Structure and Thermotropic Properties of Mixed-Chain Phosphatidylcholine Bilayer Membranes†

E. N. Serrallach, G. H. de Haas, and G. G. Shipley*

ABSTRACT: The structure and thermotropic properties of hydrated 1-myristoyl-2-palmitoyl-L-phosphatidylcholine (MPPC) and 1-palmitoyl-2-myristoyl-L-phosphatidylcholine (PMPC) have been studied by X-ray diffraction and differential scanning calorimetry (DSC). After prolonged storage at −3 °C, hydrated multilamellar dispersions of both MPPC and PMPC show two endothermic transitions of comparable enthalpies on initial heating. MPPC undergoes transitions at 26 °C (ΔH = 8.0 kcal/mol of MPPC) and 34 °C (ΔH = 8.1 kcal/mol of MPPC); PMPC exhibits transitions at lower temperatures, 17 °C (ΔH = 7.7 kcal/mol of PMPC) and 27 °C (ΔH = 7.8 kcal/mol of PMPC). For both MPPC and PMPC, only the higher temperature transition is readily reversible; the enthalpy associated with the lower temperature transition is progressively recovered with increasing incubation time at low temperatures, suggesting conversions, albeit kinetically complex, between metastable and stable phases. X-ray diffraction data show the presence of bilayer structures and a similar pattern of structural changes on heating for hydrated (50 wt %) MPPC and PMPC. Below the low-temperature transition a "crystalline" bilayer L_c phase [bilayer periodicity d = 57 Å (MPPC) and d = 57 Å (PMPC)] with an ordered hydrocarbon chain packing mode is present. Between the two transitions, a rippled gel phase $P_{\beta'}$ [bilayer periodicity d = 66 Å (MPPC) and d = 65 Å (PMPC); ripple periodicity ~210 Å (MPPC) and ~180 Å (PMPC)] with hexagonal chain packing is observed. Above the high-temperature transition, a liquid-crystalline L_α bilayer phase [bilayer periodicity d = 64 Å (MPPC) and d = 64 Å (PMPC)] with melted chains is present. On cooling from the L_α phase, both MPPC and PMPC convert back to the $P_{\beta'}$ phase. On further cooling, a *different* bilayer gel phase [d = 63 Å (MPPC), d = 63 Å (PMPC)] forms, which exhibits a time- and temperature-dependent conversion to the L_c phase. In contrast to the identical-chain palmitoyl (DPPC) compound, MPPC and PMPC do not form an $L_{\beta'}$ phase on heating from the L_c phase, and the structural changes associated with the sub- and pretransitions of DPPC are combined in the low-temperature transition of MPPC and PMPC.

The structure and properties of 1,2-diacyl-L-phosphatidylcholines (PC)[1] containing identical fatty acyl chains in the 1 and 2 positions on the glycerol backbone have been studied in detail by a wide variety of biophysical methods. A combination of scanning calorimetry and X-ray diffraction methods has been particularly valuable in describing the thermotropic transitions exhibited by hydrated PCs and elucidating the structural changes accompanying these phase changes. For hydrated 1,2-dipalmitoyl-L-phosphatidylcholine (DPPC) and 1,2-dimyristoyl-L-phosphatidylcholine (DMPC), for example, the two reversible transitions at approximately 35 and 41 °C for DPPC and 13 and 24 °C for DMPC correspond to gel → gel ($L_{\beta'} \rightarrow P_{\beta'}$) and gel → liquid-crystal ($P_{\beta'} \rightarrow L_\alpha$) bilayer transformations (Chapman et al., 1967; Tardieu et al., 1973; Mabrey & Sturtevant, 1976; Levine et al., 1968; Janiak et al., 1976, 1979) [for phase nomenclature, see Tardieu et al. (1973)]. More recently, Chen et al. (1980) have shown by scanning calorimetry that the DPPC–water $L_{\beta'}$ phase is metastable at low temperatures and that slow conversion to a different low temperature form occurs. The structural changes accompanying this transition have been studied by X-ray diffraction (Füldner, 1981; Ruocco & Shipley, 1982a,b), spectroscopic (Cameron & Mantsch, 1982), and dilatometric (Nagle & Wilkinson, 1982) methods. It is clear that this low-temperature transformation ($L_{\beta'} \rightarrow L_c$) is accompanied by both increased lateral order (chain packing) and dehydration and can be considered as a two-dimensional (or possibly three-dimensional) crystallization of hydrated DPPC bilayers (Ruocco & Shipley, 1982b).

Since PCs present in cell membranes are usually heterogeneous with respect to fatty acyl chain composition, in both chain length and degree of unsaturation, it is clearly important to examine the properties of mixed-chain phosphatidylcholines. As a first step, the synthesis of mixed *saturated* chain PCs has been achieved, and a number of differential scanning calorimetry (DSC) studies of a variety of hydrated mixed-chain PCs have now been reported (Keough & Davis, 1979; Stümpel et al., 1981; Chen & Sturtevant, 1981; Mason et al., 1981a,b). Due to difficulties associated with acyl migration during the synthesis, some of these studies were complicated by the presence of significant amounts of the positional isomer [see Keough & Davis (1979) and Chen & Sturtevant (1981)]. In most cases, at least two transitions were observed, perhaps related to the pretransition and main transition exhibited by DPPC for example (see above). However, the variability in the transition temperature and enthalpy associated with the low-temperature transition suggested alternative explanations. In light of our previous experiences with low-temperature metastable forms of DPPC (Ruocco & Shipley, 1982a,b), 1,3-dipalmitoyl-PC [β-DPPC; see Serrallach et al. (1983)], sphingomyelin (Estep et al., 1980), cerebrosides (Ruocco et al., 1981), and phosphatidylethanolamines (Mulukutla & Shipley, 1984), we have made a detailed study of the structure, metastability, and thermotropic properties of the positional isomers 1-myristoyl-2-palmitoyl-L-phosphatidylcholine (MPPC) and 1-palmitoyl-2-myristoyl-L-phosphatidylcholine (PMPC).

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[1] Abbreviations: PC, phosphatidylcholine; MPPC, 1-myristoyl-2-palmitoyl-L-phosphatidylcholine; PMPC, 1-palmitoyl-2-myristoyl-L-phosphatidylcholine; DPPC, 1,2-dipalmitoyl-L-phosphatidylcholine; DMPC, 1,2-dimyristoyl-L-phosphatidylcholine; DSC, differential scanning calorimetry.

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Materials and Methods

1-Myristoyl-2-palmitoyl-L-phosphatidylcholine (MPPC) and 1-Palmitoyl-2-myristoyl-L-phosphatidylcholine (PMPC). Samples of MPPC and PPPC were synthesized as follows. DPPC and DMPC were prepared from *sn*-glycero-3-phosphocholine and acyl chlorides with pyridine, triethylamine, or diaminopyridine as catalysts. Conversion of DPPC and DMPC to the 1-acyl-*sn*-glycero-3-phosphocholine used the procedure described by Hanahan (1952). Reacylation of the lysolecithins to yield the mixed-acid lecithins MPPC and PMPC was carried out by using the method described by de Haas & van Deenen (1960). Phospholipase A_2 degradation of MPPC and PMPC indicated a maximum level of 6% contributed by the positional isomer.

Differential Scanning Calorimetry (DSC). Hydrated multilamellar dispersions were prepared by adding distilled, deionized water directly into the stainless steel DSC pans containing weighed amounts of MPPC or PMPC. After the DSC pan was sealed, proper mixing was ensured by heating and cooling the sample to temperatures above and below the transition temperature of the lipid. For the studies concerning the thermotropic behavior of PCs in excess water, a weight ratio of 1:3 (PC to H_2O) was used. The samples were stored at −3 °C, without water freezing, until use.

DSC measurements were performed on a Perkin-Elmer DSC-2 instrument (Norwalk, CT) over the temperature range of −6 to 50 °C and at a heating and cooling rate of 5 °C/min. Transition temperatures were determined from the position of the transition peak. Transition enthalpies were determined from the area under the peak measured with a planimeter and calibrated with a gallium standard.

X-ray Diffraction. Hydrated multilamellar dispersions were prepared by adding weighed amounts of MPPC or PMPC directly into a thin-walled quartz capillary, followed by centrifugation to ensure that the lipid reached the bottom of the capillary. Doubly distilled water was added to the capillary and the tube recentrifuged, weighed, and then flame sealed. To obtain homogeneous mixing, the sample was heated and cooled to temperatures above and below the transition temperature of the lipid. The specimens were stored at −3 °C until use.

X-ray diffraction patterns were recorded with either photographic film or position-sensitive proportional-detector (PSD) methods. For film recording, nickel-filtered Cu Kα radiation (λ = 1.5418 Å) from an Elliot GX-6 rotating anode X-ray generator (Elliot Automation, Borehamwood, U.K.) was collimated by double-mirror optics into a point source. For counter recording, Cu Kα radiation from a microfocus X-ray generator (Jarrel-Ash, Waltham, MA) was line focused (100 μm × 14 mm) by a single mirror and collimated with the slit optical system of a Luzzati-Baro camera. X-ray diffraction data were recorded by using a linear position sensitive detector (Tennelec, Oak Ridge, TN) and associated electronics (Tracor Northern, Middleton, WI). In all cases, samples were contained in thin-walled quartz capillary tubes (Charles Supper Co., Inc., Natick, MA; internal diameter 1.0 mm) and mounted in a variable-temperature sample holder with a temperature stability of ±1 °C.

Results

Thermotropic Properties of Hydrated MPPC. Following storage at −3 °C for several months, the initial DSC heating curve of fully hydrated (75 wt % H_2O) MPPC recorded at 5 °C/min exhibits two endothermic transitions (Figure 1a). A rather broad, skewed transition is present with a peak maximum at 26 °C (ΔH = 8.0 kcal/mol of MPPC), followed by a sharper transition at 34 °C (ΔH = 8.1 kcal/mol of MPPC). It should be noted that following prolonged incubation at −3 °C, the enthalpies associated with the two transitions are comparable. On cooling to −3 °C at 5 °C/min, only a single transition is observed at 31 °C, ΔH = 7.5 kcal/mol of MPPC (Figure 1b). Immediate reheating shows evidence of a broad, low-enthalpy transition centered at ~20 °C, followed by the high-temperature transition at 34 °C (Figure 1c). Thus, although the high-temperature transition at 34 °C is readily reversible, irreversibility of the low-temperature transition at 26 °C suggests the presence of low-temperature metastable forms of MPPC.

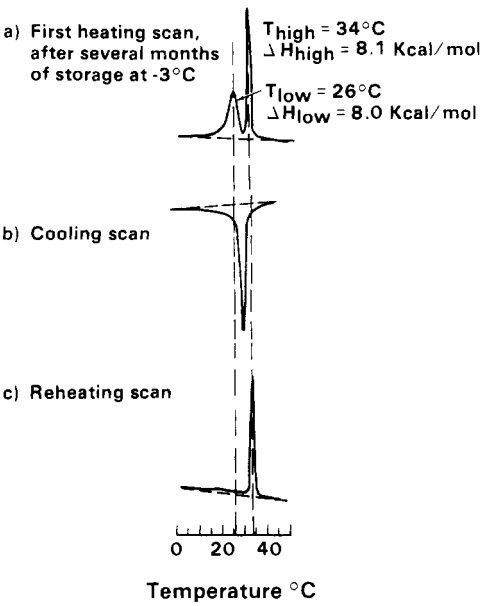


FIGURE 1: DSC heating and cooling curves of hydrated (75 wt % H_2O) MPPC. (a) Initial heating scan following several months storage at −3 °C. (b) Cooling scan immediately following (a). (c) Heating scan immediately following (b). Heating/cooling rates 5 °C/min.

Since other PCs exhibit slow conversion between different low-temperature forms [see Chen et al. (1980), Füldner (1981), and Ruocco & Shipley (1982a,b)], calorimetric studies following incubation for different periods of time at −3 and 7 °C were performed. For this study, MPPC samples were heated to ~50 °C (i.e., above the high-temperature transition), cooled rapidly at 20 °C/min to −3 °C, and then heated to 7 °C at 5 °C/min. Initial DSC heating curves following incubation at 7 °C and then cooling to −3 °C (5 °C/min) are shown in Figure 2. With no incubation at 7 °C, a broad low-enthalpy transition at ~20 °C is followed by the usual high-temperature transition at 34 °C (Figure 2a). After 30-min incubation at 7 °C a low-temperature transition is clearly present at ~23 °C (Figure 2b). With the incubation time at 7 °C increasing up to 9 h (Figure 2b–e), the transition temperature increases slightly, reaching a value of 26 °C, and notably, the transition enthalpy increases significantly, reaching a value of ~7 kcal/mol of MPPC after 9 h.

The enthalpies of the two transitions are plotted as a function of incubation time in Figure 3. Clearly, the enthalpy associated with the high-temperature transition (8.1 kcal/mol of MPPC) is independent of incubation time, indicating complete reversibility of this transition. In contrast, the enthalpy associated with the low-temperature transition depends on the incubation conditions. On both cooling and immediate reheating (Figures 1b, c and 2a), a small transition enthalpy (~1.5) kcal/mol of MPPC) is observed (Figure 3). Incubation at 7 °C results in a fast kinetic process, the low-tem-

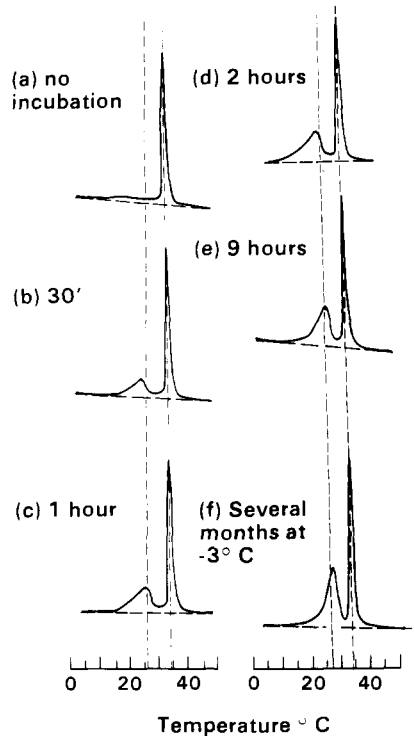


FIGURE 2: DSC heating curves of hydrated (75 wt %) MPPC following incubation at 7 °C for different times. (a) Immediate reheating; (b) 30 min; (c) 1 h; (d) 2 h; (e) 9 h. For the sample stored several months at −3 °C, the initial heating curve is shown in (f). Heating rates 5 °C/min.

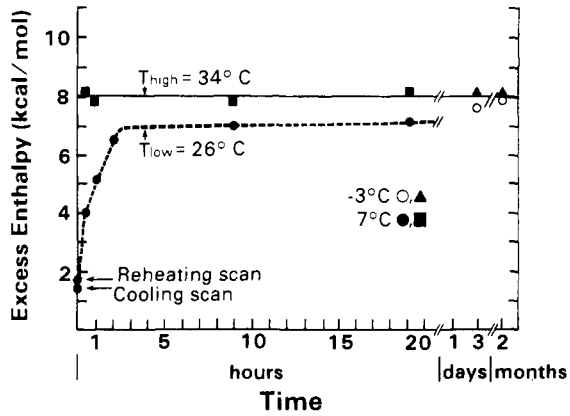


FIGURE 3: Transition enthalpy associated with the high- and low-temperature transitions of MPPC following incubation at 7 [(■) and (●), respectively] and −3 °C [(▲) and (○), respectively]. Heating rates 5 °C/min.

perature transition enthalpy increasing to ~7 kcal/mol of MPPC in the first 2 h, followed by a slower process with only small changes in this transition enthalpy occurring on incubation up to 19 h (Figure 3). For longer term incubation studies, the hydrated MPPC sample was removed from the calorimeter and stored at −3 °C. After the sample was stored for several months at −3 °C, the first DSC heating curve showed a sharper low-temperature transition at 26 °C with an enthalpy of 8.0 kcal/mol of MPPC (Figure 2f). As shown in Figure 3, longer incubation times and/or the lower incubation temperature result in an increase in the enthalpy associated with the low-temperature transition, the limiting value being 8.0 kcal/mol of MPPC.

X-ray diffraction patterns of hydrated (50 wt % H_2O) MPPC were recorded as a function of increasing (Figure 4, right) and decreasing (Figure 4, left) temperature. Following prolonged incubation of hydrated MPPC at −3 °C, an initial X-ray diffraction pattern was recorded at −5 °C. At −5 °C, the low-angle region shows four diffraction spacings with periodicities in the ratio $1:{}^1/_2:{}^1/_3:{}^1/_4$. This lamellar diffraction pattern indicates the presence of a hydrated phospholipid bilayer structure of periodicity, MPPC bilayer plus intercalated water, d = 57 Å. In addition, the wide-angle region shows the presence of a number of reflections in the angular range 1/3.5 to 1/6.0 ($Å^{-1}$). This complex diffraction pattern in the wide-angle region provides clear evidence for a low-temperature phase with specific lateral intermolecular interactions. Clearly, the hydrocarbon chain packing is not of the "hexagonal" type associated with gel-state PC bilayers, which shows primarily a strong reflection at ~1/4.2 $Å^{-1}$ (Tardieu et al., 1973; Janiak et al., 1979). Rather, the wide-angle data strongly suggest that lateral crystallization of the chains into an ordered chain packing mode described by one of the specific, complex subcells has occurred. Similar observations have been made for the stable, low-temperature L_c bilayer phase of DPPC (Füldner, 1981; Ruocco & Shipley, 1982a,b).

As shown in Figure 4 (right), this "crystalline" bilayer phase of MPPC is present at all temperatures up to 25 °C. At 28 and 31 °C, corresponding to temperatures between the low- and high-temperature transitions (see Figure 1), the diffraction pattern changes in three ways: (i) the bilayer periodicity increases to 66 Å; (ii) additional low-angle reflections characteristic of a two-dimensional rippled bilayer phase are present as revealed by high-resolution low-angle diffraction studies (see inset to Figure 4, right); (iii) only a single diffraction line at 1/4.2 $Å^{-1}$ is observed in the wide-angle region. Thus, in the region between the two thermal transitions, the hydrated MPPC transforms to a rippled bilayer phase of ripple period ~210 Å, presumably of the $P_{\beta'}$ (or P_{β}) type originally described by Tardieu et al. (1973). In this intermediate phase, the lateral chain packing is that of the hexagonal type.

At temperatures >34 °C, the bilayer periodicity decreases to 64 Å and the broad, diffuse reflection at 1/4.5 $Å^{-1}$ indicates the presence of the usual L_α bilayer phase with "melted" hydrocarbon chains at temperatures above the high-temperature transition. Thus, the X-ray diffraction data demonstrate the structural changes accompanying the two transitions. The low-temperature endotherm represents a transition between L_c and $P_{\beta'}$ bilayer phases and is accompanied by an increased bilayer periodicity, an altered, less specific chain-packing mode, and bilayer rippling. The high-temperature $P_{\beta'} \rightarrow L_\alpha$ transition reflects the chain-melting transition between bilayer phases.

The behavior on cooling hydrated MPPC from the L_α bilayer phase (Figure 4, left) differs from that observed on heating (see above). The changes in the X-ray diffraction pattern accompanying the $P_{\beta'} \rightarrow L_\alpha$ bilayer transition are reversible on cooling; note the increase in bilayer periodicity to 66 Å and the appearance of the 1/4.2 $Å^{-1}$ reflection at temperatures <34 °C. High-resolution studies of the low-angle region confirm the presence of additional reflections characteristic of the rippled $P_{\beta'}$ phase. This phase is present at temperatures down to ~15 °C, below which a nonrippled bilayer phase of slightly smaller bilayer periodicity, 63 Å, exists. High-resolution diffraction patterns do not show the additional low-angle reflections characteristic of the rippled phase (data not shown). This bilayer phase, presumably $L_{\beta'}$ (or L_β), is present at temperatures down to ~7 °C, below which further changes in the diffraction pattern occur. Below 7 °C, the wide-angle X-ray diffraction pattern changes as the stable low-temperature, L_c, bilayer phase begins to form. In particular, the sharp wide-angle reflection at ~1/4.5 $Å^{-1}$ becomes progressively stronger as the temperature is lowered.

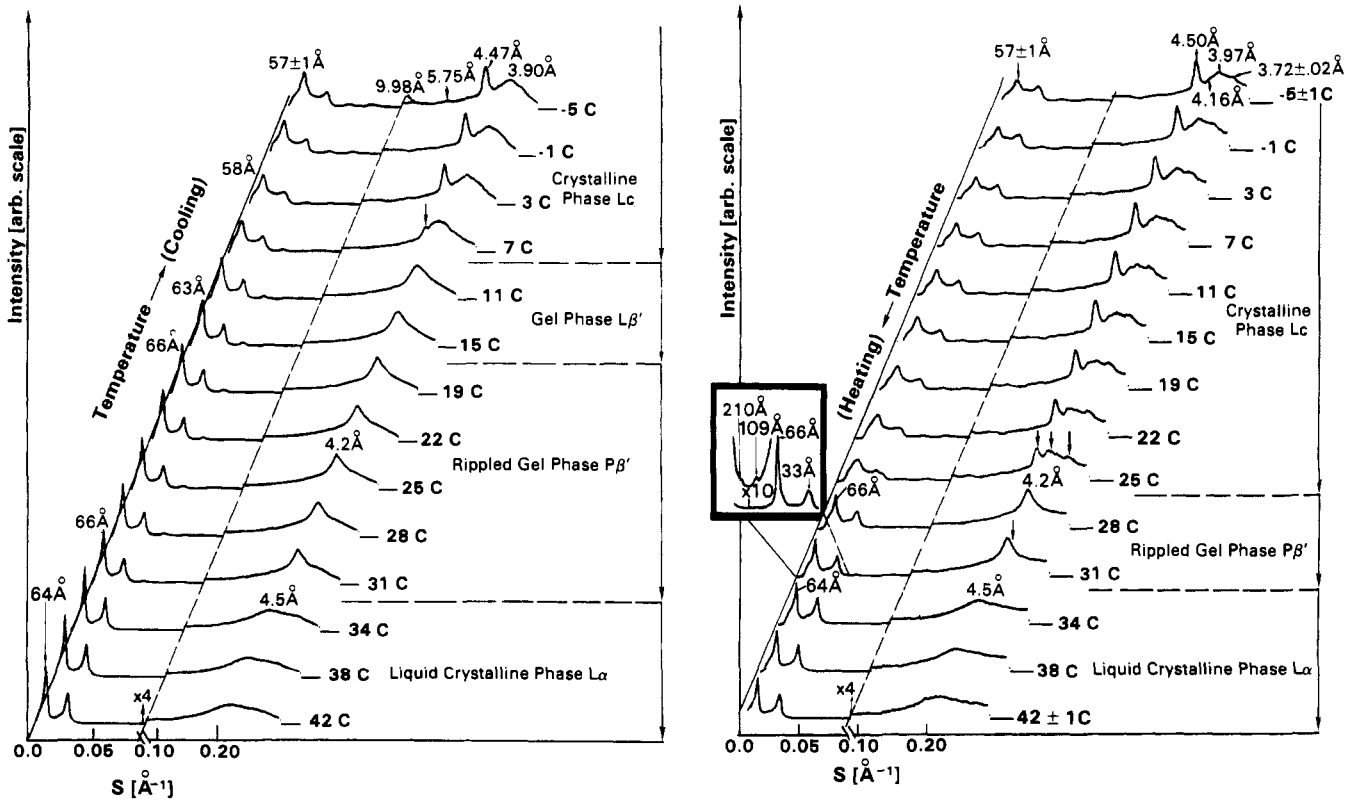


FIGURE 4: X-ray diffraction patterns of hydrated (50 wt % H_2O) MPPC as a function of increasing (right) and decreasing (left) temperature. Intensity data were recorded by using the linear position sensitive detector; sample to detector distance = 123 mm. The acquisition time was 1 h at each temperature. The inset shows the low-angle region at 31 °C collected with a sample to detector distance of 380 mm.

Additional wide-angle reflections characteristic of the L_c phase also appear (see Figure 4, top left), and the bilayer periodicity decreases, reaching a limiting value of 57 Å. As shown clearly in Figure 4, the X-ray diffraction pattern of hydrated MPPC at −5 °C following cooling strongly resembles that of the original low-temperature, equilibrated sample. However, it should be recognized that the changes occurring in the X-ray diffraction pattern at low temperatures probably represent a combination of temperature *and* time effects as the stable L_c bilayer phase forms.

Given that the low-temperature conversion to the stable L_c phase is relatively slow, we have attempted to follow the structural changes accompanying this process. Hydrated MPPC was cooled rapidly from 31 to −3 °C and heated to 7 °C and its X-ray diffraction pattern recorded as a function of time (Figure 5). During the initial 3-min accumulation, the low-angle region is complex with a number of poorly resolved and overlapping diffraction lines in the angular range 30–70 Å, providing evidence for more than one bilayer phase (see Figure 5). At this stage, the dominant, albeit somewhat broad, peak at $1/4.2$ Å^{-1} resembles that present at 31 °C in the gel $P_{\beta'}$ phase. With increasing time at 7 °C, the low-angle pattern becomes progressively less complex and after 2 h resembles that of the L_c phase in both periodicity (57 Å) and intensity distribution. In addition, during this time period the sharp reflection at $1/4.5$ Å^{-1} becomes dominant, and other wide-angle reflections characteristic of the L_c phase are apparent. After 24 h, the diffraction pattern is essentially identical with that of an MPPC sample that had undergone long-term (2-week) low-temperature equilibration at −3 °C.

Thermotropic Properties of Hydrated PMPC. Fully hydrated (75 wt % H_2O) PMPC following prolonged storage at −3 °C also exhibits two endothermic transitions of comparable enthalpies in the initial DSC heating curve (Figure 6a). The broad, skewed transition (peak maximum, 17 °C; ΔH = 7.7

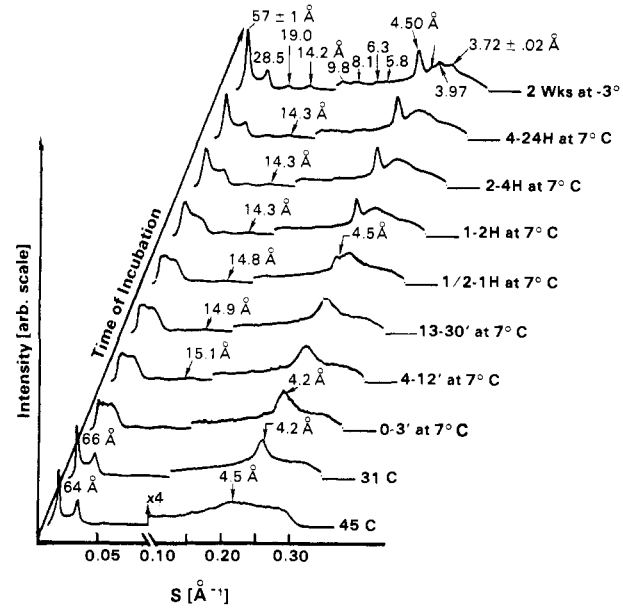


FIGURE 5: Kinetics of formation of the stable L_c phase of hydrated (50 wt % H_2O) MPPC. X-ray diffraction patterns were recorded at 45 and 31 °C (bottom), and then the sample was cooled rapidly to −3 °C and then incubated at 7 °C. Diffraction patterns were then recorded at 7 °C as a function of increasing time. Sample to detector distance = 123 mm; acquisition times as indicated.

kcal/mol of PMPC) is followed by a sharper high-temperature transition at 27 °C (ΔH = 7.8 kcal/mol of PMPC). On cooling (Figure 6b) at 5 °C/min, only the high-temperature transition is observed (T_c = 26 °C, ΔH = 6.9 kcal/mol of PMPC). On immediate heating (Figure 6c), the lower transition is not readily observed; the transition temperature and enthalpy of the upper transition are identical with those observed on initial heating (cf. Figure 6a).

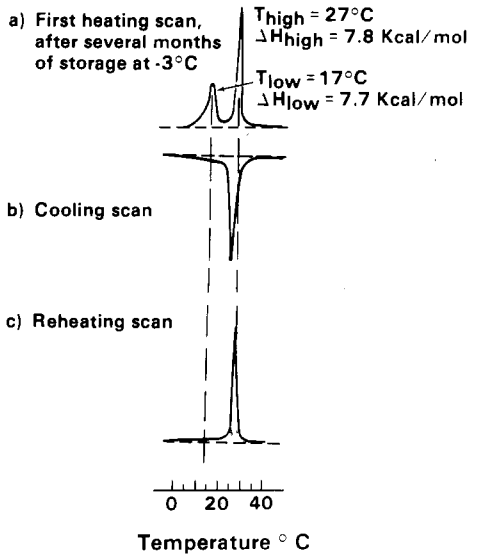


FIGURE 6: DSC heating and cooling curves of hydrated (75 wt % H_2O) PMPC. (a) Initial heating scan following several months storage at −3 °C. (b) Cooling scan immediately following (a). (c) Heating scan immediately following (b). Heating/cooling rates 5 °C/min.

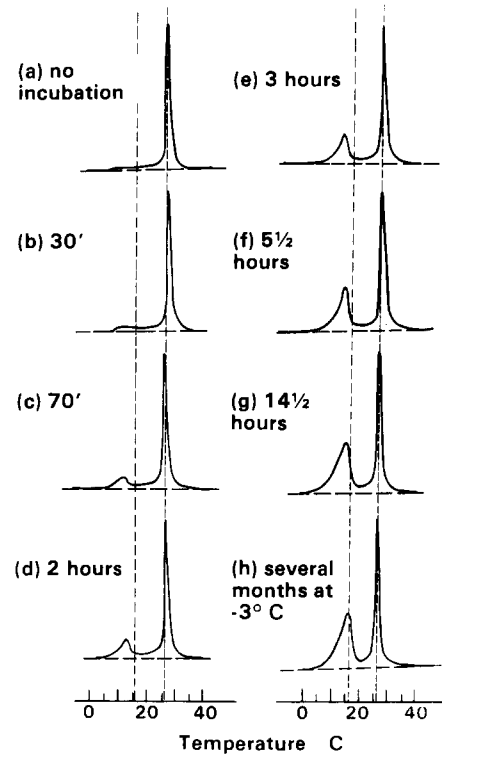


FIGURE 7: DSC heating curves of hydrated (75 wt % H_2O) PMPC following incubation at −3 °C for different times. (a) Immediate reheating; (b) 30 min; (c) 70 min; (d) 2 h; (e) 3 h; (f) 5.5 h; (g) 14.5 h. For the sample stored several months at −3 °C, the initial heating curve is shown in (h). Heating rates 5 °C/min.

The recovery of the low-temperature transition (reflecting the kinetics of formation of the low-temperature phase) was monitored following incubation of PMPC at different temperatures for increasing times. For example, the DSC heating curves following rapid cooling from the high-temperature phase (50 °C) and incubation at −3 °C for increasing periods of time are shown in Figure 7. With increasing time at −3 °C, a broad low-enthalpy transition progressively sharpens, increases in transition temperature, and markedly increases in transition enthalpy (Figure 7a–g). Qualitatively, at least, the DSC heating curve followed 14.5-h incubation at −3 °C (Figure 7g) resembles that of hydrated PMPC following prolonged low-

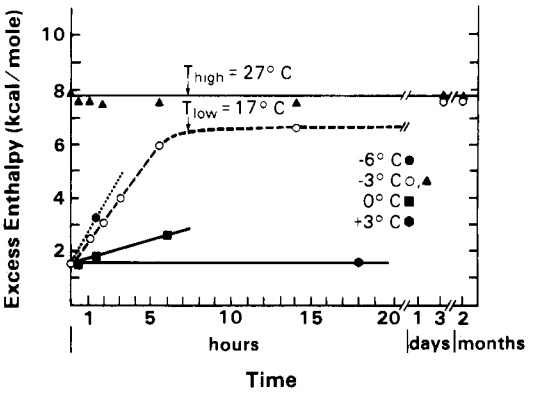


FIGURE 8: Transition enthalpy associated with the high- and low-temperature transition of hydrated (75 wt % H_2O) PMPC following incubation at different temperatures. High-temperature transition enthalpy following incubation at −3 °C (▲); low-temperature transition following incubation at 3 (●), 0 (■), −3 (○), and −6 °C (●). Heating rates 5 °C/min.

temperature equilibration (see Figures 6a and 7h). Similar, albeit less extensive, DSC studies were used to follow the kinetics of recovery of the low-temperature transition at other temperatures, i.e., −6, 0, and 3 °C.

The enthalpy data for the low- and high-temperature transitions as a function of incubation time are summarized in Figure 8. For hydrated PMPC, the enthalpy associated with the high-temperature transition is essentially independent of storage time at −3 °C (ΔH = 7.8 kcal/mol of PMPC). In contrast, at −3 °C at least three kinetic processes appear to be associated with the formation of the stable low-temperature form as monitored by the enthalpy of the low-temperature transition: (i) a rapid, essentially reversible process that accounts for ~1.5 kcal/mol of PMPC; (ii) a slower, approximately linear process (up to ~6 h) that corresponds to recovery of ~6 kcal/mol of PMPC; (iii) an even slower process (days) whereby the total enthalpy associated with the low-temperature transition (7.7 kcal/mol of PMPC) is recovered. As shown in Figure 8, the overall kinetic process is extremely sensitive to the equilibration temperature. At 3 °C, only the initial fast step is observed, and after 18 h the enthalpy associated with the low-temperature transition remains at ~1.5 kcal/mol of PMPC, suggesting that the other two processes do not occur significantly at this temperature. At 0 °C, the initial fast process occurs, but the kinetics of the second process are much slower than at −3 °C. Finally, at −6 °C, the initial process is followed by what appears to be a slightly faster secondary kinetic process. Thus, on the basis of the data presented in Figure 8, it would appear that at least one of the steps (ii, above) involved in the formation of the stable low-temperature form is quite temperature dependent.

X-ray diffraction data have been recorded as a function of temperature for PMPC at different hydrations. The most detailed study is that for PMPC containing 25 wt % water (Figure 9). Following prolonged incubation at −3 °C, the PMPC sample was cooled to −9 °C and the initial X-ray diffraction recorded (Figure 9, top right). The low-angle region shows a series of lamellar reflections, h = 1–4, of periodicity 57 Å. The wide-angle region shows a number of reflections, the most prominent appearing at 1/9.5, 1/6.73, 1/4.47 and a rather broad reflection at 1/3.96 $Å^{-1}$, again indicative of an ordered crystalline L_c bilayer phase. This diffraction pattern remains essentially unchanged up to 12 °C, at which temperature the bilayer periodicity increases to 62 Å, the intensity distribution of the low-angle reflections changes, and only a single reflection at 1/4.2 $Å^{-1}$ is observed

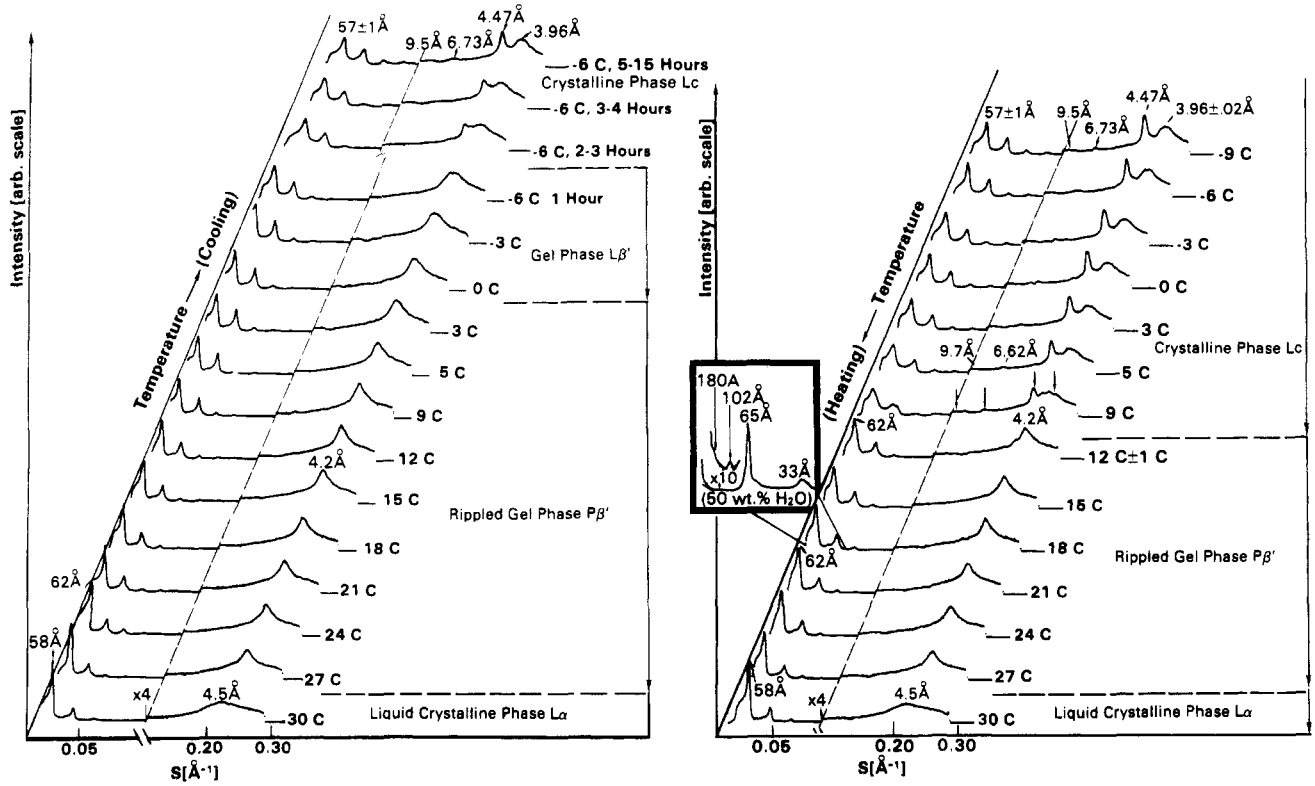


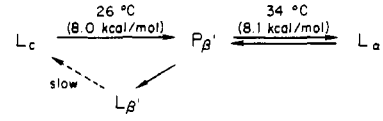
FIGURE 9: X-ray diffraction patterns of hydrated (25 wt % H_2O) PMPC as a function of increasing (right) and decreasing (left) temperature. Intensity data were recorded by using the linear position sensitive detector; sample to detector distance = 123 mm. Inset shows the low-angle diffraction pattern of hydrated (50 wt % H_2O) PMPC at 18 °C collected with a sample to detector distance of 380 mm.

in the wide-angle region. This diffraction pattern is observed from 12 to 27 °C, corresponding to the temperature range where the rippled $P_{\beta'}$ (or P_β) phase is present (see below). At 30 °C, above the high-temperature transition, the diffraction pattern corresponds to that of a lamellar L_α bilayer phase (bilayer periodicity = 58 Å; broad reflection at $\sim 1/4.5$ Å^{-1}). On cooling (Figure 9, left), the gel → liquid-crystal transition of PMPC is reversible, the diffraction pattern corresponding to the gel phase reappearing at 27 °C. This phase exhibits supercooling and is present down to ~3 °C. At lower temperatures, conversion to a different bilayer $L_{\beta'}$ (or L_β) gel phase (see below) is followed by a time- and temperature-dependent conversion back to the original L_c crystalline form. After 15 h at −6 °C, the X-ray diffraction pattern is almost identical with that observed following prolonged incubation at −3 °C (cf. Figure 9, top left and top right).

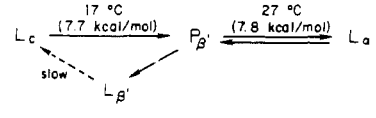
A similar series of experiments was performed on PMPC dispersions containing 50 wt % water. A similar pattern of behavior is observed. The diffraction pattern of the L_c phase (d = 57 Å) is identical with that observed at lower hydration (25 wt % water, see above). On heating, at 12 °C the L_c phase converts to a bilayer phase of periodicity 65 Å. Higher resolution studies at 18 °C show clearly the presence of additional low-angle reflections characteristic of a two-dimensional $P_{\beta'}$ (or P_β) structure (see Figure 9, inset). The wavelength or period of the ripple is ~180 Å. At >27 °C, a liquid-crystalline L_α phase is present (d = 64 Å). On cooling, a similar pattern of structural changes to that observed at lower (25 wt %) hydration (see above) occurs. The rippled $P_{\beta'}$ phase reappears on cooling, and then at lower temperatures, the $P_{\beta'}$ phase converts to a nonrippled bilayer gel phase (d = 63 Å). The conversion from the bilayer gel phase to the low-temperature, stable L_c phase is much slower than at 25 wt % H_2O.

Discussion

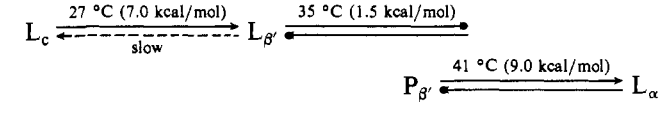
Our detailed calorimetric and X-ray diffraction study of the two positional isomers of PC containing C-14 and C-16 fatty acyl chains demonstrates that samples of hydrated MPPC and PMPC incubated at −3 °C exhibit similar thermotropic and structural behavior. However, differences are found in the transition temperatures and in the kinetics of formation of the low-temperature crystalline phase L_c. Compared to DPPC, the effect of replacing a C-16 chain with a C-14 chain at either the *sn*-1 (MPPC) or *sn*-2 (PMPC) position is as follows. A reduction in the chain length of the *sn*-2 fatty acid has a greater effect on lowering the chain melting transition temperature than does a comparable reduction at the *sn*-1 position: T_c(DPPC) (41 °C) > T_c(MPPC) (34 °C) > T_c(PMPC) (27 °C) > T_c (DMPC) (24 °C). However, the two transition enthalpies of MPPC are comparable to those of PMPC. Perhaps, surprisingly, given the different molecular geometries (see Figure 10), the overall thermotropic behavior and structures formed by MPPC and PMPC are quite similar and can be summarized as follows. For MPPC



For PMPC



In comparison, DPPC shows the following sequence of phase changes:



The subtransition at 27 °C was observed following incubation at −3 °C for 1 year.

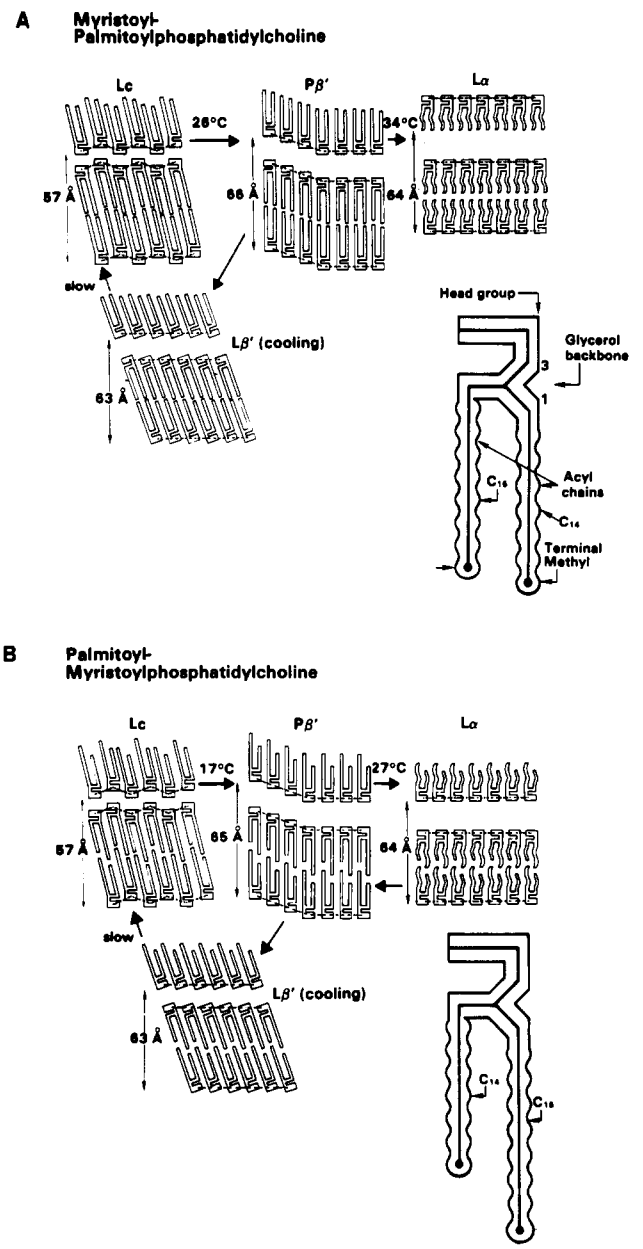


FIGURE 10: Schematic models and summary of the structural changes exhibited by hydrated MPPC (A) and PMPC (B) with increasing and decreasing temperature. Bilayer parameters are given for 50 wt % H_2O dispersions.

In contrast to DPPC, at the low-temperature transition the ordered L_c bilayer phase of both MPPC and PMPC transforms directly to the rippled $P_{\beta'}$ phase without going through the "planar" bilayer $L_{\beta'}$ gel phase. Thus, this transition corresponds to the structural changes associated with the combined effects of a sub- and pretransition of DPPC. By analogy with our earlier analysis of DPPC transitions (Ruocco & Shipley, 1982b), the specific chain packing modes (or subcells) stabilizing the L_c phases of MPPC and PMPC transform directly to that of the hexagonal subcell. On heating, the "distorted" hexagonal or orthorhombic chain packing mode characteristic of the $L_{\beta'}$ phase is not observed. On cooling, the behavior of MPPC and PMPC more closely resembles that of DPPC. In both cases, the $L_\alpha \rightarrow P_{\beta'}$ transition is readily reversible; however, the $P_{\beta'}$ phase exhibits significant supercooling. Although our data are not extensive, there is evidence that under the slow cooling conditions of the X-ray diffraction experiments structural changes occur at ~15 and 0 °C for MPPC and PMPC, respectively. In both cases, the decrease in bilayer periodicity and lack of nonlamellar reflections *suggest* that a conventional $L_{\beta'}$ bilayer gel structure is formed and that it is this $L_{\beta'}$ phase not the rippled $P_{\beta'}$ phase that slowly converts to the ordered L_c bilayer phase. Three kinetic processes appear to be involved in the formation of the L_c phase of both MPPC and PMPC (see Figures 3 and 8). Upon cooling, a fast process occurs almost reversibly and is probably related to the $P_{\beta'} \rightarrow L_{\beta'}$ transition. The two slower events have half-lives of hours and days. A similar pattern of kinetic behavior accompanies the formation of the L_c phase in hydrated α-DPPC. The slow, temperature-dependent processes (see Figures 3 and 8) presumably account for the variability in the transition enthalpies reported for the subtransition of α-DPPC, range 3.5–7 kcal/mol of DPPC (Chen et al., 1980; Füldner, 1981; Ruocco & Shipley, 1982a,b), and the low-temperature transition of both MPPC and PMPC, range 0–9 kcal/mol) (Keough & Davis, 1979; Chen & Sturtevant, 1981; Stümpel et al., 1981, 1983). The X-ray diffraction patterns in the wide-angle region for the low-temperature crystalline phase L_c from MPPC and PMPC are very similar to each other and to the diffraction pattern of the corresponding L_c phase in DPPC (Füldner, 1981; Ruocco & Shipley, 1982a,b) with a dominant sharp reflection at $\sim 1/4.5$ Å^{-1} and a broad shoulder at about $1/3.9$ Å^{-1}. This suggests that the chain packing modes, at least, may be closely related for all three PCs in the L_c phase. It is also worth noting that the total enthalpy and, particularly, entropy changes on going from the L_c to L_α phases (expressed per CH_2 group per molecule) are almost identical for MPPC [ΔH = 0.62 kcal/mol; ΔS = 2.04 cal/(mol K)], PMPC [ΔH = 0.60 kcal/mol; ΔS = 2.04 cal/(mol K)], and DPPC (ΔH = 0.62 kcal/mol; ΔS = 2.03 cal/(mol K)]. Thus, in spite of different pathways (see above), the overall thermodynamic changes between the crystalline-chain and melted-chain bilayers are essentially identical.

Recently, Stümpel et al. (1983) have reported calorimetric and X-ray diffraction data for an extensive range of identical-chain, mixed-chain, and β-phosphatidylcholines. In all cases except DMPC, they identify an ordered low-temperature phase similar to the L_c phase described in this study. On the basis of their data, Stümpel et al. (1983) have suggested a unified general pattern of thermotropic behavior for all of these compounds. Our more detailed kinetic and structural studies show that although MPPC, PMPC, and β-DPPC (Serrallach et al., 1983) show some similarities, in particular their ability to form ordered low-temperature bilayer phases, they do differ in terms of the structures formed in the temperature region between the two transitions. β-DPPC exhibits an interdigitated "monolayer" structure (Serrallach et al., 1983) and not the rippled $P_{\beta'}$ structure as suggested by the general scheme of Stümpel et al. (1983), whereas the mixed-chain compounds MPPC and PMPC form rippled bilayer phases, similar to those of DMPC and DPPC, in the intermediate temperature range. Thus, although there are similarities in the overall thermotropic properties of all of these compounds, the β-PCs do follow a somewhat different pattern of behavior, clearly reflecting their different molecular conformation around the glycerol region [see Seelig et al. (1980), Büldt & de Haas (1982), and Serrallach et al. (1983)].

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Registry No. MPPC, 69525-80-0; PMPC, 69441-09-4.

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Spontaneous Phosphatidylcholine Exchange between Small Unilamellar Vesicles and Lipid-Apolipoprotein Complexes. Effects of Particle Concentrations and Compositions†

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ABSTRACT: We investigated the spontaneous exchange of phosphatidylcholine (PC) between model lipoproteins and small unilamellar vesicles. The model lipoproteins were recombinants of bovine apolipoprotein A-I, egg PC, and cholesterol. Vesicles contained egg PC and cholesterol in varying molar ratios. Lipid exchange was followed by incubating radiolabeled vesicles ([^{3}H]PC plus cholesteryl [^{14}C]oleate as a nonexchangeable marker) with unlabeled lipid-apolipoprotein complexes at a constant temperature of 37 °C. Incubation mixtures were fractionated on Bio-Gel A-5m columns at 5 °C, and the ^{3}H cpm/^{14}C cpm ratio under the vesicle peak was determined. Comparison of this ratio with that of vesicles before incubation was used to calculate the proportion of radiolabel transferred to complexes at various times. The results show that no net transfer of PC occurs under our experimental conditions; 70% of vesicle PC exchanges with complexes, indicating that only the outer monolayer of the vesicle is available for exchange; in contrast, all of the complex PC is exchangeable. Exchange is temperature dependent with an activation energy of 22.9 ± 2.0 kcal/mol. Under our experimental conditions the rate of PC exchange is linearly dependent upon both vesicle and complex PC concentrations with a rate constant of 6.9 ± 0.7 μM^{-1} h^{-1}, and the rate constant varies inversely with the cholesterol content of vesicles or complexes. These results imply that a complete kinetic model of spontaneous lipid exchange must account for the dependence of exchange rates not only on the concentration and composition of the donor particles but on the concentration and chemical nature of the acceptors, as well.

Exchange and transfer of protein and lipid components among lipoprotein classes or between lipoproteins and cell membranes are essential processes which determine the steady-state composition, structure, and metabolism of lipoproteins (Bell, 1978; Smith et al., 1978; Miller & Gotto, 1982). One aspect of these processes is the exchange and transfer of phospholipids, involving high-density lipoproteins (HDL)[1] (Scanu et al., 1982). Influx of phospholipids, particularly

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[1] Abbreviations: PC, phosphatidylcholine; HDL, high-density lipoproteins; apo A-I, apolipoprotein A-I; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; SDS, sodium dodecyl sulfate; DPPC, dipalmitoylphosphatidylcholine; SUV, small unilamellar vesicles.

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