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Calorimetric and fatty acid spin label study of subgel and interdigitated gel phases formed by asymmetric phosphatidylcholines

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Several saturated asymmetric and symmetric phosphatidylcholines were studied by ESR spectroscopy and differential scanning calorimetry in order to determine the behavior of a fatty acid spin labeled near its terminal methyl, 16-doxylstearate, in the mixed interdigitated gel phase and the L_c subgel phase and other properties of these lipids. This spin label was motionally restricted in the mixed interdigitated gel phases of 18:10PC and 18:12PC. The motional restriction was similar to that reported earlier for fully interdigitated phases. This spin label was motionally restricted almost to the same degree in 10:18PC suggesting that this asymmetric lipid may also form a mixed interdigitated bilayer. In contrast the spin label had more motion in the gel phase of 18:14PC than in symmetric forms of PC, consistent with conclusions from X-ray diffraction studies that this less asymmetric lipid does not form a mixed interdigitated phase. The spin label was partially frozen out of the L_c subgel phases of symmetric forms of PC and 18:14PC formed by storage at low temperature. The phase behavior of the other asymmetric lipids also depended on the sample history. Storage at low temperature caused 10:18PC and 18:12PC to go into ordered phases. The enthalpy of the transition of these ordered phases to the liquid-crystalline phase was 2–2.4-times greater than that of the transition of the gel phase formed on cooling back from the liquid-crystalline phase. The temperature of this high enthalpy transition was 0.8 K below that of the lower enthalpy gel to liquid-crystalline phase transition for 18:12PC, but 4.6 K higher for 10:18PC. The spin label was frozen out of these ordered phases, as it was out of the L_c subgel phases, suggesting that 18:12PC and 10:18PC may also form an L_c phase. 18:10PC was not observed to form an ordered phase although storage of the sample at low temperatures did affect the temperature of its transition from the liquid-crystalline phase back to the gel phase upon cycling through its phase transition.

Abbreviations: DSC, differential scanning calorimetry; ESR, electron spin resonance; 18:18PC, L-α-distearoylphosphatidylcholine; 16:16PC, L-α-dipalmitoylphosphatidylcholine; 14:14PC, L-α-dimyristoylphosphatidylcholine; PC, phosphatidylcholine; 18:10PC, 1-stearoyl-2-caproyl-*sn*-glycero-3-phosphocholine; 18:12PC, 1-stearoyl-2-lauroyl-*sn*-glycero-3-phosphocholine; 18:14PC, 1-stearoyl-2-myristoyl-*sn*-glycero-3-phosphocholine; 10:18PC, 1-caproyl-2-stearoyl-*sn*-glycero-3-phosphocholine.

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Introduction

Lipid phase behavior has been shown in recent years to be more complex than previously thought, even for synthetic saturated forms of phosphatidylcholine (PC). Asymmetric forms of PC and sphingolipids can form interdigitated bilayers in which the van der Waals interactions between the unequal chains are maximized [1–5]. This interdigitation may take two forms. If the difference in chain length is great enough, as for 18 : 10PC, 18 : 12PC [1,2], and lignoceroyl-sphingomyelin [3], a mixed interdigitated phase is formed in which the two short chains of lipid on each side of the bilayer are packed end to end while the longer chain from lipid on both sides completely spans the bilayer width. If the difference in chain length is smaller as for 18 : 14PC, a partially interdigitated bilayer might form in which the shorter chain of lipid on one side of the bilayer is packed end to end with the longer chain of lipid on the other side of the bilayer [6]. This type of structure has not yet actually been established by X-ray diffraction for any lipid. However, changes in the chain conformation of lignoceroyl-sphingomyelin with temperature, detected by Raman spectroscopy, are consistent with a transition from a mixed interdigitated to a partially interdigitated phase [3].

X-ray diffraction studies have shown that in the presence of amphiphilic compounds such as glycerol, polymixin, acetylcholine, chlorpromazine and alcohols, symmetric forms of PC and other phospholipids can form a fully interdigitated phase in which both fatty acid chains of lipid on both sides of the bilayer completely span the bilayer [7–12]. Interdigitation compensates for the lipid expanding effects of these amphiphilic substances and maximizes the van der Waals interactions between the chains. Based on the behavior of a fatty acid spin label it was suggested that a fully interdigitated structure may also form in the presence of myelin basic protein [13].

In addition to these interdigitated phases and the non-interdigitated L_β gel phase, both symmetric and asymmetric forms of PC can form a more stable, more closely packed, and less hydrated L_c subgel phase after prolonged storage at a low temperature [14–21].

Studies of these symmetric and asymmetric saturated lipids by calorimetry [14,18,22–25] and spectroscopic [3,20,21,26–29] techniques have provided further information about their properties, have been useful at suggesting the structure of the phases formed, and have detected transitions between interdigitated and non-interdigitated phases and between the subgel and gel phases. However, in general, X-ray diffraction [1,2,4,6–12,15–19] has been necessary to establish the structure of the various phases formed. In order to evaluate the predictive and diagnostic ability of calorimetric and spectroscopic techniques it is necessary to use them to study lipid phases whose structures have been established by X-ray diffraction and correlate the information provided by these techniques with the structure of the lipid phase.

We found earlier that the maximum hyperfine splitting, T_{max}, of the spectrum of a fatty acid spin labeled near the terminal methyl, 16-doxylstearate, is significantly greater in fully interdigitated lipid bilayers than in non-interdigitated gel phase lipid bilayers [28]. The lipid systems studied were symmetric forms of PC and PG in the presence of glycerol and polymixin, which had been shown by X-ray diffraction to be fully interdigitated. In these interdigitated bilayers the T_{max} value of 16-doxylstearate was similar to that of a fatty acid spin labeled closer to the head group region, 5-doxylstearate, while it is considerably less in non-interdigitated bilayers. This result is to be expected for a fully interdigitated bilayer since the terminal methyls of the fatty acid chains of lipid on one side of the bilayer are located near the bilayer-aqueous interface on the other side of the bilayer. A similar effect on 16-doxylstearate should occur in a mixed interdigitated bilayer. However, the effect of partial interdigitation on the spectrum of 16-doxylstearate is less easy to predict.

In the present study several saturated asymmetric and symmetric phosphatidylcholines have been studied by ESR spectroscopy and differential scanning calorimetry, in order to determine the behavior of 16-doxylstearate in the mixed interdigitated gel and the L_c subgel phases, and other properties of these lipids.

Materials and Methods

Materials. The asymmetric forms of PC were synthesized as described [30]. The symmetric forms, 14:14PC, 16:16PC, and 18:18PC were purchased from Sigma Chemical Co. All lipids were chromatographically pure. Fatty acid analysis of the asymmetric forms was performed as described [25] and all were at least 98% isomerically pure. The fatty acid spin labels, 5-doxylstearate and 16-doxylstearate were purchased from Syva, Palo Alto, CA.

Preparation of lipid dispersions for calorimetry. The lipid was dispersed in distilled water at a concentration of 7 mg/1.6 ml, by vortex mixing at a temperature above the lipid phase transition temperature. The suspension was divided into five aliquots. Each was centrifuged in an Eppendorf bench centrifuge for 5 min and the wet pellet was loaded into an aluminum DSC pan. One pan was scanned on the DSC before freezing while the rest were frozen at −20°C and scanned after storage for varying periods of time. The samples were run on a Perkin-Elmer DSC-2 equipped with a Perkin-Elmer Data Station. The frozen samples were kept frozen while transferring to the DSC sample holder set at 3°C. They were left at this temperature for 10 min to allow the ice to thaw. They were then cooled to −8°C briefly (the water does not freeze under these conditions) and scanned at heating rates of 10 K/min and 5 K/min. Cooling scans at similar rates were also performed. The temperature of maximum heat absorption or release was defined as the phase transition temperature, T_m for the main transition from the gel to liquid-crystalline phase, T_s for the transition from the subgel phase, and T_p for the premelt transition. The transition temperatures obtained on cooling were corrected for instrumental hysteresis. For heating and cooling rates of 5 K/min, the correction factor was found to be 3.3 K. The enthalpies of these transitions, ΔH_m, ΔH_s, and ΔH_p, were calculated by obtaining the areas of the peaks using the data station and the amount of lipid in the pan as described [28].

Preparation of lipid dispersions for ESR spectroscopy. Samples labeled with spin label were prepared similarly except that the lipid and spin label were first dissolved together at a 150:1 mole ratio in chloroform/methanol (1:1, v/v), the solvent was evaporated and the lipid was dispersed as described above. Several aliquots were loaded into 100 μl capillary tubes for ESR measurement, sealed at one end with a flame and centrifuged at 2000 rpm. The other end was sealed with parafilm and some of the samples were stored in the freezer at −20°C for varying periods of time. ESR spectra were measured on a varian E-104B spectrometer equipped with a Varian temperature controller and a DEC LSI-11 based microcomputer system. The maximum hyperfine splitting, $2T_{max}$, of the ESR spectra, order parameter S, and motional parameter, τ_0, were measured as described earlier [13]. The microwave power used was 10 mW.

Results

18:10PC and 18:12PC

These two greatly asymmetric species of PC were predicted to be interdigitated on the basis of the change in entropy during their gel to liquid-crystalline phase transitions [25] and were subsequently shown to form mixed interdigitated phases by X-ray diffraction [1,2]. The transition profiles obtained on heating and cooling are shown in Fig. 1 and the corresponding thermodynamic parameters are listed in Table I. Thermodynamic parameters from heating scans of these lipids were reported earlier [25] but not from cooling scans. The temperatures and enthalpies of these two lipids are more similar to each other than those reported earlier.

The phase behavior depended on the sample history. Prolonged storage of 18:10PC in the freezer (−20°C) had no effect on heating scans but increased the phase transition temperature observed on cooling. Cooling scans of a freshly prepared sample and a sample stored at −20°C are shown in Fig. 1b and c, respectively. The freshly prepared sample refroze at a temperature about 4 K below the melting temperature (after correction for instrumental hysteresis) but with a similar enthalpy, while most of the sample which had been stored at −20°C refroze at nearly the same temperature as the melting temperature (Fig. 1c). A small portion of the sample froze at the lower temperature. (See Table I for transition

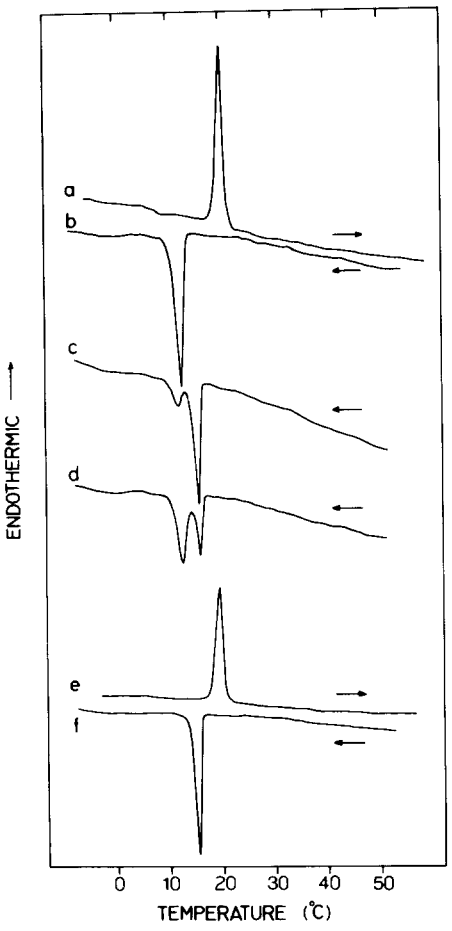

Fig. 1. DSC heating and cooling thermograms of (a–d) 18:10PC and (e, f) 18:12PC. (a) Heating scan of a freshly prepared sample of 18:10PC; (b) subsequent cooling scan; (c) cooling scan after storage of sample for several days at −20°C (heating scan was similar to that in (a)); (d) cooling scan after cycling through phase transition several times after (c); (e) heating scan of freshly prepared sample of 18:12PC; (f) subsequent cooling scan. Heating and cooling rates were 5 K/min. Sensivity settings in mcal/sec were (a–d) 0.5 and (e, f) 1.0. The phase transition temperatures on cooling are 3.3 K below those on heating at a rate of 5 K/min due to instrumental hysteresis. The values for cooling scans in Table I have been corrected for this difference.

temperatures corrected for hysteresis on heating and cooling; the scans shown in the figures have not been corrected.) The appearance of the scan in Fig. 1c did not depend on the cooling rate. However, after repeated cycling through the phase transition, the height of the lower temperature peak on cooling increased at the expense of the higher temperature one as shown in Fig. 1d and more of the sample refroze at the lower temperature, similar to the freshly prepared sample.

18:12PC normally had only one transition on heating and cooling (Fig. 1e, f) with an enthalpy of 7.9 kcal/mol. At a heating and cooling rate of 5 K/min the T_m on cooling was 1 K lower than that on heating. This difference was greater at a rate of 10 K/min indicating a kinetic barrier to reformation of the gel phase. Although prolonged storage of the sample in the freezer had no effect on its heating or cooling scans, incubation under more controlled conditions in the DSC at −18°C for 1–2 h caused it to go into a phase whose transition to the liquid-crystalline phase occurred at a temperature 0.8 K lower and with an enthalpy two times greater than that of the gel phase (Table I). The phase formed after incubation at low temperatures is designated phase II while that giving the lower enthalpy transition is phase I. Cycling through the high enthalpy phase transition gave phase I.

The ESR spectrum of 16-doxylstearate in 18:10PC is compared to that in 18:18PC and 18:12PC and to that of 5-doxylstearate in 18:10PC at 4°C in Fig. 2. The spectral parameters are given in Table II. The T_{max} value of 16-doxylstearate in 18:10PC is only 1.7 G less than that of 5-doxylstearate in this lipid and considerably greater than that of 16-doxylstearate in 18:18PC. The T_{max} value of 16-doxylstearate in phase I of 18:12PC is similar to that in 18:10PC. These large values of T_{max} are as expected for a mixed or fully interdigitated bilayer and distinctly different from a non-interdigitated bilayer. However, the appearance of the spectra in 18:10PC and 18:12PC (Fig. 2A, E) and the values of T_{max}, which are slightly below 30 G, indicate that 16-doxylstearate is not as well-ordered or motionally restricted in the asymmetric lipids as in the fully interdigitated phases of symmetric forms of PC and PG with glycerol or polymixin [28]. This may be due to the smaller temperature interval between the temperature of measurement, 4 K, and the T_m values of the asymmetric lipids compared to the symmetric lipids.

Differences in the phase transition temperature on cooling of fresh and stored samples of 18:10PC, similar to those observed by calorime-

TABLE I

THERMODYNAMIC PARAMETERS OF PHASE TRANSITIONS OF ASYMMETRIC AND SYMMETRIC FORMS OF PHOSPHATIDYLCHOLINE [a]

T in °C; ΔH in kcal/mol; ΔS in cal/mol per K.

	Heating							Cooling [e]			
	T_p	T_s	T_m	ΔH_p	ΔH_s	ΔH_m	ΔS_m	T_1	T_2	ΔH_1	ΔH_2
18:10PC			20.0			8.2±0.6	28.0	15.8 [f]	19.3 [f]	8.5 [g]	6.1 [h]
18:12PC											
1st scan [b]		18.9			15.8						
rescan			19.7			7.9±0.9	26.0		18.7		7.8±0.9
18:14PC											
1st scan [b]		25	31		9.4 [c]	6.0±0.5	19.7				
rescan	17.8		31	2.0 [c]		6.0±0.5		12.4	30.7	1.1	5.6±0.3
10:18PC											
1st scan [b]		14.4 [d]			13.6±0.3 [c,d]						
rescan			9.8			5.7±0.4	20.2		9.9		5.8±0.5
18:18PC	50.9		55.2	1.3±0.4		10.6±0.7	32.3		55.2		10.8±0.6

[a] At heating and cooling rates of 5 K/min.
[b] After freezing of sample.
[c] Maximum observed when no other peaks other than main transition present.
[d] This transition may be equivalent to subgel transition even though it occurs at a higher temperature than T_m (see text).
[e] Cooling temperatures corrected for instrumental hysteresis by addition of 3.3 K. Where 2 peaks are observed, T_1 is the lower temperature peak and T_2 the higher; T_2 is equivalent to T_m on heating.
[f] Ratio varies with repeated heating.
[g] Value when only peak 1 present.
[h] Maximum value observed. Some of peak 1 always present.

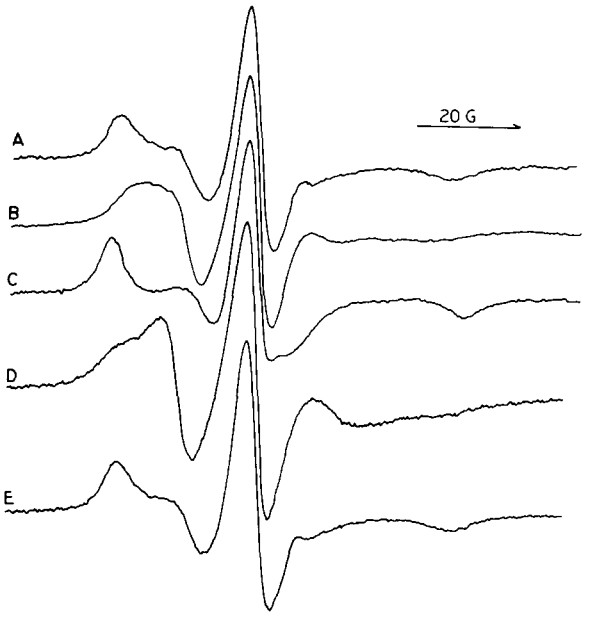

Fig. 2. ESR spectra of 16-doxylstearate at 4°C in (A) 18:10PC, freshly prepared sample; (B) 18:18PC, freshly prepared; (D) 18:12PC, after storage at −20°C (phase II); (E) 18:12PC after cycling through the phase transition once (phase I). The spectrum of 5-doxylstearate in 18:10PC at 4°C is shown in (C). (D) is plotted out amplified 2.5-times compared to (E). Thus the height of the middle peak in (D) is 0.43-times that of (E) due to exchange broadening in (D).

try, could be detected from changes in the motion of the spin label (not shown). However, the spectral parameters of the lipid above and below the phase transition in both cases were identical suggesting that the lipid forms the same phases in both cases but simply has a different freezing temperature after storage at −20°C than when prepared fresh. Storage of 18:12PC at −20°C, however, had a large effect on the spectrum of 16-doxylstearate as shown in Fig. 2D. The spectrum of phase II had two components, both of which were characteristic of more motion or greater disorder than the spectrum in phase I in Fig. 2E. The height of the spectrum was also reduced by over 50% after storage in spite of the greater disorder and motion. This indicates that the spectrum in Fig. 2D is partially exchange broadened due to clustering of the spin label into

TABLE II

ESR SPECTRAL PARAMETERS OF FATTY ACID SPIN LABELS IN ASYMMETRIC AND SYMMETRIC FORMS OF PHOSPHATIDYLCHOLINE

n.d., not determined.

		5-Doxyl-stearate	16-Doxyl-stearate
Below phase transition temperature			
	Temp. (°C) [a]	T_{max} (gauss)	T_{max} (gauss)
14:14PC	4	ND	23.1
16:16PC	4	30.8	25.2
18:18PC	4	29.9	25.1
18:10PC	4	30.8	29.0
18:12PC	4	30.8	29.1
18:14PC	4	31.5	26.6, 21.9 [b]
10:18PC	4	30.6	28.7
Above phase transition temperature			
	Temp. (°C) [a]	S	τ_0 (ns)
14:14PC	29	n.d.	1.07
16:16PC	46	0.43	0.82
18:18PC	64	[b]	0.48
18:10PC	29	0.49	0.95
18:12PC	34	0.47	0.97
18:14PC	39	0.37	0.90
10:18PC	29	0.50	1.00

[a] Temperature at which spectrum measured.
[b] Two components present.

its own domains. Thus the spin label is partially frozen out of phase II. Cycling through the high enthalpy phase transition gave the spectrum in Fig. 2E which is identical to that in phase I of freshly prepared samples.

18:14PC

This less asymmetric lipid has been reported to have its main transition at about 30°C [23–25,18] with a smaller change in enthalpy and entropy during the gel to liquid-crystalline phase transition than the more asymmetric forms, leading to the suggestion that it forms a more disordered gel phase which may be only partially interdigitated [25]. The greater disorder has been confirmed by Raman spectroscopy [26]. X-ray diffraction shows that it does not form the mixed interdigitated phase, but has not yet revealed what structure is formed [1]. Several groups have also reported a low enthalpy pretransition at about 19–20°C [23–25], while one of these groups later reported that 18:14PC which had been stored at 4°C for a prolonged time had instead a high enthalpy transition at 24°C in addition to the main transition [18]. They did not report further details about the reversibility of these transitions. This effect of storage at low temperature is similar to that which had been reported earlier for symmetric PCs [14]. X-ray diffraction indicated that the gel phase of 18:14PC after storage at 4°C was similar to that of the L_c phase of symmetric forms of PC [18].

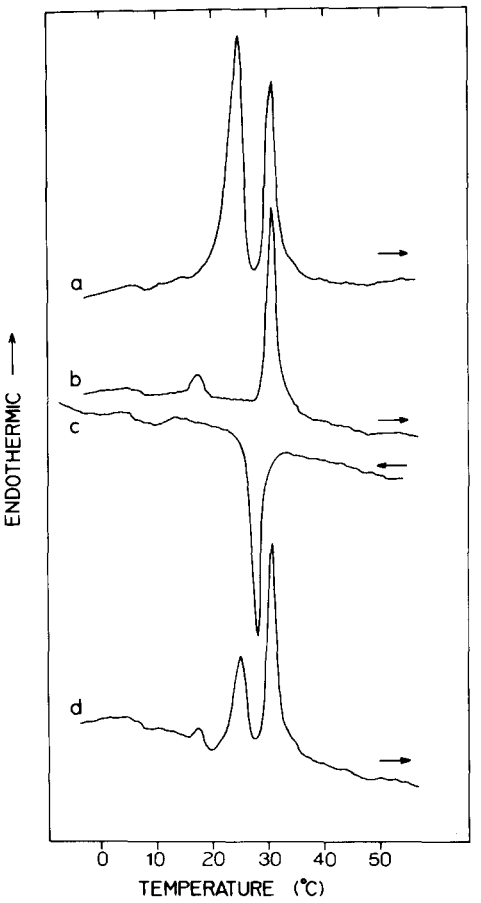

Fig. 3. DSC heating and cooling thermograms of 18:14PC. (a) first heating scan after storage at −20°C; (b) second heating scan; (c) cooling scan; (d) first heating scan after incubation of the sample in the DSC at −13°C for 30 min. Heating and cooling rates 5 K/min. Sensivity setting for all scans was 0.3 mcal/s. The low-enthalpy transitions at 17°C on heating in (b) and at 10°C on cooling in (c) were reproducible. Other details as in caption to Fig. 1.

This subgel phase of 18 : 14PC has not been studied further. Although, in a study using Raman spectroscopy, the lipid was stored at 0°C for 3 days, a lower temperature transition below the main transition was not detected using this technique suggesting that the lipid sample had not gone into its subgel phase.

Heating and cooling thermograms for a sample of 18 : 14PC which had been stored at −20°C for several days are shown in Fig. 3. On the first heating scan (Fig. 3a) the sample has the high enthalpy transition at 25°C and the main transition at 31°C. The premelt transition is absent. The enthalpy of the 25°C transition varied but could be as much as 1.6-times that of the main transition (Table I). On reheating after cooling the sample to −8°C (Fig. 3b), the subgel transition did not occur but the low enthalpy pretransition at about 18°C was observed instead, in addition to the main melting transition at 31°C. Both of these transitions were reversible on cooling (Fig. 3c). Storage of the sample at −20°C overnight or freezing even briefly in the DSC at −13°C caused the subgel phase to partially reform, restoring the 25°C transition as shown for a sample incubated in the calorimeter at −13°C for 30 min in Fig. 3d. A premelt transition is also present indicating that part of the sample is probably in the $L_{\beta'}$ phase and part is in the L_c phase at low temperature.

ESR spectra of 16-doxylstearate at 4°C in the subgel phase (after storage of the sample at −20°C) and in the gel phase (after heating to 40°C and cooling back down) are shown in Fig. 4 A and B, respectively. The spectrum in the subgel phase is reduced in amplitude compared to that in the gel phase suggesting that the spin label has been frozen out of the subgel phase causing exchange broadening. Exchange broadening disappears above 25°C and a spectrum characteristic of anisotropic motion (not shown) and an order parameter of 0.35 is obtained at 26°C, lower than that of 0.41 in the gel phase of 18 : 18PC at 44°C. The spectrum in the gel phase of 18 : 14PC at 4°C in Fig. 4B has two components, one with a similar T_{max} value to 18 : 18PC and one smaller (Table II). Both components are considerably more mobile than in the mixed interdigitated phases of the more asymmetric PCs. Above the pretransition temperature the spectrum becomes anisotropic as shown for a spectrum at 19°C in Fig. 4C. The order parameter is 0.38.

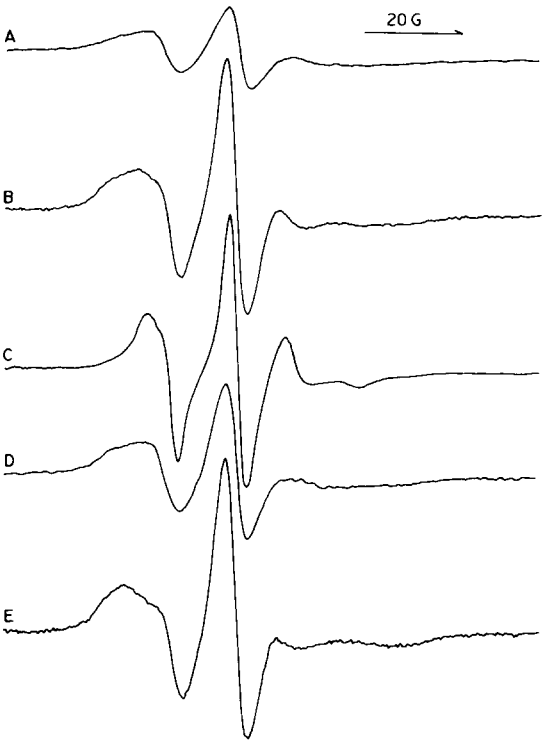

Fig. 4. ESR spectra of 16-doxylstearate in (A–C) 18 : 14PC and (D, E) 16 : 16PC. (A) At 4°C before heating of 18 : 14PC which had been stored at −20°C (subgel phase); (B) at 4°C after heating to 40°C and cooling back down (probably $L_{\beta'}$ phase); (C) at 19°C after the scan in (B) ($P_{\beta'}$ phase); (D) At 4°C in L_c phase of 16 : 16PC; and (E) at 4°C in $L_{\beta'}$ phase, after cycling through phase transition once. (A) and (B) are plotted at the same instrument sensitivity so the heights can be directly compared. (C) is plotted at 0.5-times that scale. (D) and (E) are also plotted out at the same instrument sensitivity relative to each other.

The behavior of the spin label in the subgel phase of 18 : 14PC is similar to that in the subgel phase of 16 : 16PC. The spectra of 16-doxylstearate in 16 : 16PC at 4°C in the subgel L_c phase (obtained by storage at −20°C for several months) and the $L_{\beta'}$ gel phase (obtained after heating above the phase transition temperature and cooling back down) are shown in Fig. 4D and E, respectively. The sample stored at −20°C gave the usual subgel transition at 13°C in addition to the pretransition at 35°C and the main transition at 42°C when scanned by DSC (data not shown).

As occurred for 18 : 14PC, the spectrum in the L_c phase of 16 : 16PC (Fig. 4D) is broadened and reduced in amplitude compared to that in the $L_{\beta'}$ phase (Fig. 4E) indicating freezing out of the spin label from the L_c phase, causing exchange broadening. Note that the behavior of the spin label in the L_c phase is distinctly different from that in the mixed or fully interdigitated phases.

10 : 18PC

The effective asymmetry of this lipid, in which the 2-chain is longer than the 1-chain, is not as great as that of 18 : 10PC or 18 : 12PC because of the fact that the two chains are out of register making the bilayer-penetrating length of the 2-chain 1.5 carbon bonds less than its actual length [31]. This lipid has been studied by calorimetry previously but X-ray diffraction or spectroscopic studies on it have not yet been reported. It is not known if it forms an interdigitated state, but it has been predicted to do so on the basis of its thermodynamic parameters [25].

In the previous calorimetric study of this lipid, the sample had not been stored at a low temperature before scanning. A freshly prepared sample has a phase transition at about 10°C with a low enthalpy of 5.7 kcal/mol (Ref. 25 and Table I). The gel phase of a freshly prepared sample is designated phase I. Storage at −20°C converts the sample to another phase, phase II, which has a higher temperature, higher enthalpy transition. Fig. 5a shows the thermogram obtained on heating of a sample which had been stored at −20°C for several days. An exothermic transition is followed by a small peak at 10°C and then a high enthalpy transition at 14.4°C. On cooling to −8°C (Fig. 5b), only a single peak with similar T_m and enthalpy to the transition of phase I on heating is observed. On reheating from this temperature, two endothermic transitions are observed at 10°C and 14.4°C. The height of the lower temperature one increases at the expense of the higher temperature one with repeated cycling through the phase transition as shown for the second and fourth heating scans in Fig. 5c and d, respectively. Eventually only a single peak at 10°C is observed similar to that for a freshly prepared sample which has not been frozen. No transition corresponding to reformation of phase II was ever seen on cooling, even though the heating scans indicate that the sample is still partially in phase II through several heating and cooling cycles. Therefore reformation of phase

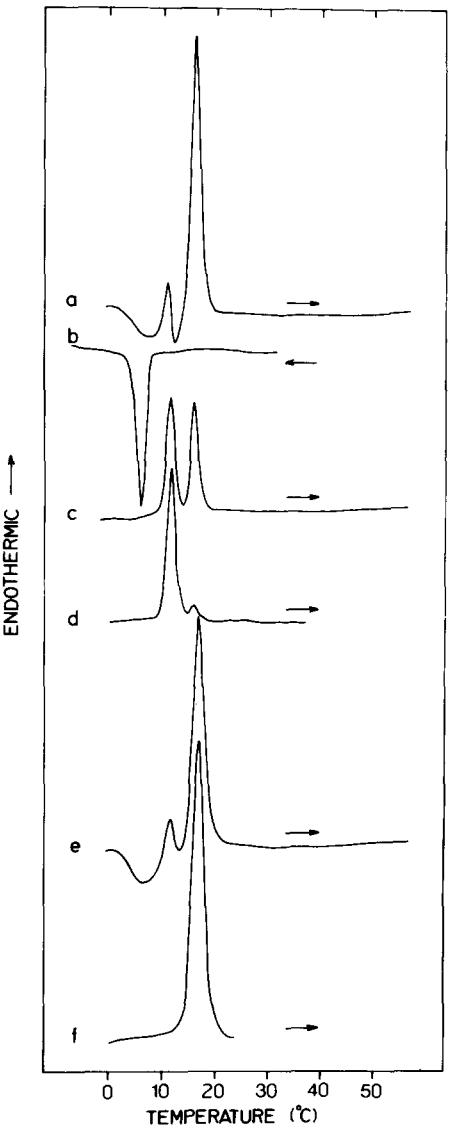

Fig. 5. Heating and cooling thermograms of 10 : 18PC. (a) First heating scan after storage at −20°C (phase II); (b) cooling scan; (c) second heating scan; (d) fourth heating scan (phase I); (e) first heating scan after again storing sample at −20°C for 5 days (subsequent heating scans resemble those in (c, d); (f) heating scan after cycling through phase transition until scan resembling (d) obtained, then incubating in DSC at −18°C for 30 min. Heating and cooling rates 10 K/min. The phase transition temperatures on cooling are normally 4.6 K below those on heating at this rate. The temperatures given in Table I were obtained at 5 K/min and were corrected for instrumental hysteresis.

II for scans such as that shown in Fig. 5c must take place slowly and non-cooperatively at temperatures below 10°C while cooling and reheating.

Storage of this sample again at −20°C for 5 days caused it to revert to phase II as shown in Fig. 5e. Even brief freezing caused complete restoration of phase II as shown in Fig. 5f for the same sample which had been recycled through its phase transition several times until it gave a scan similar to that in Fig. 5d, and then incubated in the DSC at −18°C for 30 min.

ESR spectra of 16-doxylstearate in phases I and II of 10:18PC at 4°C are shown in Fig. 6 B and A, respectively. The spectrum in phase II is reduced in amplitude by 50% relative to that in phase I, suggesting exchange broadening, and resembles that in the subgel phases of 18:14PC and 16:16PC and phase II of 18:12PC. In phase I, a spectrum typical of restricted motion is obtained. The T_{max} value is 28.7 G, a little less than that for 18:12PC and 10:10PC. The T_{max} value of 5-doxylstearate in 10:18PC is similar to that in the other asymmetric and symmetric forms (Table II) and about 2 G greater than that of 16-doxylstearate.

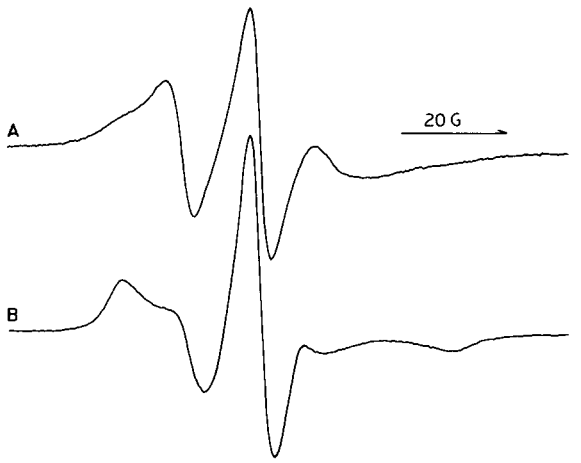

Fig. 6. ESR spectra of 16-doxylstearate at 4°C in 10:18PC. (A) sample which had been stored at −20°C (phase II); (B) after cycling through the phase transition once (phase I). (A) is plotted at 1.6-times greater instrument sensitivity than (B). Thus the height of the center peak (A) is actually 0.5-times that of (B) due to exchange broadening in (A).

Liquid-crystalline phase of asymmetric forms of PC

Above the phase transition temperature, the spectrum of 16-doxylstearate in all the asymmetric forms of PC is isotropic, as in the liquid-crystalline phase of the symmetric forms, and reflects considerably more motion than for 5-doxylstearate which gives an anisotropic spectrum. The motional parameter of 16-doxylstearate is greater (indicating less motion) in the liquid-crystalline phases of all the asymmetric forms than in 18:18PC but similar to that in 16:16PC and 14:14PC (Table II). This does not support significant interdigitation in the liquid-crystalline phase of these asymmetric forms. The motional parameter of 16-doxylstearate and the order parameter of 5-doxylstearate in the liquid-crystalline phase of 18:14PC are smaller than those in the other asymmetric forms suggesting that 18:14PC is more disordered than the other lipids in the liquid-crystalline phase as well as the gel phase.

Discussion

The thermotropic behavior and structures formed by the different asymmetric forms of PC, as deduced from earlier X-ray diffraction studies and the present calorimetric and spin label results, are summarized in Fig. 7.

The fatty acid spin label, 16-doxylstearate, is significantly more motionally restricted in the mixed interdigitated phases of 18:10PC and 18:12PC than in non-interdigitated bilayers of symmetric forms of PC and the less asymmetric 18:14PC. This supports the conclusion from our earlier study on interdigitated bilayers of PC and PG in the presence of glycerol and polymixin [28] that this spin label may be useful for detection of mixed interdigitated and fully interdigitated bilayers. The motional restriction of the spin label in phase I of 10:18PC, although not quite as great as for 18:10PC and 18:12PC, suggests that this lipid probably also forms a mixed interdigitated phase (Fig. 7). The match between the long chain and two short chains packed end to end is not as close for 10:18PC as for 18:10PC and 18:12PC so that if it interdigitated fully it would not be surprising if it were more disordered than the other two lipids.

Davis and Keough [32,33] recently showed that

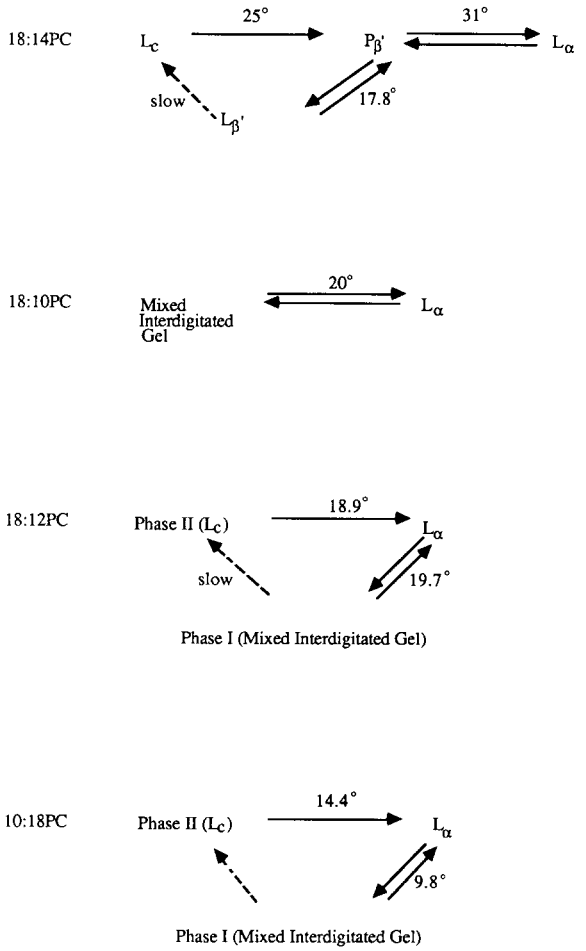

Fig. 7. Schematic representation of thermotropic phase behavior of different asymmetric forms of PC, based on earlier X-ray diffraction studies and the present calorimetric and spin label results. The scheme for 18:14PC is according to that of Serrallach et al. [19] except that the $L_{\beta'}$ to $P_{\beta'}$ transition is shown to be reversible as indicated by the calorimetric results. See Discussion for explanation of the suggestion that phase II of 18:12PC and 10:18PC is similar to the L_c phase and that phase I of 10:18PC is a mixed interdigitated gel phase like that of 18:10PC and 18:12PC. See Refs. 1, 2, and 6 for a diagrammatic representation of this phase.

both 10:18PC and 18:10PC were exceptions to a pattern followed by the T_m values of a number of less asymmetric PCs, also suggesting that 10:18PC as well as 18:10PC were organized differently from the less asymmetric lipids. The T_m values of the less asymmetric PCs depended on the length of the shorter chain as well as the chain length difference. However, the T_m values of more asymmetric lipids which form the mixed interdigitated phase may depend more on the effective length of the longer chain than on the shorter chain. Thus 18:10PC and 18:12PC have almost the same T_m, while 10:18PC, in which the effective bilayer penetrating length of the 2-C(18) chain is decreased by 1.5 carbon bonds, has a lower T_m.

Interestingly, the T_m of 18:10PC is significantly less than that of the fully interdigitated bilayer of 18:18PC (in the presence of glycerol) even though their bilayer thicknesses are similar. Results with symmetric PCs showed that full interdigitation has little effect on the T_m and enthalpy relative to the non-interdigitated form of the same lipid [28]. The significantly lower T_m of the mixed interdigitated bilayer is probably caused by the perturbing effect of the bulky fatty acid terminal methyl group [34]. The presence of two methyl groups meeting end-to-end in the center of a mixed interdigitated bilayer such as 18:10PC must decrease the van der Waals interactions between the fatty acid chains.

The spin label is more disordered in the gel state of 18:14PC than in the gel phase of symmetric forms of PC in agreement with Raman spectroscopic results [26]. This is consistent with a relatively non-interdigitated bilayer in which a number of *gauche* isomers would have to occur in the longer chain for it to fit into a bilayer of thickness determined mainly by the shorter chain [1,22,26]. However, it might also be consistent with partial interdigitation.

Although calorimetric results have been reported on these lipids previously, data from cooling scans has not been nor have the higher enthalpy transitions on heating of 10:18PC and 18:12PC after storage at low temperature. We have also shown that the lower temperature, high enthalpy subgel transition of 18:14PC can be partially restored after brief incubation below 0°C and is abolished after heating above the lipid phase transition temperature once. This supports the suggestion by Serrallach et al. [19] that the $P_{\beta'}$ phase of asymmetric PCs transforms to the $L_{\beta'}$ phase on cooling and that it is the $L_{\beta'}$ phase which slowly transforms into the L_c phase at low temperatures (Fig. 7).

The high enthalpy transitions of the various asymmetric forms of PC occur 6 K below the T_m

for 18 : 14PC, 0.8 K below for 18 : 12PC, but 4.6 K above the T_m for 10 : 18PC. The difference between T_m and the temperature of the transition observed after storage of symmetric forms of PC at low temperature has been found to decrease with decrease in chain length until, for dilauroylphosphatidylcholine, this transition occurs at a higher temperature than the T_m [35]. Although not yet established by X-ray diffraction, the phase undergoing this transition may be the L_c phase. Phase II of 10 : 18PC and 18 : 12PC may then be similar to the L_c phase of symmetric forms of PC and 18 : 14PC (Fig. 7). The fatty acid spin label is frozen out of phase II of 10 : 18PC and 18 : 12PC as for the L_c phases of symmetric PCs and of 18 : 14PC, suggesting a similarly high degree of order as in the L_c phase.

Phase II of 10 : 18PC is not completely eliminated by heating through its phase transition. Only repeated cycling through the upper phase transition gradually eliminates this transition. Repeated cycling through the phase transition also lowered the freezing temperature of 18 : 10PC to below its melting temperature, similar to the behavior of a freshly prepared sample. Incubation of the sample below 0°C prior to reheating caused its freezing temperature on the subsequent cooling scan to be increased to a value similar to its melting temperature. The persistence of these effects of low temperature incubation even after heating the sample above its phase transition temperature several times might be related to changes in the distribution of water or to changes in the morphology of the sample in the DSC pan. Breakdown of large multilayered vesicles of 18 : 14PC into small vesicles at the premelt transition has been observed by electron microscopy [1]. The changes may affect the rate of formation of different phases. After repeated heating, the behavior found for freshly prepared samples is restored.

The formation by 10 : 18PC of a highly ordered phase with a higher phase transition temperature and enthalpy in which the spin label probe is insoluble, resembles that of asymmetric forms of cerebroside sulfate [36]. Other asymmetric sphingolipids, sphingomyelin and cerebroside, also have similar phase behavior [37,38]. These highly ordered phases of asymmetric forms of PC and sphingolipids may have structural features in common.

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