabilize the DOPE liposomes (Fig. 8). Again the lyso PC showed weaker activities than the the PC. The lyso PC-stabilized liposomes were also leaky, losing quenched calcein fluorescence in approximately 4 hours. Comparing the two lyso PC and two lyso PG, shorter-chain amphiphile showed a relatively higher stabilization activity than the one with a longer chain (Fig. 7 & 8).

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

CHAPTER IV

DISCUSSION

Liposomes composed of unsaturated PE, such as DOPE, have become popular as a drug delivery [for review, see Collins & Huang, (1988)] and a diagnostic reagent [for review, see Ho & Huang, (1988)]. This is because of the high tendency of unsaturated PE to aggregate into non-bilayer phases (Cullis & de Kruijff, 1979; Gruner et al., 1985). Non-bilayer structures such as the isotropic phase or the lipidic particles are intermediate structures of membrane fusion (Siegel, 1986) which is a prerequisite of liposomal drug delivery. Thus, it is important to understand the stabilization and destabilization of the bilayer phase of the unsaturated PE. In the present study we have examined the structural requirement of a stabilizer molecule which can be combined with unsaturated PE to form stable liposomes. We have chosen PG as a model stabilizer because many structural analogs are available. High-sensitivity DSC is used to study the stabilization of the L_α phase of DEPE. Liposomes composed of DOPE and a variety of different PG 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 PG become active stabilizers. This is because the H_{II} phase formation requires close bilayer contact (Ellens et al., 1984). PE bilayers

containing a relatively high concentration of a charged stabilizer cannot come to a close contact 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 (Fig. 3) is due to the same charge repulsion effect, since the head group of PC and lyso PC is 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 the long-chain PG such as DPPG and DSPG at low mole % are actually destabilizers, whereas the shorter-chain PG such as DLPG and DMPG are active stabilizers at the same concentrations (Fig. 1). The destabilization effect is apparently proportional to the 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 and Gruner, 1987). This is due to the fact that long chain hydrocarbons can fill in the 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 and Gruner, 1987). The longer chain amphiphiles may also reduce the "intrinsic radius of curvature", R_o , of the H_{II} tubes (Tate & Gruner, 1987). The destabilization effect of DPPG and DSPG must be related to this chain length effect. For the stabilization of DOPE liposomes, DSPG is also a weaker stabilizer than the other shorter-chain PG (Fig. 5). The same chain length effect must also control the stability of the liposomes. At

higher concentrations of DPPG and DSPG, the headgroup hydration effect 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 (Fig. 2), nor for the stability of the DOPE liposomes (Fig. 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 is 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 PG, 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 liposome, the relative order of DOPG > DLOPG > DSPG is again observed (Fig. 6), indicating the same physical parameters also control the liposome stability.

Madden and Cullis (1982) have reported that lyso PC is a stabilizer for the L_α 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 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 PE having a cone-shaped configuration are prone to form H_{II} phase, whereas lysophospholipids having an inverted-cone shape favor the micellar phase [for review, see Gruner et al., (1985)]. 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; Gruner et al., 1985). It is thus not surprising that the data in Fig. 3 indicate a stronger L_α phase stabilization effect for the lyso PG and lyso PC than the corresponding PG and PC. What is surprising is that the opposite effect was observed for the DOPE liposome stabilization effect (Figs. 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 hours at room temperature. The reason for the discrepancy is not clear. One possibility is that the $L\alpha$ phase for the binary mixture of DOPE and lysoPG or lysoPC only appears within a narrow range of the lysoPG or lysoPC mole% 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 mole % DMPG (Figs. 3 and 5). This could be due to the fact that DOPE has a much higher tendency to form 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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LIST OF REFERENCES

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APPENDIX

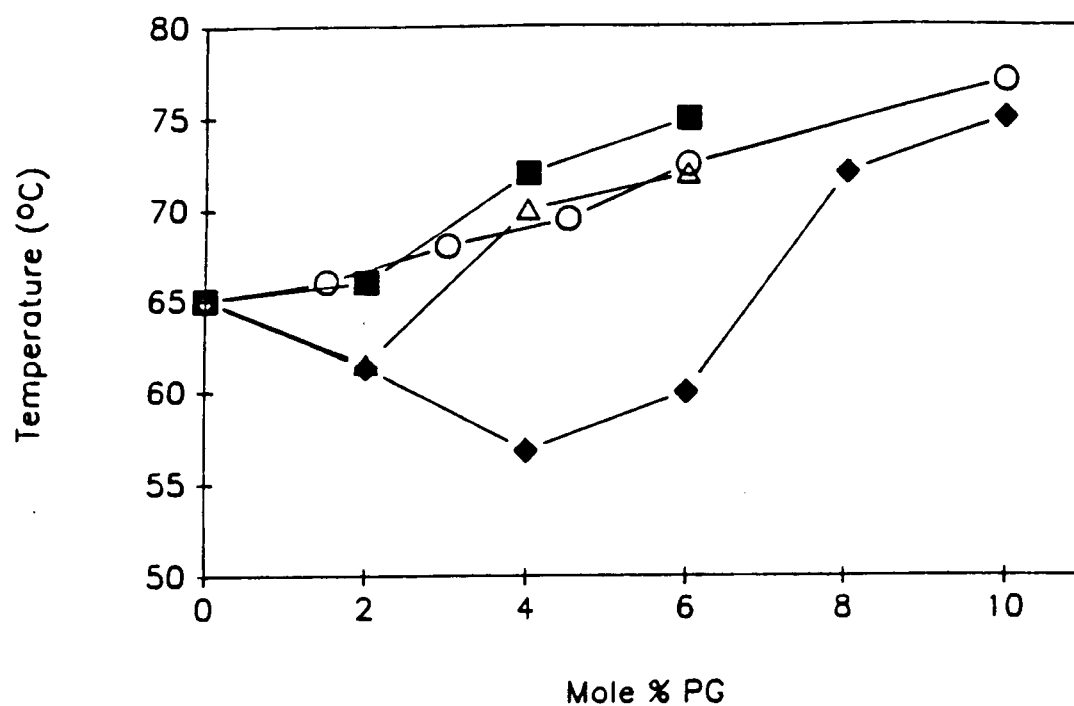


Figure 1. Chain length effect on the $L\alpha \rightarrow H_{II}$ phase transition temperature (T_h) of DEPE. T_h were determined by DSC and plotted vs mole % PG. Symbols: ($\square$) DLPG, ($\circ$) DMPG, ($\triangle$) DPPG, ($\diamond$) DSPG. Filled symbols indicate the existence of phase separation from $L\beta \rightarrow L\alpha$ transition in the corresponding scans. Also the T_h of 12 mole % DMPG in DEPE was not detected up to 85°C.

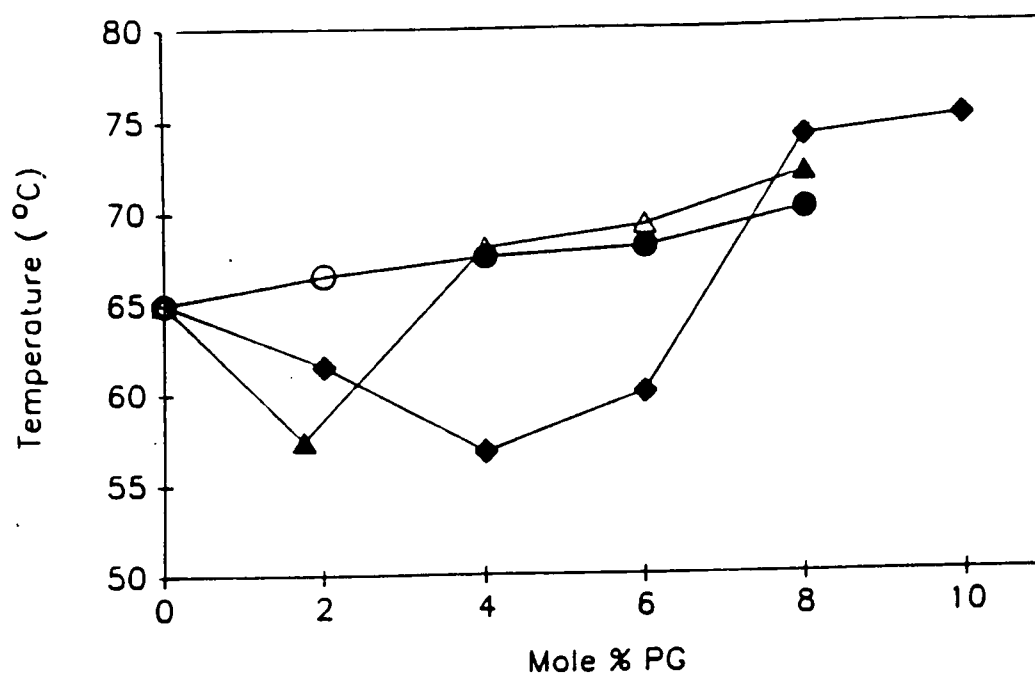


Figure 2. Cis-unsaturation effect of C18PG on the $L\alpha \rightarrow H_{II}$ phase transition temperature (T_h) of DEPE. Symbols: (○) DOPG, (△) DLOPG. Filled symbols indicate the existence of phase separation from $L\beta \rightarrow L\alpha$ transition in the corresponding scans. Data for (◇) DSPG are also represented here for comparison.

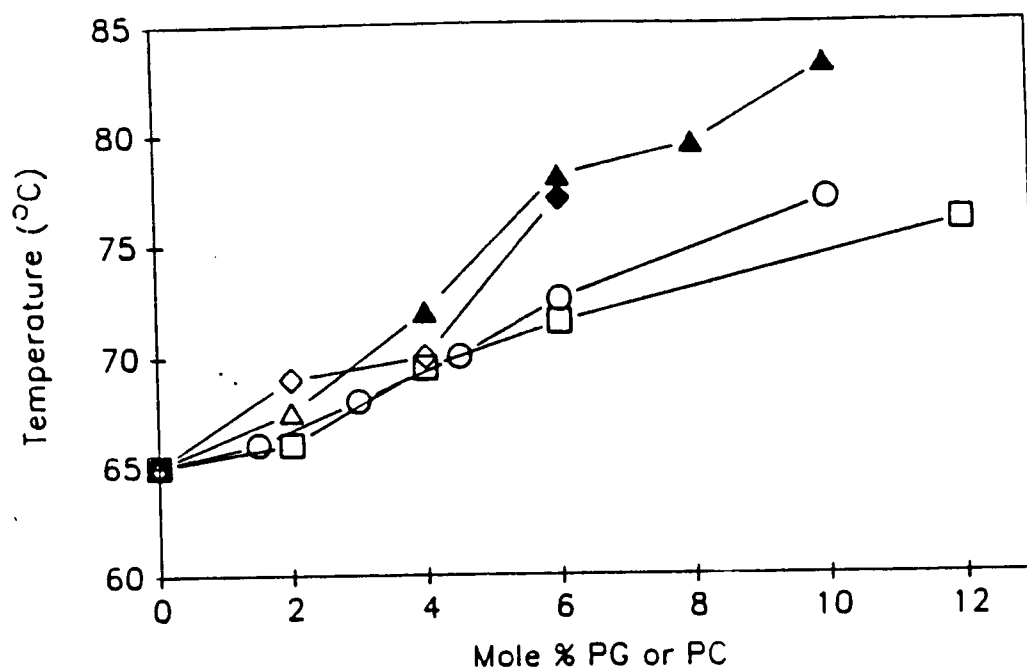


Figure 3. Chain number effect of PG or PC on the $L\alpha \rightarrow H_{II}$ transition temperature (T_h) of DEPE. . Symbols: ($\circ$) DMPG, (Δ) MMPC, ($\square$) DMPC, ($\diamond$) MMPC. Filled symbols indicate the existence of phase separation from $L\beta \rightarrow L\alpha$ in the corresponding scans.

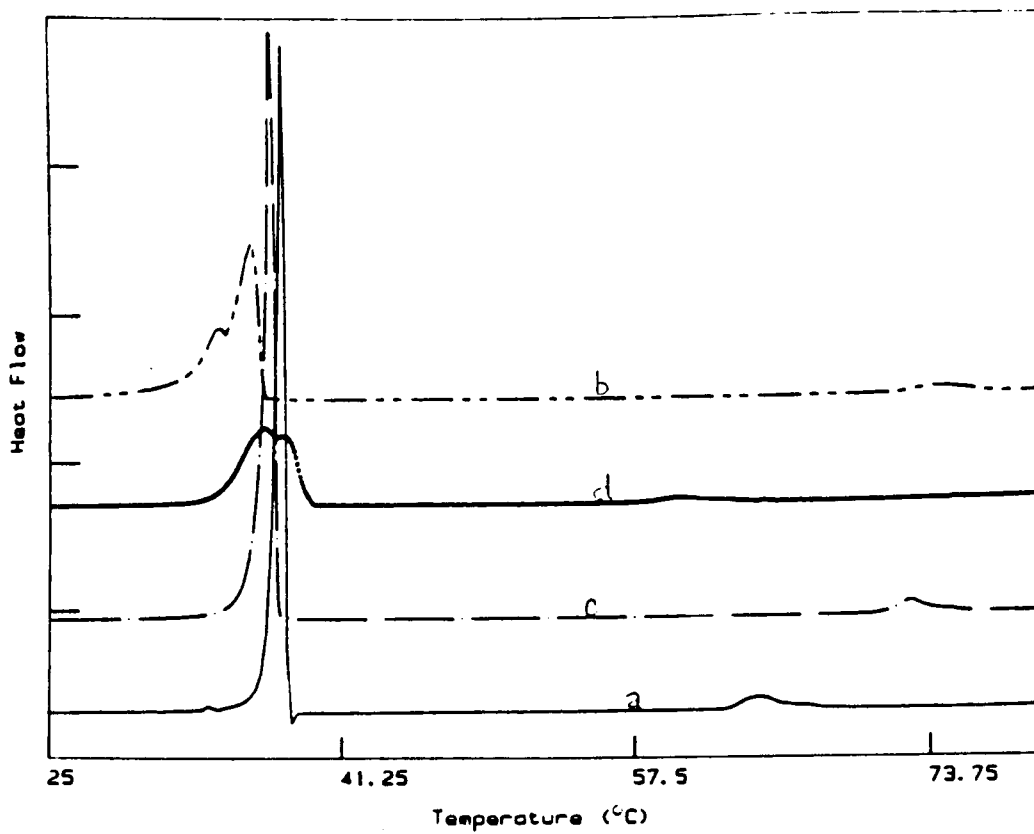


Figure 4. Representative DSC scans of (a) pure DEPE, (b) 6 mole % DLPG, (c) 6 mole % DMPG or (d) 6 mole % DSPG in DEPE.

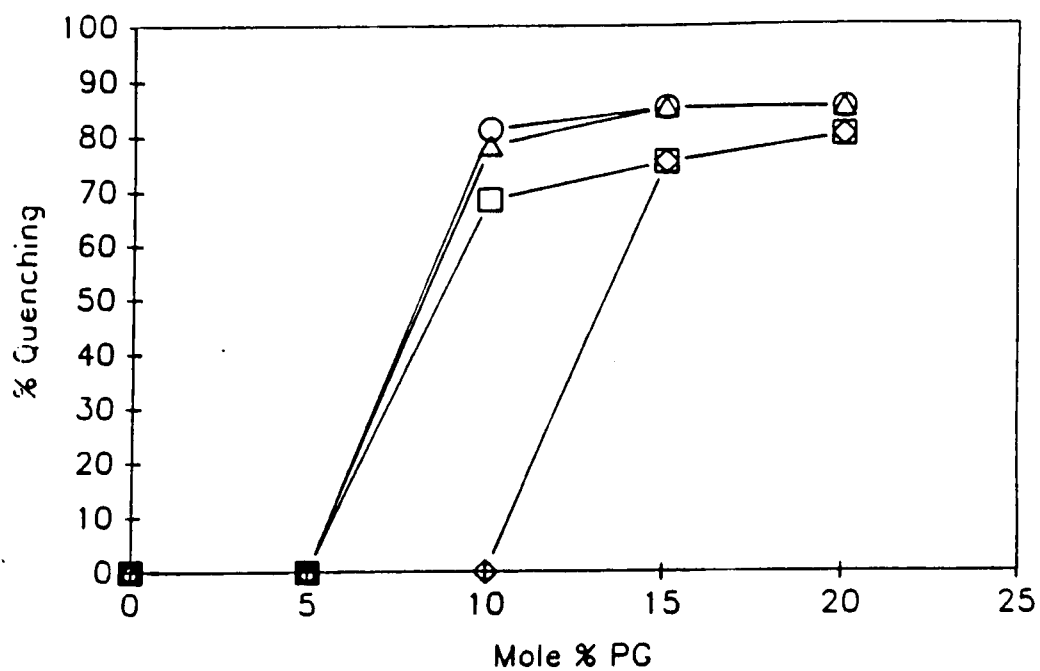


Figure 5. Chain length effect on DOPE liposome stabilization. % quenching was plotted vs mole % PG. Symbols: ($\square$) DLPG, ($\circ$) DMPG, ($\triangle$) DPPG, ($\diamond$) DSPG.

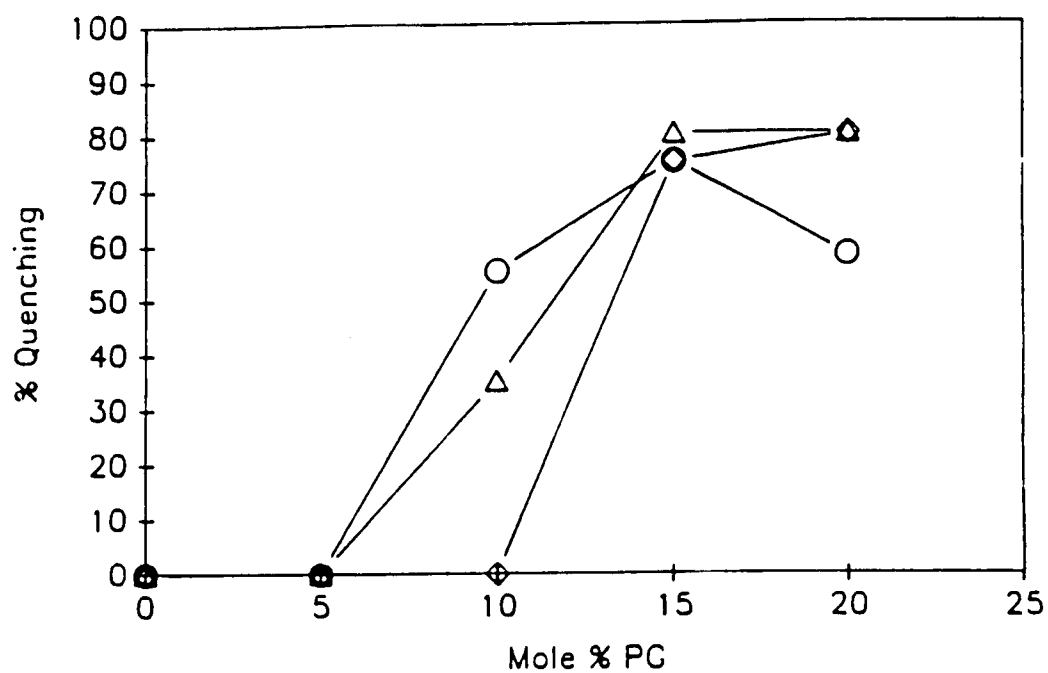


Figure 6. Cis-unsaturation effect of C18PG on DOPE liposome stabilization. % Quenching was plotted vs mole % PG. Symbols: ($\bigcirc$) DOPG, (Δ) DLOPG. Data for ($\diamond$) DSPG are also shown here for comparison.

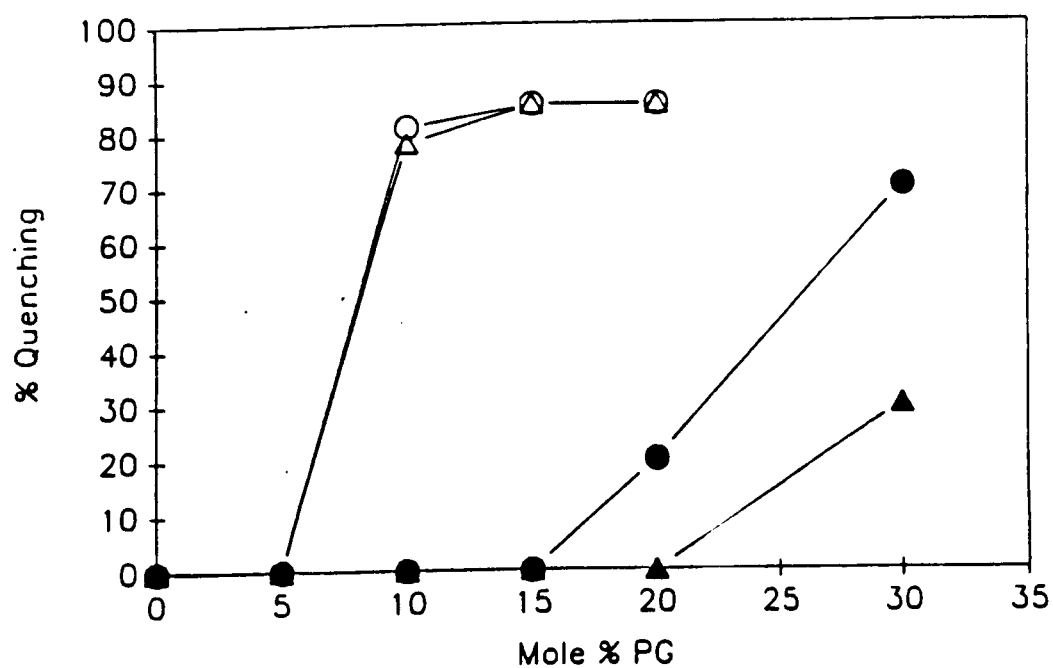


Figure 7. Chain number effect of PG on DOPE liposome stabilization. % Quenching was plotted vs mole % PG. Symbols: (O) DMPG, (●) MMPG, (△) DPPG, (▲) MPPG.

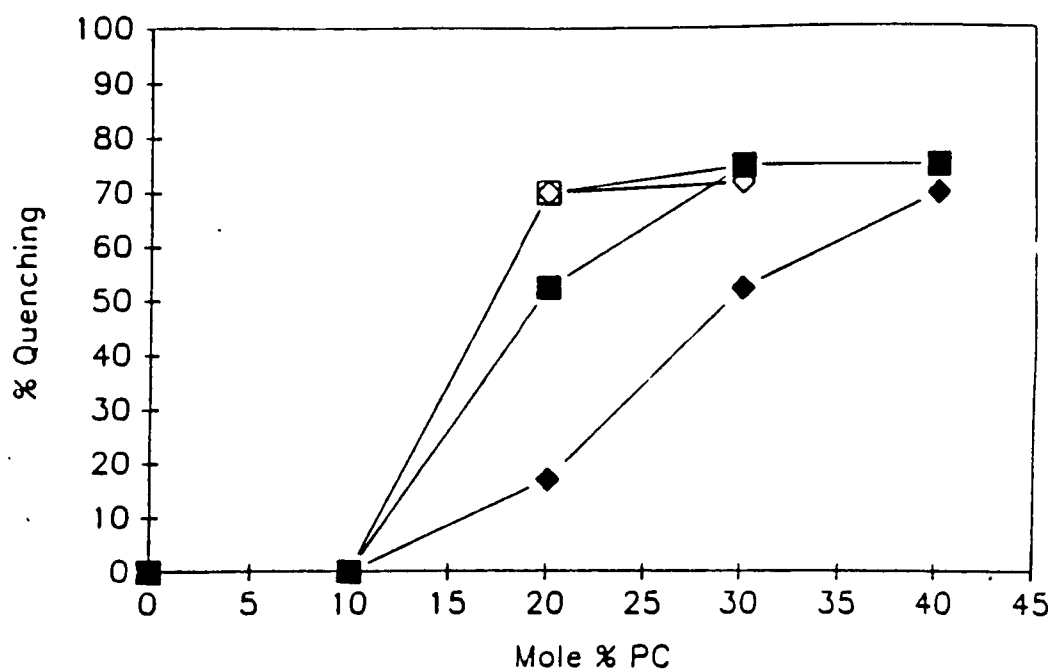


Figure 8. Chain number effect of PC on DOPE liposome stabilization. % Quenching was plotted vs mole % PC. Symbols: ($\square$) DMPC, ($\blacksquare$) MMPC, ($\diamond$) DPPC, ($\blacklozenge$) MPPC.

VITA

Ana Maria Tari was born in Macao on July 13, 1968. After her high school graduation, she came to the United States to further her education. She received her Bachelor of Science in Chemistry and Mathematics from Lincoln Memorial University in June 1984.

She then enrolled in the University of Tennessee, Knoxville. In May of 1989, she received the Master of Science in Biochemistry.