brief communication

On the modulation of a high-enthalpy pretransition in binary mixtures of DMPC and DMPG by polar headgroup interaction

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ABSTRACT Employing high-sensitivity differential scanning calorimetry (DSC), we discovered a pretransition in binary mixtures of dimyristoyl phosphatidylcholine and dimyristoyl phosphatidylglycerol, the main feature of which is its extraordinarily high transition enthalpy of 6.3 Kcal/mol, nearly an order of magnitude higher than those values previously found for such transitions. Using DSC, deuterium nuclear magnetic resonance, and electron microscopy, it is shown that the energetic origin of this type of pretransition is caused by interactions between the phospholipids in their headgroup region. The most likely interaction involves the formation of a hydrogen bond between the headgroups of the two phospholipid species in the gel ($L_{\beta'}$) phase which is disrupted at the transition to the "ripple" ($P_{\beta'}$) phase. The finding that this large pretransition is unique for mixtures of phosphocholine and phosphoglycerol with myristoyl chains indicates a dependence of the headgroup long range order of such mixtures in the gel phase on the acyl chain length.

INTRODUCTION

Binary mixtures of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and 1,2-dimyristoyl-*sn*-glycero-3-phospho-rac-glycerol (DMPG) exhibit nearly ideal mixing behavior over the whole composition range (Findlay and Barton, 1978) and, in contrast to most other saturated phospholipid mixtures, retain a pretransition, detectable by high-sensitivity differential scanning calorimetry (DSC).

This pretransition represents the transformation of smooth, flat bilayers with the acyl chains tilted and in an all-*trans* conformation ($L_{\beta'}$ phase) to the $P_{\beta'}$ phase which exhibits the well known ripple structure and a reduction of the chain tilt angle (Janiak et al., 1976; Tardieu et al., 1973; Rand et al., 1975). In contrast to the extensively studied main phase transition ($P_{\beta'}$ to L_α transition) the energetic origin of the pretransition is only poorly understood. It is generally assumed that the pretransition enthalpy (which amounts to ~10% of the main transition enthalpy) results from acyl chain tilt changes and alterations of the order in the phospholipid headgroup region (Janiak et al., 1976; Mabrey and Sturtevant, 1978).

In this study we demonstrate that in binary mixtures of DMPC and DMPG the pretransition enthalpy can be as high as that of the main transition and can be modulated by the composition and the pH value. The data provide evidence that headgroup interactions can be crucial for the characteristics of the transition from the $L_{\beta'}$ to the $P_{\beta'}$ phase. This work is complementary to a monolayer study of DMPC/DMPG mixtures using the technique of specular reflection of neutrons (Bayerl et al., manuscript submitted for publication).

MATERIALS AND METHODS

All phospholipids were obtained from Avanti Polar Lipids, Inc. (Birmingham, AL) and were used without any further purification.

For all multilamellar vesicle preparations, the phospholipids were weighed at the corresponding proportion into glass test tubes, dissolved in chloroform (2 mg/ml), dried under a stream of nitrogen followed by overnight vacuum desiccation. The lipid film was then redispersed in buffer solution at a concentration of 2 mg/ml and incubated at 50°C for 30 min under gentle vortexing. After that, the tube with the lipid dispersion was transferred to a water bath with a temperature of 4°C and incubated for 1 h at this temperature before the measurements. Longer incubation times (up to 4 h at 50°C and 36 h at 4°C) did not affect the results.

For all experiments except the pH variation study, the lipid mixtures were dispersed in a 50-mM Hepes buffer, pH - 7.5, and 2 mM EDTA. For samples of pure DMPG this buffer contained additionally 50 mM NaCl. For ^{2}H-NMR measurements, deuterium depleted water obtained from Sigma Chemical Co. (St. Louis, MO) was used for the buffer preparation and the DMPC-d_{54} concentration was 40 mg/ml.

For the DSC experiments performed as a function of the pH value, the following buffers were used in the pH range given in parentheses: 50 mM Mes Buffer (pH 5.2–6.7); 50 mM Hepes buffer (pH 6.3–7.9); 50 mM Tris buffer (pH 7.4–9.0); phosphate buffer (pH 7.0–8.0); carbonate buffer (pH 9.2–10.9). Each buffer contained 2 mM EDTA.

DSC measurements

High-sensitivity DSC measurements were performed with an MC-2 microcalorimeter (MicroCal, Inc., Amherst, MA) as reported elsewhere (Bayerl et al., 1988).

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The samples were transferred from the 4°C cooling bath into the sample cell which was preequilibrated at this temperature. After an additional 30 min equilibration time the heating scan was started with a scan rate of 20°C/h and a 15-s time increment (filter constant) between each data acquisition. Control measurements were also performed at a 10°C/h scan rate and gave identical results.

^{2}H-NMR measurements

Deuterium NMR experiments were performed at 46 MHz using a home built NMR spectrometer described by Sternin (1985). All spectra were obtained using the quadrupolar echo technique with a pulse separation of 50 μs and two 90° pulses of 4 μs duration. At a spectral width of 250 KHz 10,000 scans were accumulated. All experiments were done on resonance with an eight cyclops pulse cycling sequence and no phase corrections were performed. The temperature was varied in an ascending manner in 1°C steps (20 min equilibration time between each step), controlled by a temperature control unit (Bruker Instruments, Inc., Billerica, MA).

Electron microscopy

Three samples were prepared for the microscopy experiments: pure DMPC (pH 7.5, Hepes buffer) and equimolar DMPC/DMPG mixtures at pH 5.2 (Mes buffer) and at pH 7.5 (Hepes buffer). Small droplets (0.5–1.0 μl) of the lipid/water mixture deposited on gold planchettes were frozen in liquid propane, cooled by liquid nitrogen from two temperatures which corresponded to the $L_{\beta'}$ phase (T_{cryo} = 10°C) and the $P_{\beta'}$ phase (T_{cryo} = 20°C). The samples were processed in a Balzers 400 T freeze-etch device (etch time 1 min) and replicated by evaporating 1.5 nm platinum carbon mixture from an electrode at a 45° angle followed by a 15-nm thick film of carbon at a 90° angle to increase the mechanical stability of the replica. The replicas were examined in an electron microscope (model 1200 EX; JEOL USA, Electron Optics Div., Peabody, MA).

RESULTS

Fig. 1 shows two hs-DSC endotherms of multilamellar vesicles (MLV) of an equimolar mixture of DMPC and DMPG. The pretransition of this binary mixture exhibits an enthalpy of ΔH_{pre} = 6.3 kcal/mol, which is similar to that of the main transition (ΔH_m = 6.5 kcal/mol). However, this extraordinarily large pretransition can only be observed for the first heating scan of the sample. A second heating scan performed after cooling the sample in the calorimeter from 30°C down to 4°C and restarting it 1.5 h after the end of the first scan gives an endotherm with unchanged main transition (ΔH_m = 6.5 kcal/mol) but without any pretransition (Fig. 1, dotted endotherm). However, prolonged incubation of this sample at 4°C for at least 36 h before the DSC scan restores the large pretransition completely. In contrast, stopping the first scan right after the completion of the pretransition (i.e., at 18.2°C for an equimolar mixture) and doing the second scan under the same conditions as described above, gives an identical high enthalpy pretransition as observed for the first scan. This demonstrates the sensitivity of this particular pretransition about incubation of the sample in the main phase transition temperature region.

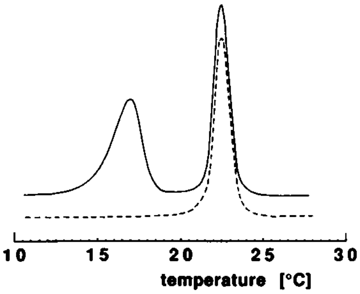

FIGURE 1 DSC endotherms of an equimolar DMPC/DMPG mixture at pH 7.5 (Hepes buffer). Upper trace: first heating scan; lower (*dotted*) trace: second heating scan obtained shortly after the first scan (see text).

The pure components of this mixture exhibit under identical conditions (pH and ionic strength) endotherms with pretransition enthalpies ΔH_{pre} which are nearly an order of magnitude lower than that for the mixture (DMPC: ΔH_{pre} = 0.85 kcal/mol, ΔH_m = 6.5 kcal/mol: DMPG: ΔH_{pre} = 0.80 kcal/mol, ΔH_m = 6.3 kcal/mol) and do not change for repeated scanning.

The disappearance of the pretransition of the mixture after the first scan is probably caused by the spontaneous transition of the MLV to smaller, rather unilamellar vesicles upon slowly traversing the main phase transition of the mixture (T_m = 23.6°C). This instability of DMPC/DMPG mixtures when incubated near T_m was recently reported by Madden et al. (1988). A comparison of electron microscopy replicas of the mixture taken before and after the first scan shows that the MLV with initial diameters of 0.5–3 μm spontaneously transform to vesicles with an average diameter of 150 nm after slowly traversing T_m. In contrast, micrographs taken from samples where the scan was stopped right after the completion of the pretransition give no indication for a disruption of the MLV structure.

For this reason, all experiments reported below were conducted with new samples for each scan and special care was exercised to avoid incubating the samples near T_m before the DSC measurements.

Fig. 2 shows a plot of the pre- and main transition enthalpies (ΔH_{pre} and ΔH_m) of DMPC/DMPG mixtures as well as their pretransition temperatures (T_{pre}) as a function of the composition. It is obvious that ΔH_{pre} depends drastically on the proportion of the components and reaches a maximum at equimolar mixture whereas ΔH_m does not depend on this parameter. Interestingly, the pretransition temperature T_{pre} depends in the same manner on the composition as ΔH_{pre}, whereas the main

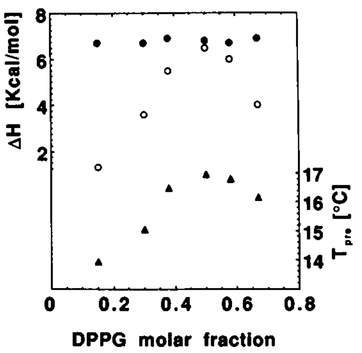

FIGURE 2 Plot of the enthalpy of the pretransition, ΔH_{pre} (○), and of the main transition, ΔH_m (●) as well as of the pretransition temperature, T_{pre} (▲), as a function of the composition of the DMPC/DMPG mixture at pH = 7.5 (Hepes buffer).

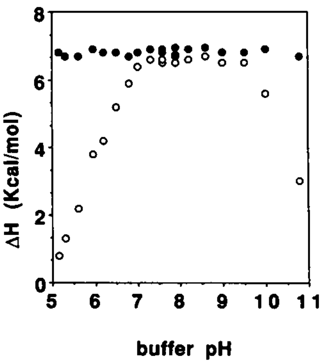

FIGURE 3 Plot of the enthalpy of the pretransition, ΔH_{pre} (○), and of the main transition, ΔH_m (●), of an equimolar DMPC/DMPG mixture as a function of the pH value of the buffer. Multiple data points at the same abscissa value around pH = 8.0 are due to the use of different buffers; see Materials and Methods for a complete listing of the buffers used.

transition temperature $T_m = 23.6 \pm 0.3$°C is constant for all compositions. These measurements were performed up to a molar fraction of DMPG $X_{DMPG} = 0.7$ in the mixture. Measurements at $X_{DMPG} > 0.7$ are obscured by sample instability and were not considered.

The reported behavior of the pretransition enthalpy is largely independent of the ionic strength of the buffer medium, as an increase of the NaCl concentration up to 0.5 M does not change the initial value of ΔH_{pre}. As the two phospholipids possess identical chain lengths and differ in their structure of the headgroups only, the dependence of ΔH_{pre} on the composition indicates that headgroup interactions might be the origin of the excess pretransition enthalpy. Therefore, hs-DSC measurements of an equimolar mixture of DMPC and DMPG were performed as a function of the pH value of the MLV dispersion. The results demonstrate a striking dependence of ΔH_{pre} on the pH value in the pH range 5.0–10.0 with a maximum of ΔH_{pre} between pH = 7.0–9.5 (Fig. 3). At pH = 5.0 the value of $\Delta H_{pre} = 0.9$ kcal/mol is comparable with that obtained for pure DMPC. As DMPC is insensitive for pH changes in the range studied (its pK value of the phosphate group [Eibl and Woolley, 1979] is <2), the modulation of ΔH_{pre} of the mixture is likely to be caused by changes of the headgroup interaction of the negatively charged DMPG.

Electron microscopy pictures of the replicas of these mixtures at pH = 5.2 and 7.5, respectively, frozen from 10°C show for both pH values multilamellar vesicles with smooth surfaces and with diameters between 0.3 and 4.0 μm, consistent with an $L_{\beta'}$ phase. Those samples frozen from 20°C exhibit for both pH values the well known ripple phase ($P_{\beta'}$) with two ripple wavelengths: 13 ± 3 nm and 25 ± 4 nm, respectively (pictures not shown). A comparison with a pure DMPC sample frozen under similar conditions reveals no morphological differences between the former and the binary mixtures of DMPC/DMPG, neither in the $L_{\beta'}$ phase nor in the $P_{\beta'}$ phase.

The same pretransition characteristics as reported above can be observed when DMPC is replaced by its acyl chain perdeuteriated analogue, DMPC-d_{54}. The only difference is the pretransition temperature, which is 2.7°C lower than for the corresponding 1:1 mixture of nondeuteriated phospholipids.

To obtain information about the order of the acyl chains in the pretransition region, ^{2}H-NMR measurements were conducted in the 4–18°C temperature range. An equimolar mixture of DMPC-d_{54}/DMPG at pH 7.5 was studied and compared with a sample of pure DMPC-d_{54}. The ^{2}H-NMR spectra exhibit the well known gel phase features reported by Davis (1979) and are similar for the mixture and for pure DMPC-d_{54} (spectra not shown). As the first moment M_1 of these spectra represent a measure of the mean of the distribution of the order parameters (cf. Davis, 1979, for discussion of the ^{2}H-NMR spectral moments), M_1 is plotted versus the temperature for both samples in Fig. 4. M_1 decreases with increasing temperature in the same way, indicating a decrease of the average quadrupolar splitting due to an increasing reorientation rate of the molecules about their long axes. The fact that M_1 exhibits a very similar behavior for both samples even in the pretransition range effectively rules out any considerable contributions of the acyl chains to the large pretransition observed for the mixture. This result is in good agreement with the DSC results, which stressed the significance of the headgroup interactions for the pretransition enthalpy. However, it should be emphasized that the reported large pretransition seems to be a unique feature of mixtures with myristoyl chains. In equimolar (DPPC/DPPG) and mix-

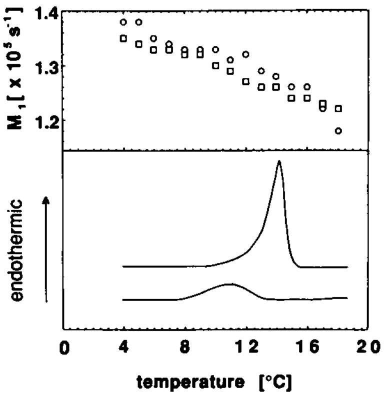

FIGURE 4 Deuterium NMR first moments (M_1) of an equimolar DMPC-d_{54}/DMPG mixture (□) and of pure DMPC-d_{54} (○) at pH = 7.5 (Hepes buffer) as a function of the temperature in the pretransition temperature range. The corresponding two DSC endotherms of these samples are shown in the lower half of the figure (*upper trace*: DMPC-d_{54}/DMPG; *lower trace*: DMPC-d_{54}).

tures only the normal pretransitions in the order of ΔH_{pre} = 1 kcal/mol can be observed.

DISCUSSION

The most surprising result of this study is the existence of a pretransition in binary mixtures of DMPC and DMPG which exhibits an enthalpy ΔH_{pre} similar to that of the main transition. It is shown that this pretransition (*a*) can be modulated in its enthalpy ΔH_{pre} by the composition of the mixture and by the pH value of the buffer medium and reaches a maximum ΔH_{pre} = 6.3 kcal/mol at equimolar composition and pH = 7.5; (*b*) is largely independent in its enthalpy ΔH_{pre} of the NaCl concentration in the buffer medium; (*c*) does not change the order of the fatty acyl chains as compared with pure DMPC-d_{54}, as viewed from the ^{2}H-NMR first moments; (*d*) is a unique feature of a binary mixture with myristoyl chains while longer chained mixtures with palmitoyl or stearoyl chains do not show this behavior.

The results of the present work provide evidence that in addition to the 'normal' pretransition, a second more powerful mechanism exists in DMPC/DMPG mixtures giving rise to an up to sevenfold higher value of ΔH_{pre}. The origin of this 'anomalous' pretransition is most likely a drastic change of the headgroup interactions. This is demonstrated by the reported pH dependence of ΔH_{pre} (Fig. 3) as well as by its composition dependence. The latter shows a correlation between ΔH_{pre} and T_{pre} which both reach a maximum at equimolar mixture (Fig. 2). Hence, the increase of the DMPG component in the binary mixture up to equimolar ratio stabilizes the $L_{\beta'}$ phase. Obviously, the presence of DMPG establishes a headgroup interaction between the two components of the mixture in the $L_{\beta'}$ phase, which prevents the formation of a ripple ($P_{\beta'}$) phase until sufficient energy is provided to break this interaction. The energy barrier is an order of magnitude higher than for any pretransition reported yet. As the effect is largely independent on the NaCl concentration, electrostatic interactions between the single negatively charged DMPG headgroups and the zwitterionic DMPC headgroups are unlikely to account for this behavior. Moreover, the finding that the large pretransition can be reproduced in consecutive scans under conditions that the scans do not extend beyond the completion temperature of this pretransition, rules out that this feature reflects the energy required for the disruption of the MLV structure. Similarly, due to the above mentioned reproducibility of the large pretransition it appears unlikely that this feature is caused by a subtransition which can be observed for pure DMPC dispersions (Finegold and Singer, 1986; Lewis et al., 1987). The formation of the Lc phase requires at least 3 d of annealing of the DMPC sample at low temperature whereas the large pretransition can be reproduced 1 h after the first scan. Hence, three other mechanisms which could account for this pretransition are open for discussion: (*a*) Stereochemical effects due to the chirality of the DMPG headgroup, resulting in a long range ordering of the headgroups in the $L_{\beta'}$ phase. (*b*) A bilayer interdigitation in the $L_{\beta'}$ phase due to the presence of DMPG. (*c*) The formation of a hydrogen bond between the DMPC and DMPG headgroups in the $L_{\beta'}$ phase.

Stereochemical effects are well known to affect the pretransition temperature of DMPC (Eklund et al., 1984) and the chirality of the DMPG headgroup gives rise to different Na$^+$ affinities of the two enantiomers (Eklund et al., 1987). The DMPG used in this study is a racemic mixture of both headgroup enantiomers. Although the formation of two different long range ordered headgroup conformations in the $L_{\beta'}$ phase is conceivable, this can hardly explain the high pretransition enthalpy and the dependence of T_{pre} on the composition. It would also be in contradiction to the observed independence of ΔH_{pre} on the NaCl concentration.

The formation of interdigitated bilayer structures, which were reported for pure DPPG dispersed in Tris buffer (Wilkinson et al., 1987) and for DPPG in the presence of some proteins (for review see Slater and Huang, 1988), is unlikely to account for the reported behavior. Interdigitation tends to suppress the pretransition measured by DSC. Moreover, the observed maximum ΔH_{pre} at pH 7.5 does not depend on the presence of Tris buffer but can be obtained with phosphate buffer and Hepes buffer as well.

The headgroup of DMPG is well known for its potential to form hydrogen bonds (Boggs, 1987; Pascher et al., 1987). The assumption of formation of an H bond between the terminal C-OH group of DMPG and the phosphate group of DMPC in the $L_{\beta'}$ phase is the most unambiguous and easiest explanation for the present results. This rather strong binding with an average lifetime of 10^{-6}–10^{-10} s is sufficiently strong to stabilize the $L_{\beta'}$ phase against the $P_{\beta'}$ phase. The enthalpy of association of a medium strength O-H...O hydrogen bond in biopolymers was estimated to be ~5.5 kcal/mol (Hofacker, 1983). This value compares favorably with the maximum value of ΔH_{pre} obtained in equimolar mixtures of DMPC/DMPG at pH = 7.5, taking into account that the underlying 'normal' pretransition enthalpy is ≈1 kcal/mol. Thus, each DMPG headgroup forms on the average just one H bond with a DMPC headgroup. Under this condition, the number of such bonds is highest at equimolar composition, which explains the dependence of ΔH_{pre} on the composition in terms of the average number of H bonds formed in the $L_{\beta'}$ phase. The change of the chain tilt at the $L_{\beta'} - P_{\beta'}$ transition and the resulting increasing spacing between the phospholipids is likely to prevent such H bonds in the $P_{\beta'}$ phase. The observed pH dependence of ΔH_{pre} is likely to be caused by a modulation of the electric charge state of the DMPG headgroup, which prevents the formation of H bonds in the $L_{\beta'}$ phase below pH = 5 and above pH ~ 11. Although the apparent pK value of the phosphate group of phosphatidylglycerol in the L_α phase is normally in the range 3–3.5 (van Dijck et al., 1978), the intrinsic value could be higher in a mixture with DMPC, in particular in the presence of H bonds. Another explanation is an alteration of the average distance between the headgroups by pH changes which eventually prevents the formation of H bonds between them. Similarly, the increased area per headgroup in vesicular dispersions as compared with MLV due to the lower packing density of the phospholipids in the former is the most likely explanation for the disappearance of the (large) pretransition after the disruption of the MLV structure. Another indication for the involvement of hydrogen bonds comes from the isotopic substitution of the buffer of equimolar DMPC/DMPG mixtures by buffered heavy water. This has a significant effect on both the pretransition enthalpy and the transition temperature. The pretransition enthalpy is 20% higher in the presence of buffered D_2O than in H_2O buffer. The subtraction of the pretransition temperature of pure DMPC in D_2O and H_2O, respectively, from that obtained for the mixture at pH 7.2 gives for the D_2O buffer 0.8°C and for the H_2O buffer 2.2°C. The different binding strength of a deuterium bond compared with that of a hydrogen bond is likely to be the reason for this remarkable difference.

The finding, that the reported behavior of ΔH_{pre} can be observed exclusively in DMPC/DMPG mixtures is less obvious. One can speculate that the packing density of such mixtures in the $L_{\beta'}$ phase differs for mixtures with myristoyl chains from those with longer fatty acid chains. This could be accomplished by a decrease of the tilt angle with the chain length.

CONCLUSIONS

This study demonstrates that the pretransition enthalpy and temperature in DMPC/DMPG mixtures can be modulated over a considerable range by changing the headgroup interactions between these phospholipids. The most likely reason for this behavior is the formation of hydrogen bonds between the DMPC and DMPG headgroups in the $L_{\beta'}$ phase. To provide more evidence for the existence of H bonds under such conditions, further experiments such as ^{2}H-NMR measurements using headgroup selectively deuteriated DMPG and comparative DSC measurements with the two headgroup enantiomers of DMPG separately in mixture with DMPC will be performed. Moreover, x-ray measurements are required to compare the bilayer thickness in the $L_{\beta'}$ phase of DMPC/DMPG and DPPC/DPPG mixtures to obtain information about possibly different packing densities.

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REFERENCES

Bayerl, T. M., C. F. Schmidt, and E. Sackmann. 1988. Kinetics of symmetric and asymmetric phospholipid transfer between small sonicated vesicles studied by high-sensitive differential scanning calorimetry, NMR, electron microscopy and dynamic light scattering. *Biochemistry.* 27:6078–6085.

Boggs, J. M. 1987. Lipid intermolecular hydrogen bonding: influence on the structural organization and membrane function. *Biochim. Biophys. Acta.* 906:353–404.

Davis, J. H. 1979. Deuterium magnetic resonance study of the gel and liquid crystalline phases of dipalmitoyl phosphatidylcholine. *Biophys. J.* 27:339–358.

Eibl, H., and P. Woolley. 1979. Electrostatic interactions at charged lipid membranes. Hydrogen bonds in lipid membrane surfaces. *Biophys. Chem.* 10:261–265.

Eklund, K. K., J. A. Virtanen, and P. K. J. Kinnunen. 1984. Involvement of phospholipid stereoconfiguration in the pretransition of dimyristoyl phosphatidylcholine. *Biochim. Biophys. Acta.* 793:310–312.

Eklund, K. K., J. A. Virtanen, J. Patrikainen, and P. J. J. Kinnunen. 1987. Role of the polar headgroup stereoconfiguration in the cation-induced aggregation of dimyristoyl phosphatidylglycerol vesicles. *Biochemistry.* 26:7542–7545.

Findlay, E. J., and P. G. Barton. 1978. Phase behaviour of synthetic phosphatidylglycerols and binary mixtures with phosphatidylcholines in the presence and absence of calcium ions. *Biochemistry.* 17:2400–2408.

Finegold, L., and M. Singer. 1986. The metastability of saturated phosphatidylcholines depends on the acyl chain length. *Biochim. Biophys. Acta* 855:417–420.

Hofacker, G. L. 1983. Intra- and intermolecular interactions in biophysics. *In* Biophysics. W. Hoppe, W. Lohmann, H. Markl, and H. Ziegler, editors. Springer Verlag, Berlin. 235–237.

Janiak, M. J., D. M. Small, and G. G. Shipley. 1976. Nature of the thermal pretransition of synthetic phospholipids: dimyristoyl- and dipalmitoyl-lecithin. *Biochemistry.* 15:4575–4580.

Lewis, R. N. A. H., N. Mak, and R. N. McElhaney. 1987. A differential scanning calorimetric study of the thermotropic phase behaviour of model membranes composed of phosphatidylcholines containing linear saturated fatty acyl chains. *Biochemistry.* 26:6118–6126.

Mabrey, S., and J. Sturtevant. 1978. High sensitive differential scanning calorimetry in the study of biomembranes and related model systems. *In* Methods of Membrane Biology. E. D. Korn, editor. Plenum Press, New York. 9:237–274.

Madden, T. D., C. P. S. Tilcock, K. Wong, and P. R. Cullis. 1988. Spontaneous vesiculation of large multilamellar vesicles composed of saturated phosphatidylcholine and phosphatidylglycerol mixtures. *Biochemistry.* 27:8724–8730.

Pascher, I., S. Sundell, K. Harlos, and H. Eibl. 1987. Conformation and packing properties of membrane lipids: the crystal structure of sodium dimyristoyl phosphatidylglycerol. *Biochim. Biophys. Acta.* 896:77–88.

Rand, R. P., D. Chapman, and K. Larson. 1975. Tilted hydrocarbon chains of dipalmitoyl lecithin become perpendicular to the bilayer before melting. *Biophys. J.* 15:1117–1124.

Slater, J. L., and C. Huang. 1988. Interdigitated bilayer membranes. *Prog. Lipid Res.* 27:325–359.

Sternin, E. 1985. Data acquisition and processing: a systems approach. *Rev. Sci. Instrum.* 56:2043–2049.

Tardieu, A., V. Luzatti, and F. C. Reman. 1973. Structure and polymorphism of the hydrocarbon chains of lipids: a study of lecithin-water phases. *J. Mol. Biol.* 75:711–733.

Van Dijck, P. W. M., B. DeKruijff, A. J. Verkleij, and L. L. M. Van Deenen. 1978. Comparative studies on the effects of pH and Ca^{2+} on bilayers of various negatively charged phospholipids and their mixtures with phosphatidylcholine. *Biochim. Biophys. Acta.* 512:84–96.

Wilkinson, D. A., D. A. Tirell, A. B. Turek, and T. J. McIntosh. 1987. Tris buffer causes acyl chain interdigitation in phosphatidylglycerol. *Biochim. Biophys. Acta.* 905:447–453.