

Structure and Thermotropic Properties of Phosphatidylethanolamine and Its *N*-Methyl Derivatives[†]

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ABSTRACT: The structure and thermotropic properties of hydrated bilayers of 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE) and its *N*-monomethyl (mmDMPE) and *N,N*-dimethyl (dmDMPE) derivatives have been investigated by differential scanning calorimetry and X-ray diffraction. For DMPE, mmDMPE, and dmDMPE, multilamellar dispersions (~50 wt % water) show chain melting bilayer gel → bilayer liquid-crystal transitions (onset) at 49.2, 42.3, and 30.7 °C, respectively, with the corresponding value for 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine occurring at 23 °C. Thus, the bilayer chain melting transition decreases with increasing *N*-methylation, as originally reported for the corresponding palmitoyl series [Vaughan, D. J., & Keough, K. M. (1974) *FEBS Lett.* 47, 158-161]. This transition is reversible on cooling, and DMPE, mmDMPE, and dmDMPE

form the original bilayer gel phase with the rotationally disordered hydrocarbon chains packed in a hexagonal lattice. Following prolonged incubation at -4 °C, the bilayer gel phase is shown to be metastable, and conversion to a low-temperature "crystalline" phase occurs with the hydrocarbon chains adopting a specific packing mode. For DMPE, mmDMPE, and dmDMPE, either a single or a double endothermic transition occurs as the "crystal" bilayer phase converts to the bilayer gel phase. A similar pattern of behavior is observed for the palmitoyl series. The relatively slow kinetic conversion of the metastable bilayer gel phase with hexagonally packed hydrocarbon chains to a bilayer phase in which the chains have "crystallized" appears to be a general property of membrane phospholipids and sphingolipids.

The glycerol-based phospholipids phosphatidylethanolamine (PE)¹ and phosphatidylcholine (PC) are major constituents of the lipid bilayer compartment of most mammalian plasma membranes (Rouser et al., 1968; Ansell & Spanner, 1982). In some mammalian tissues, particularly liver, these two major phospholipid species are directly related metabolically, some of the PC being derived from the sequential methylation of PE using *S*-adenosylmethionine (Bremer & Greenberg, 1961; Gibson et al., 1961). The addition of the three methyl groups appears to be enzymatically regulated by two membrane-located methyl transferases (Hirata et al., 1978). Recently, Axelrod and co-workers have implicated this PE → PC methylation process in the regulation of a number of membrane receptor mediated events [for a review, see Hirata & Axelrod (1980)], perhaps as a result of altered membrane fluidity (Hirata & Axelrod, 1978).

Although much is known about the structure and properties of PE and PC, relatively few physicochemical studies of the two *N*-methylated intermediates, monomethyl-PE and dimethyl-PE, have been performed. Vaughan & Keough (1974) showed by scanning calorimetry that the monomethyl and dimethyl derivatives of dipalmitoyl-PE (DPPE) exhibit chain melting transitions at temperatures intermediate between those for DPPE and dipalmitoyl-PC (DPPC). In addition, neither of the *N*-methylated intermediates showed evidence of transitions at lower temperatures, such as the pretransition found for DPPC (Chapman et al., 1967; Tardieu et al., 1973; Janiak et al., 1976, 1979). Recently, it has been shown that both PC (Chen et al., 1980; Fuldner, 1981; Ruocco & Shipley, 1982a,b; Cameron & Mantsch, 1982; Nagle & Wilkinson, 1982) and PE (Wilkinson & Nagle, 1981; Seddon et al., 1983; Chang & Epand, 1983; Mantsch et al., 1983) exhibit metastable behavior at low temperatures, allowing the conversion of metastable bilayer gel phases to more stable crystalline bilayer

phases. In this study, we have investigated the structure, metastability, and thermotropic properties of hydrated dimyristoyl-PE (DMPE) and its monomethyl (mmDMPE) and dimethyl (dmDMPE) derivatives by using differential scanning calorimetry (DSC) and X-ray diffraction. Similar DSC studies on the dipalmitoyl-PE series (i.e., DPPE, mmDPPE, and dmDPPE) have also been performed. These data and their comparison with similar data on dimyristoyl-PC (DMPC) and DPPC enable the influence of polar group modification (methylation) on bilayer structure and stability to be evaluated.

Materials and Methods

Synthetic DMPE, DPPE, mmDPPE, and dmDPPE were obtained from Calbiochem (La Jolla, CA). Synthetic mmDMPE and dmDMPE were obtained from R. Berchtold (Berne, Switzerland). Samples of mmDMPE, DPPE, mmDPPE, and dmDPPE were shown to be pure by thin-layer chromatography (TLC) using chloroform/methanol/water/acetic acid (65:25:4:1). DMPE and dmDMPE were purified on a silicic acid column after which they were shown to be pure by TLC using the same solvent system described above.

Hydrated multilamellar dispersions of the lipids were prepared by centrifuging weighed amounts of lipid and water through a narrow constriction in a sealed glass tube at 60 °C. Lipid dispersions were then transferred to stainless-steel calorimetry pans and to X-ray diffraction capillary tubes (internal diameter 1.0 mm). The DSC pans and capillary tubes were sealed and then stored at -4 °C for varying periods of time. Additional calorimetry samples were prepared by addition of weighed amounts of lipid and water directly into

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¹ Abbreviations: PE, phosphatidylethanolamine; PC, phosphatidylcholine; DMPE, 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine; mmDMPE, 1,2-dimyristoyl-*sn*-glycero-3-phospho-*N*-methylethanolamine; dmDMPE, 1,2-dimyristoyl-*sn*-glycero-3-phospho-*N,N*-dimethylethanolamine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; mmDPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-*N*-methylethanolamine; dmDPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-*N,N*-dimethylethanolamine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DSC, differential scanning calorimetry; TLC, thin-layer chromatography.

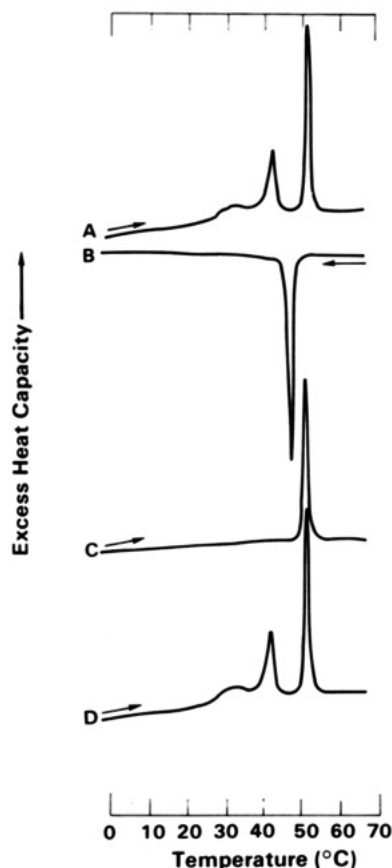


FIGURE 1: DSC of hydrated (51.4 wt % H_2O) DMPE: (A) initial heating scan after equilibration at -4°C for 53 days; (B) cooling scan immediately following first heating scan; (C) second heating scan; (D) initial heating scan after subsequent storage at -4°C for 48 days.

stainless-steel pans, which were then sealed immediately.

Differential scanning calorimetry (DSC) studies were conducted in a Perkin-Elmer DSC-2 differential scanning calorimeter (Norwalk, CT) calibrated with indium and gallium. Pans were removed from storage and transferred at low temperature (0°C) to the calorimeter equilibrated at -3°C prior to the transfer. Samples were studied at heating and cooling rates of $5^\circ\text{C}/\text{min}$. Each sample was heated and cooled repeatedly between -3 and 77°C . Transition enthalpies were determined from the area under the transition peak by comparison with that for a known standard, gallium. For each transition, both the onset temperature (determined by extrapolation to the base line) and the peak temperature were recorded.

For X-ray diffraction studies, nickel-filtered $\text{Cu K}\alpha$ X radiation ($\lambda = 1.5418 \text{ \AA}$) from an Elliott GX-6 rotating anode generator (Elliott Automation, Borehamwood, England) was collimated by toroidal optics (Elliott, 1965), and X-ray diffraction patterns were recorded by photographic film methods. X-ray diffraction patterns were recorded for hydrated DMPE, mmDMPE, and dmDMPE at various temperatures by means of a variable-temperature sample holder. For the low-temperature (2°C) recordings obtained after sample storage at -4°C , samples were transferred at 0°C to the sample holder pre-equilibrated at 2°C .

Results

DMPE. The DSC heating curve of hydrated (51.4 wt % H_2O) DMPE following low-temperature (-4°C) storage for 53 days is shown in Figure 1A. The curve shows three peaks, the first two of which are centered at approximately 31°C (broad) and 41°C (sharp); these two low-temperature peaks

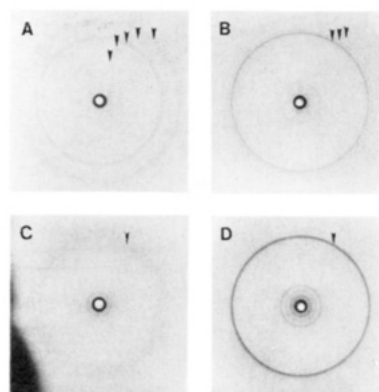


FIGURE 2: X-ray diffraction patterns of hydrated (49.2 wt % H_2O) DMPE: (A) at 2°C after storage at -4°C for 60 days; (B) at 43°C (after heating from 2 to 43°C); (C) at 61°C (after heating from 43 to 61°C); (D) at 43°C (after cooling from 61 to 43°C). Sample to film distance = 62.9 mm .

(T_{L1} and T_{L2}) have a combined enthalpy of 6.2 kcal/mol of DMPE. The third endothermic transition (T_M) with a peak at 50.4°C has an enthalpy (ΔH) of 5.6 kcal/mol of DMPE. The cooling curve recorded immediately after the initial heating scan shows only a single transition exotherm with a peak at 47.9°C (Figure 1B). The heating scan immediately following this cooling scan shows only a single transition with a peak at 50.4°C (Figure 1C), corresponding to the original T_M (see Figure 1A). The two low-temperature transitions (T_{L1} and T_{L2}) are regained upon prolonged storage (48 days) at -4°C (Figure 1D).

X-ray diffraction experiments to determine the structural changes of hydrated DMPE were performed at temperature points below T_{L1} , between T_{L2} and T_M , and above T_M . The diffraction pattern of hydrated (49.2 wt % H_2O) DMPE at 2°C after equilibration for 60 days at -4°C is shown in Figure 2A. This form is lamellar with a long spacing $d = 50.9 \text{ \AA}$ although only a strong first-order reflection and a weak fourth-order reflection are observed. Five diffraction lines are visible in the wide-angle region (arrows in Figure 2A). A strong, sharp reflection is present at $1/4.56 \text{ \AA}^{-1}$, while a rather broad reflection is centered at $1/3.69 \text{ \AA}^{-1}$; three weaker reflections are present at $1/7.35$, $1/5.13$, and $1/4.08 \text{ \AA}^{-1}$. After the sample is heated to 43°C , the diffraction pattern shown in Figure 2B is observed. This phase is characterized in the low-angle region by four sharp lamellar reflections, $d = 55.5 \pm 0.3 \text{ \AA}$. The wide-angle region shows a strong reflection at $1/4.21 \text{ \AA}^{-1}$ and two weaker reflections at $1/4.00$ and $1/3.79 \text{ \AA}^{-1}$ (arrows in Figure 2B). Above T_M , at 61°C , the diffraction pattern exhibits four sharp lamellar reflections, $d = 46.9 \pm 0.8 \text{ \AA}$, along with a diffuse reflection in the wide-angle region centered at $\sim 1/4.60 \text{ \AA}^{-1}$ (arrow in Figure 2C). Cooling from 61 to 43°C (below T_M) results in a diffraction pattern (Figure 2D) which is similar to but not identical with that shown in Figure 2B. Although the bilayer periodicity is identical (55.4 \AA), it differs in that it lacks the two weak wide-angle reflections at $1/4.00$ and $1/3.79 \text{ \AA}^{-1}$ that are present in Figure 2B. Only a sharp diffraction line at $1/4.21 \text{ \AA}^{-1}$ is observed (arrow in Figure 2D).

mmDMPE. The DSC data for hydrated mmDMPE (52.1 wt % H_2O) are shown in Figure 3. The initial heating scan recorded after storage at -4°C for 58 days is shown in Figure 3A. Two endothermic transitions are evident; the broad low-temperature transition endotherm, T_L , has an enthalpy of $\Delta H = 5.4 \text{ kcal/mol}$ of mmDMPE and is centered at $\sim 21^\circ\text{C}$, while the second endotherm, T_M , occurs with a peak at 46.0°C ($\Delta H = 6.3 \text{ kcal/mol}$ of mmDMPE). The cooling scan

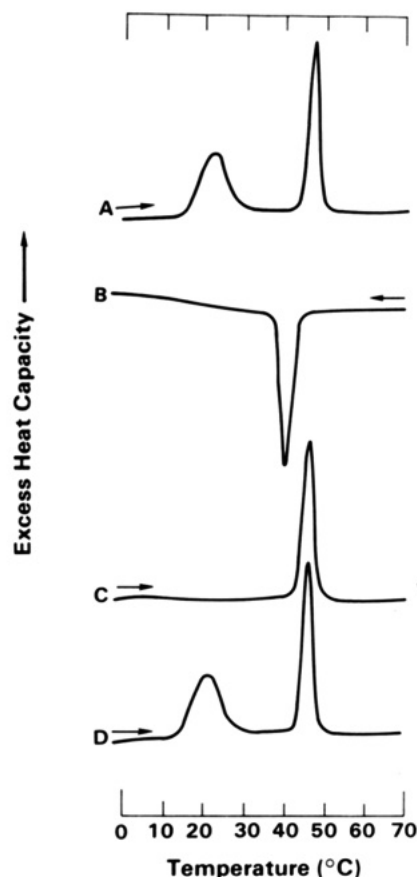


FIGURE 3: DSC of hydrated (52.1 wt % H₂O) mmDMPE: (A) initial heating scan after equilibration at -4°C for 58 days; (B) cooling scan immediately following first heating scan; (C) second heating scan; (D) initial heating scan after subsequent storage at -4°C for 60 days.

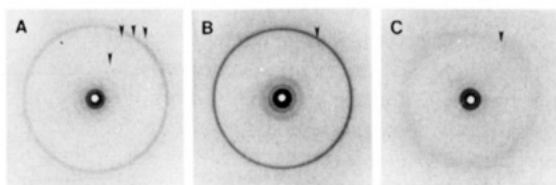


FIGURE 4: X-ray diffraction patterns of hydrated (50.8 wt % H₂O) mmDMPE: (A) at 2°C after storage at -4°C for 65 days; (B) at 37°C (after heating from 2 to 37°C); (C) at 55°C (after heating from 37 to 55°C). Sample to film distance = 62.9 mm.

immediately following the initial heating run (Figure 3B) shows only a single exotherm at $\sim 40^{\circ}\text{C}$ corresponding to the higher transition, T_M . The heating curve recorded immediately after the cooling scan also shows only a single transition, T_M , with a peak at 46.0°C (Figure 3C). The low-temperature transition, T_L , is regained following storage at -4°C for 60 days (Figure 3D).

Following storage at -4°C for 65 days, the diffraction pattern shown in Figure 4A is observed for hydrated mmDMPE (50.8 wt % H₂O) at 2°C . The three low-angle reflections index on a lamellar geometry, $d = 63.8 \pm 0.8 \text{ \AA}$. Four lines are present in the wide-angle region (arrows in Figure 4A): one is a strong, albeit broad reflection centered at $1/4.05 \text{ \AA}^{-1}$, while the other three are weaker, sharper reflections at $1/7.89$, $1/4.39$, and $1/3.75 \text{ \AA}^{-1}$. After being heated to 37°C (between T_L and T_M), the lamellar diffraction pattern (periodicity = $66.4 \pm 0.8 \text{ \AA}$) shown in Figure 4B is observed. The wide-angle region is characterized by a single strong line at $1/4.22 \text{ \AA}^{-1}$ (arrow in Figure 4B). Heating to 55°C (above T_M) again results in a lamellar diffraction pattern (periodicity = $57.7 \pm 0.8 \text{ \AA}$) and a broad reflection

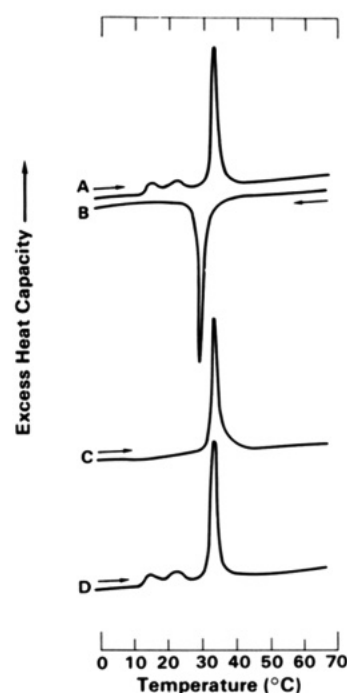


FIGURE 5: DSC of hydrated (55.5 wt % H₂O) dmDMPE: (A) initial heating scan after equilibration at -4°C for 51 days; (B) cooling scan immediately following first heating scan; (C) second heating scan; (D) initial heating scan after subsequent storage at -4°C for 45 days.

in the wide-angle region centered at $1/4.47 \text{ \AA}^{-1}$ (arrow in Figure 4C). Cooling the sample to 37°C results in a pattern identical with that shown in Figure 4B.

dmDMPE. The DSC data for hydrated (55.5 wt % H₂O) dmDMPE are shown in Figure 5. The initial heating scan after storage at -4°C for 51 days shows three endothermic peaks (Figure 5A). The two low-temperature transitions (T_{L1} and T_{L2}) centered at 14 and 21°C have a combined enthalpy of only 1.5 kcal/mol of dmDMPE. The main transition peak, T_M , occurs at 33.8°C ($\Delta H = 6.7 \text{ kcal/mol}$ of dmDMPE). The subsequent cooling and heating curves show only the high-temperature transition, T_M , at ~ 30 and $\sim 34^{\circ}\text{C}$, respectively (Figure 5B,C). Prolonged storage (45 days) at -4°C results in the reappearance of the two low-temperature, low enthalpy transitions, T_{L1} and T_{L2} (Figure 5D).

Some insight into the nature of the two low-temperature transitions T_{L1} and T_{L2} is obtained from the results of the study of another sample of hydrated (75.9 wt % H₂O) dmDMPE. The initial DSC heating curve of dmDMPE obtained after storage at -4°C for 7 days is shown in Figure 6A. In this case, only T_{L1} ($T = 13.4^{\circ}\text{C}$; $\Delta H = 4.1 \text{ kcal/mol}$ of dmDMPE) and T_M are observed. Following subsequent storage at -4°C for 15 days, the initial heating curve shown in Figure 6B is obtained. Both low-temperature transitions, T_{L1} (14.0°C) and T_{L2} (21.0°C), are observed with a combined enthalpy of 3.3 kcal/mol of dmDMPE. Subsequent prolonged storage at -4°C (6 months) produced the DSC heating curve shown in Figure 6C. In this case, only T_{L2} is observed at 21.2°C ($\Delta H = 6.4 \text{ kcal/mol}$ of dmDMPE), together with T_M . It is not clear why the low-temperature transitions have significantly higher enthalpies than those discussed above for the 56.5 wt % H₂O dmDMPE sample, although they probably represent different degrees of formation of the low-temperature phase(s). In the case of mixed-chain phosphatidylcholines, we have shown that the kinetics of formation of the stable low-temperature phase are both temperature and hydration level dependent (Serrallach et al., 1984).

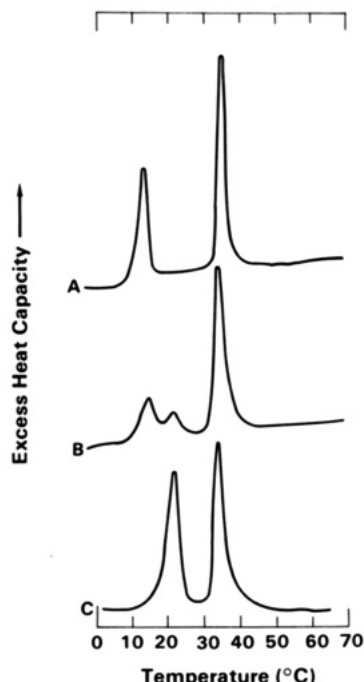


FIGURE 6: Initial DSC heating curves of hydrated (75.9 wt % H_2O) dmDMPE after storage at -4°C for varying lengths of time: (A) after storage for 7 days; (B) after storage for 15 days; (C) after storage for 6 months.

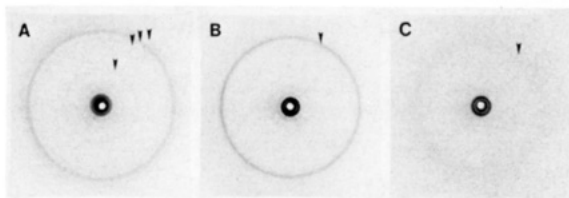


FIGURE 7: X-ray diffraction patterns of hydrated (48.2 wt %) dmDMPE: (A) at 2°C after storage at -4°C for 60 days; (B) at 27°C (after heating from 2 to 27°C); (C) at 47°C (after heating from 27 to 47°C). Sample to film distance = 62.9 mm .

Following storage at -4°C for 60 days, hydrated dmDMPE (48.2 wt % H_2O) gives at 2°C the X-ray diffraction pattern shown in Figure 7A. The low-angle reflections indicate a lamellar structure (periodicity = $64.3 \pm 0.8\text{ \AA}$). Four reflections are present in the wide-angle region (arrows in Figure 7A). These include a strong line centered at $1/4.09\text{ \AA}^{-1}$ and weaker lines at $1/7.89$, $1/4.50$, and $1/3.80\text{ \AA}^{-1}$. At 27°C (between T_{L2} and T_M), the diffraction pattern shown in Figure 7B is obtained. Again, a lamellar structure with a periodicity $d = 65.4 \pm 0.8\text{ \AA}$ is observed. The wide-angle region shows only a single strong reflection at $1/4.21\text{ \AA}^{-1}$ (arrow in Figure 7B). The diffraction pattern of dmDMPE at 47°C (above T_M) is shown in Figure 7C. A lamellar periodicity of $60.0 \pm 0.8\text{ \AA}$ is observed, and in the wide-angle region, a diffuse reflection centered at $1/4.44\text{ \AA}^{-1}$ (arrow in Figure 7C) is present. Cooling to 27°C (below T_M) results in a diffraction pattern identical with that shown in Figure 7B.

DPPE, mmDPPE, and dmDPPE. DSC data were also recorded for hydrated ($\sim 75\text{ wt } \%$ H_2O) DPPE, mmDPPE, dmDPPE, and DPPC. A pattern of thermotropic behavior similar to that of the myristoyl series is observed. The DSC data show an approximately linear decrease in the bilayer gel \rightarrow liquid-crystal transition temperature from 63.6 to 41.5°C with increasing N-methylation (Figure 8B) and a similar pattern of changes in enthalpy (Figure 8A) to the myristoyl series. Prolonged incubation at low temperatures results in additional endothermic transitions, again indicating gel phase

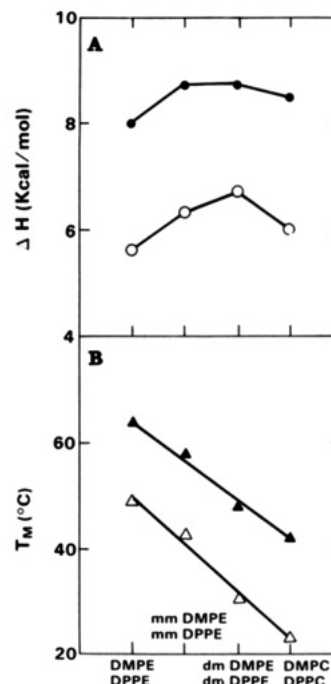


FIGURE 8: Effect of PE methylation on the enthalpy (A) and the transition temperature (B) of the main transition: (Δ) transition temperatures (T_M) for the myristoyl series; (○) enthalpy values for the myristoyl series; (▲) transition temperatures (T_M) for the palmitoyl series; (●) enthalpy values for the palmitoyl series.

metastability and the formation of a more ordered bilayer phase.

Discussion

Our combined DSC and X-ray diffraction studies show an interesting pattern of behavior for DMPE and its *N*-mono-methyl and *N,N*-dimethyl derivatives. Clearly, as reported previously by Vaughan & Keough (1974) for the palmitoyl series, the chain melting transition temperature, T_M , is sensitive to the degree of methylation. The transition temperature decreases in an approximately linear fashion from 49°C for DMPE to 23°C for DMPC, as up to three methyl groups are added (see Figure 8B). A similar linear decrease in T_M from 63.6 (DPPE) to 41.5°C (DPPC) is observed for the palmitoyl series (Figure 8B). For both series, the patterns of enthalpy changes associated with T_M are also similar. PE has the lowest enthalpy; the two methylated intermediates exhibit higher enthalpies, and the fully methylated PC has a slightly lower value (Figure 8A). However, it should be emphasized that the differences in enthalpy with increasing methylation are quite small. For a given series, the transition entropies are reasonably constant, ~ 0.020 and $\sim 0.026\text{ kcal mol}^{-1}\text{ K}^{-1}$ for the myristoyl and palmitoyl series, respectively, suggesting that the changes associated with T_M are similar in all cases (see structural data below).

In addition, the DSC data provide clear evidence that DMPE and its *N*-methyl derivatives and DPPE and its *N*-methyl derivatives exhibit metastability of their low-temperature gel phases. The DSC data show that in all cases the gel phase observed on cooling from the L_α phase slowly converts to a more stable form that exhibits an additional transition (or transitions) at $T < T_M$. Although we have not made a detailed study of the kinetics of this process, in all cases the rate of formation of the stable phase appears to be slower, at least at -4°C , than the comparable process in DPPC (Ruocco & Shipley, 1982a,b), mixed-chain PC's (Serrallach et al., 1983), and β -DPPC (Serrallach et al., 1983; E. N. Serrallach

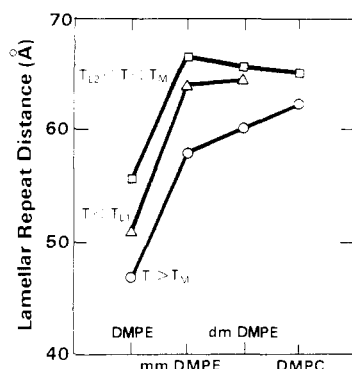


FIGURE 9: Effect of DMPE methylation on the lamellar repeat distance. Hydrated (~ 50 wt % H_2O) multilamellar dispersions: (Δ) at a temperature below the low-temperature transition(s) (note: when hydrated to 50 wt % H_2O , DMPC does not exhibit any low-temperature transition analogous to the low-temperature transitions described in this paper); (\square) at a temperature between the low-temperature transition(s) and the main transition (for DMPC, this symbol indicates a temperature between the pretransition and the main transition, $20^\circ C$); and (O) at a temperature beyond the main transition.

and G. G. Shipley, unpublished results). On the basis of the DSC data for DMPE and dmDMPE (see Figures 1 and 5), there appear to be two different low-temperature forms with different transition temperatures. Apparently, for dmDMPE, the initial phase formed ($T_{L1} = 13$ – $14^\circ C$) is itself metastable and slowly converts to a more stable, higher melting ($T_{L2} = 21^\circ C$) form (see Figure 6).

The X-ray diffraction data (see Figures 2, 4, 7, and 9) demonstrate that the structural changes occurring at the low-temperature transition(s) (T_L) and the high-temperature transition (T_M) are similar for hydrated DMPE, mmDMPE, and dmDMPE. Following long-term incubation at $-4^\circ C$, the diffraction pattern at $T < T_L$ shows clear evidence in all cases that a "crystalline" bilayer phase is present. The reflections in the wide-angle region indicate the presence of an ordered hydrocarbon chain packing arrangement, with the chains packed according to one of the precise subcell modes (Abrahamsson et al., 1978). At temperatures intermediate between T_L and T_M , a bilayer phase is again present, but in this case, the less ordered hexagonal chain packing mode is indicated by the single sharp diffraction line at $\sim 1/4.2 \text{ \AA}^{-1}$. [For DMPE at $43^\circ C$, the presence of two additional, albeit weak, diffraction lines at $1/4.00$ and $1/3.79 \text{ \AA}^{-1}$ would suggest that additional polymorphic changes of the type recently reported for dilauroyl-PE (Seddon et al., 1983) are occurring.] At temperatures greater than T_M , the X-ray diffraction data demonstrate clearly the presence of liquid-crystal bilayers with "melted" hydrocarbon chains. On cooling to temperatures below T_M (or below T_L), the diffraction pattern is essentially identical with that observed in the intermediate temperature range on heating. Thus, after low-temperature equilibration, hydrated DMPE, mmDMPE, and dmDMPE exhibit interconversions of bilayer phases with different chain packing arrangements, as summarized in Figure 10.

The bilayer periodicity (bilayer + water thickness) shows an identical pattern of behavior as DMPE, mmDMPE, and dmDMPE convert between the L_c , L_β , and L_α phases (see Figure 9). The $L_c \rightarrow L_\beta$ transition is always accompanied by an increase in bilayer periodicity, most probably due to an increase in lipid hydration [see Ruocco & Shipley (1982b)], whereas the $L_\beta \rightarrow L_\alpha$ transition results in a decrease in bilayer periodicity, most probably due to reductions in both the bilayer thickness and the water thickness as chain melting occurs. As shown in Figure 9, there is a significantly lower value for the

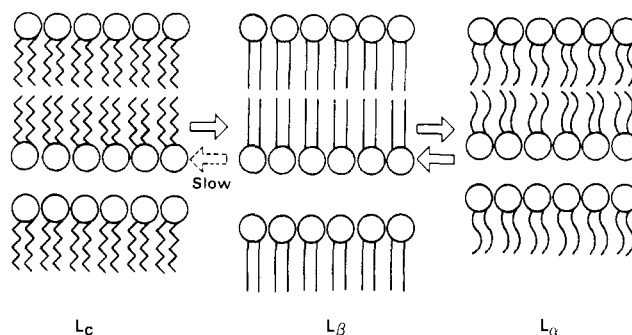


FIGURE 10: Schematic representation of the bilayer structures present at (left) $T < T_L$ (a regular zig-zag chain indicates an ordered chain packing mode), (middle) $T_L < T < T_M$ (a straight chain indicates hexagonally packed chains), and (right) $T > T_M$ (melted chains).

bilayer periodicities of DMPE in all phases, compared to the periodicities of the comparable phases of mmDMPE and dmDMPE. It might have been predicted that the effect of methylation would be to increase the volume and surface area occupied by the polar group [for differences between PE and PC, see Hitchcock et al. (1974), Elder et al. (1977), Pearson & Pascher (1979), and Hauser et al. (1981)], with progressive increases in chain tilt and a reduction in the bilayer thickness. However, the methylated DMPE intermediates and DMPC presumably favor increased hydration, and the concomitant increase in water thickness would explain the overall increase in the bilayer periodicity compared to DMPE (see Figure 9). The data suggest but do not prove that the first N -methyl group added results in a major change in lipid hydration in all the bilayer phases studied. Resolving this issue unequivocally requires a more detailed study of the hydration dependence of the bilayer periodicity of DMPE and its methylated derivatives.

Recently, several investigators have studied the metastability of hydrated phospholipid bilayers. Upon low-temperature incubation, DPPC undergoes conversion from the usual hydrated bilayer gel (L_β) to a bilayer crystalline phase (L_c), this transformation involving a decrease in bilayer hydration and an increase in lateral molecular order (Füldner, 1981; Ruocco & Shipley, 1982a,b). Upon heating, the L_c phase undergoes reconversion to the L_β phase. Thus, the nature of the metastability of DPPC is quite similar to that postulated in this paper for DMPE, mmDMPE, and dmDMPE. As already noted, however, the kinetics of the processes are quite dissimilar. Seddon et al. (1983) recently observed metastability for 1,2-dilauroyl-*sn*-glycero-3-phosphoethanolamine (DLPE) in excess water. They demonstrated that the hydrated gel bilayer L_β phase will spontaneously adopt a crystalline bilayer form (β_1), with the conversion involving increased lateral chain packing interactions and decreased hydration. Moreover, upon heating, the β_1 bilayer phase undergoes direct transformation to the L_α bilayer phase, without passing through the L_β phase. Thus, the metastability of the L_β form of DLPE is qualitatively different from the metastability observed in this study for DMPE and DPPE and their N -methyl derivatives. In addition, Seddon et al. (1983) demonstrated metastability of the L_α bilayer phase of DLPE which over a specific temperature range converts to another crystalline bilayer β_2 form. On the basis of the DSC and X-ray diffraction data (see Figures 1 and 2), it is possible that DMPE exhibits some aspects of this complex metastable behavior. However, it appears that some simplification of the metastable behavior occurs for the higher homologues of phosphatidylethanolamine.

Other investigations of the metastability of hydrated membrane phospholipids and sphingolipids have been conducted

(Harlos & Eibl, 1980; Estep et al., 1980; Freire et al., 1980; Ruocco et al., 1981). In these studies, the conversion to the stable forms involves alteration in both the hydrocarbon chain packing mode and bilayer hydration. Thus, bilayer metastability and bilayer interconversions appear to be a quite general property of membrane lipids.

Added in Proof

A recent study utilizing DSC and infrared spectroscopy provides additional information on the reversible transition (T_M) of *N*-monomethyl- and *N,N*-dimethyl-DPPE (Casal & Mantsch, 1983). The authors suggest on the basis of the infrared spectroscopic studies that a progressive increase occurs in hydrocarbon chain tilt in the gel phase from DPPE to DPPC. However, no evidence of gel phase metastability for the methylated derivatives of DPPE is presented in this study.

Acknowledgments

We acknowledge technical advice and assistance from Dr. D. Atkinson and D. Jackson. We thank Irene Miller for help in the preparation of the manuscript.

Registry No. DMPE, 998-07-2; mmDMPE, 68755-13-5; dmDMPE, 68755-14-6; DMPC, 18194-24-6; DPPE, 923-61-5; mmDPPE, 3930-13-0; dmDPPE, 3922-61-0; DPPC, 63-89-8.

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