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Lamellar-phase polymorphism in interdigitated bilayer assemblies

Jeffrey T. Mason *, Robert E. Cunningham, Timothy J. O'Leary

Department of Cellular Pathology, Armed Forces Institute of Pathology, Washington, DC 20306-6000, USA

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Abstract

Bilayers composed of 1-octadecanoyl-2-decanoyl-*sn*-glycero-3-phosphocholine (C(18)C(10)PC) adopt a mixed-interdigitated gel-phase packing where the short chains of the C(18)C(10)PC molecules pack end-to-end while their long chains span the entire hydrocarbon width of the bilayer. Calorimetric cooling scans of freshly prepared hand-shaken bilayer suspensions of C(18)C(10)PC exhibit a single exothermic phase transition at 14.6° C, whereas suspensions incubated at temperatures below 2° C for several days exhibit an additional phase-transition exotherm at 17.9° C. Calorimetric and electron microscopic evidence is presented that low-temperature incubation of C(18)C(10)PC bilayer suspensions composed of liposomes of heterogeneous size leads to the conversion of those liposomes in the suspension below about 0.2 μm in diameter into planar lamellar sheets. These lamellar sheets are the origin of the phase-transition exotherm at 17.9° C, whereas the phase-transition exotherm at 14.6° C arises from the liposomes in the suspension. We also show that phosphatidylcholine bilayer suspensions, induced to interdigitate by ethanol, exhibit a similar thermotropic behavior. The implication of these findings for the reversibility of interdigitated gel to liquid-crystalline phase transitions and the role of phospholipid molecular geometry in the formation of interdigitated bilayers are addressed.

Keywords: Phospholipid bilayer; Interdigitation; Molecular geometry; Alcohol; DSC; Electron microscopy

1. Introduction

Recently, there has been considerable interest in interdigitated phospholipid bilayers as models for biological membranes [1,2] and in conjunction with the application of liposomes to the fields of biomedicine [3] and materials science [4]. Certain saturated ether phospholipids [5] and saturated mixed-chain-length phospholipids [1] can, under appropriate conditions, self-assemble into bilayers characterized by various interdigitated chain packings. In addition, saturated symmetric-chain-length phosphatidylcholines (PCs) can be induced to form fully interdigitated bilayers by various amphipathic molecules, such as alcohols [6,7]. One of the most interesting interdigitated bilayers is formed by saturated mixed-chain-length phospholipids in which the long acyl chain of the phospholipid molecule is about twice the length of its short acyl chain. These phospholipids adopt a gel-phase organization such that the short acyl chains pack end-to-end while the long acyl chains span the entire width of the hydrocarbon core of the bilayer. This has been termed a mixed-interdigitated bilayer packing [1,8] symbolized by L_{β}^{M}.

The first mixed-chain-length phospholipid shown to form the mixed-interdigitated bilayer was 1-octadecanoyl-2-decanoyl-*sn*-glycero-3-phosphocholine (C(18)C(10)PC). Differential scanning calorimetry (DSC) studies of hand-shaken (HS) bilayer suspensions of this phospholipid revealed an unusual thermotropic behavior, in which the phase behavior seen upon cooling was dependent upon the thermal history of the sample in the gel phase [9]. If a freshly prepared HS bilayer suspension of fully hydrated C(18)C(10)PC was cooled and immediately examined by DSC, a single cooperative endothermic chain-melting phase transition was seen at 19.1° C upon heating of the sample. Upon cooling, a single exothermic phase transition was seen at 14.6° C. If the C(18)C(10)PC suspension was held at 0° C for several weeks (or at −20° C for 1–2 days), the phase behavior observed upon heating was essentially unchanged. In contrast, the cooling scan revealed two partially overlapped phase-transition exotherms, the original exotherm at 14.6° C and a new exotherm at 17.9° C.

A number of recent studies have established that this unusual thermotropic behavior is a general property of mixed-chain-length phospholipids that adopt a mixed-inter-

* Corresponding author. Fax: +1 (202) 7827164.

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digitated gel-phase packing [10–12]. It was originally proposed that this behavior results from the formation of an intermediate phase between the liquid-crystalline (L_α) and mixed-interdigitated gel phases [10,13], perhaps a partially interdigitated gel phase [10,14]. However, a recent X-ray diffraction and infrared spectroscopy study of C(10)C(22)PC HS bilayer suspensions indicated that the L_α and L_β^M phases coexist at temperatures between the two exotherms seen upon cooling of this phospholipid [12]. These observations lead to the conclusion that this unusual thermotropic behavior is not associated with any change in the packing conformation of the lipid acyl chains but may arise instead from an alteration in the size or structure of microdomains present in the liquid-crystalline bilayer phase. Consequently, it was proposed that both exothermic peaks represent $L_\alpha \rightarrow L_\beta^M$ phase transitions but that they arise from different liquid-crystalline phase domains [12].

The coexistence of two thermodynamic liquid-crystalline phases in the bilayer would be expected to give rise to isothermal freezing (3-phase coexistence) at the onset of the high-temperature exotherm when such bilayers are cooled from the L_α phase. However, the high-temperature exotherms of these mixed-chain-length phospholipids are rarely sharp enough to support an argument for isothermal freezing. Thus, in this study we have reexamined the properties of C(18)C(10)PC bilayer suspensions to determine the origin of this unusual thermotropic behavior. Evidence is presented that low-temperature incubation of C(18)C(10)PC HS bilayer suspensions composed of liposomes of heterogeneous size leads to the conversion of those liposomes in the suspension whose size is below about 0.2 μm in diameter into extended lamellar sheets. These lamellar sheets are the source of the exothermic peak at 17.9° C, whereas the closed-shell vesicles remaining in the bilayer suspension are the source of the exothermic peak at 14.6° C. We also show that symmetric-chain-length PC bilayers, induced to interdigitate by ethanol, exhibit a similar thermotropic behavior. The implication of these findings for the reversibility of interdigitated gel to liquid-crystalline phase transitions and the role of lipid molecular geometry in the formation of interdigitated bilayers are also discussed.

2. Materials and methods

2.1. Materials

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (C(16)C(16)PC) and 1,2-distearoyl-*sn*-glycero-3-phosphocholine (C(18)C(18)PC) were obtained from Avanti Polar Lipids (Alabaster, AL). C(18)C(10)PC was synthesized and purified by established procedures [15]. All of the phospholipids were precipitated three times from acetone/chloroform (95:5, v/v), dried, and stored desiccated at −20° C until used. The phospholipids were checked for purity, after the completion of the DSC and electron microscopy (EM) experiments, by thin-layer chromatography on 250-μm silica gel G plates by using a solvent system of chloroform/methanol/48% ammonium hydroxide (65:35:5, v/v) followed by visualization with iodine. In all cases, only a single spot, corresponding to authentic PC, was observed at a loading of about 1 μmol of lipid. Glass-distilled water was used in the preparation of aqueous solutions. All other reagents were of the highest grade commercially available.

2.2. Preparation of bilayer suspensions

Purified phospholipid (15–50 mg) was dissolved in chloroform, and the solvent was removed under a stream of nitrogen. The lipid film was then desiccated over calcium sulfate under reduced pressure for 12 h. HS bilayer suspensions were prepared by dispersing the dry lipid film in 50 mM KCl with vigorous agitation at a temperature 5° C above the gel to liquid-crystalline phase-transition temperature (T_m) of the phospholipid. The HS bilayer suspension was then incubated at this temperature for an additional 3 h.

Sonicated bilayer vesicles were prepared by ultrasonic irradiation of HS bilayer suspensions with a probe-tip ultrasonicator [16]. During sonication, the sonication vial was emersed in a water bath maintained at a temperature 5° C above the T_m of the phospholipid. The sonicated bilayer vesicles were isolated from residual multilamellar vesicles (MLVs) and titanium from the probe tip by centrifugation at 15 000 × g for 20 min at 38° C.

Liposomal preparations with defined size distributions [17] were prepared from HS bilayer suspensions by the polycarbonate membrane extrusion technique [17,18]. Liposomal suspensions were extruded 10 times through two (stacked) polycarbonate membranes in a water-jacketed Thermobarrel extruder (Lipex Biomembranes, Vancouver, BC, Canada). The extruder was maintained at a temperature 5° C above the T_m of the phospholipid by attachment to an external circulating water bath. The liposomal suspensions were frozen in liquid nitrogen and thawed in a water bath at the same temperature used for extrusion once during each of the first three extrusions cycles. This treatment is reported to reduce the proportion of small vesicles present in the extruded liposomal preparations [19]. Sized liposomal suspensions were prepared by using 1.0-, 0.6-, 0.1-, and 0.05-μm polycarbonate membranes (Nucleopore, Pleasanton, CA).

2.3. DSC

DSC runs were performed on a Hart model 7707 differential scanning microcalorimeter (Hart Scientific, Provo, UT) as described [10]. Samples (100–500 μl of solution) were loaded into 1-ml stainless steel vials, which were sealed and incubated in the calorimeter for 1 h at the

desired starting temperature for the DSC run. Scans were performed in both the ascending and descending temperature direction at a scan rate of 10° C/h. After baseline subtraction and correction for the instrument thermal response, the calorimetric data were analyzed to yield the phospholipid excess heat flow as a function of temperature and phase transition enthalpies (ΔH) by using software supplied by Hart. For a given phase transition, T_m was taken to be the temperature of the maximal excess heat flow, and $\Delta T_{1/2}$ was taken as the transition width at half-maximal excess heat flow.

2.4. Negative-stain EM

Carbon-coated Formvar grids (100 mesh; Ted Pella, Redding, CA) were rendered hydrophilic by placing drops of bacitracin (0.1 mg/ml) on the grids for 1 min followed by removal of the drops with filter paper. A drop of a bilayer suspension was placed on one of the grids for 2 min after which the excess sample was drawn off with filter paper. A drop of 1% ammonium molybdate stain was immediately added to the grid and drawn off after 1 min. Grids prepared in this manner were then air-dried at room temperature for 5–30 min. Samples were examined with a Zeiss model 60 electron microscope at magnifications of 2000 to 20000 × as described [17]. Samples of unstained bilayer suspensions on glass microscope slides were also examined with an Olympus BH-2 light microscope at magnifications of 200 to 2000 ×.

3. Results and discussion

3.1. Thermotropic behavior of C(18)C(10)PC HS bilayer suspensions

The dependence of the thermotropic behavior of a C(18)C(10)PC HS bilayer suspension on the thermal history of the sample in the gel phase is shown in Fig. 1. A freshly prepared C(18)C(10)PC HS bilayer suspension was held in the calorimeter at 5° C for 1 h and then scanned. A single $L_\beta^M \rightarrow L_\alpha$ phase transition was observed (Fig. 1A) with the parameters $T_m = 19.1°$ C, $\Delta T_{1/2} = 0.72°$ C, and $\Delta H = 9.7$ kcal/mol. When this sample was cooled, a single $L_\alpha \rightarrow L_\beta^M$ phase transition was observed (Fig. 1B) with the parameters $T_m = 14.6°$ C, $\Delta T_{1/2} = 0.67°$ C, and $\Delta H = 9.1$ kcal/mol. The above pattern of behavior was reversible as long as the sample was not held at low temperatures for a prolonged period of time. Fig. 1C shows the heating scan of the sample after incubation at −20° C for 72 h. The $L_\beta^M \rightarrow L_\alpha$ phase transition was unchanged except for a slight narrowing ($\Delta T_{1/2} = 0.48°$ C) of the transition profile. In contrast, the subsequent cooling scan displayed two overlapped phase-transition exotherms (Fig. 1D). The higher temperature exotherm yielded values of $T_m = 17.9°$ C, $\Delta T_{1/2} = 0.38°$ C, and $\Delta H = 6.2$ kcal/mol; the lower temperature exotherm yielded values of $T_m = 14.6°$ C, $\Delta T_{1/2} = 0.72°$ C, and $\Delta H = 2.9$ kcal/mol. Fig. 1E shows the cooling scan of the C(18)C(10)PC sample of Fig. 1D after five heating/cooling cycles through the phase-transition region (5–25° C). The higher temperature exotherm was reduced in magnitude ($\Delta H = 2.1$ kcal/mol) relative to the lower temperature exotherm ($\Delta H = 6.9$ kcal/mol), although the total enthalpy for both exotherms ($\Delta H = 9.0$ kcal/mol) remained essentially unchanged. Further cycling through the phase-transition region produced small changes in the relative enthalpies of the two exotherms; however, the higher temperature exotherm never disappeared completely.

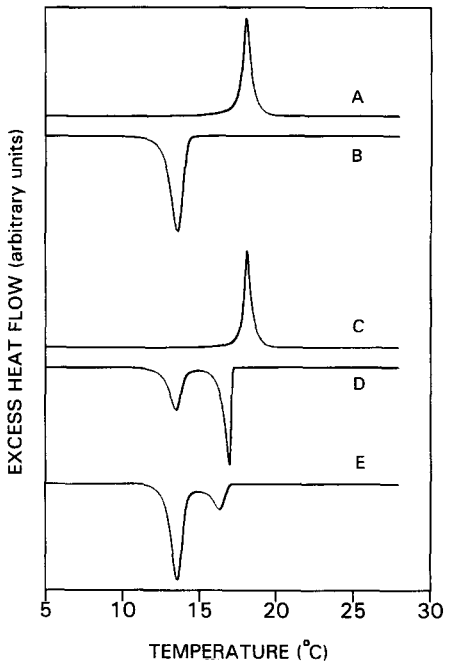

Fig. 1. DSC profiles of C(18)C(10)PC HS bilayer suspensions. Heating (A) and cooling (B) scans of a freshly prepared suspension, initial heating (C) and cooling (D) scans of a suspension held at −20° C for 72 h, and cooling scan (E) of the preceding sample after 5 cycles between 5 and 25° C are shown.

3.2. Morphology of freshly prepared C(18)C(10)PC HS bilayer suspensions

The simplest way to reconcile the observations of Lewis et al. [12] with the DSC results for C(18)C(10)PC described above is to propose that the two transition exotherms seen upon cooling of incubated C(18)C(10)PC bilayer suspensions are $L_\alpha \rightarrow L_\beta^M$ phase transitions that arise from two different lamellar morphologies. Previous negative-stain [17] and freeze-fracture [8] EM examinations of freshly hydrated C(18)C(10)PC HS bilayer suspensions revealed the presence of closed-shell unilamellar and multilamellar vesicles (liposomes). No additional lipid morphologies were detected. These observations were confirmed in the current study (not shown). Thus,

C(18)C(10)PC bilayer suspensions that exhibit the single exotherm at 14.6° C upon cooling consist exclusively of phospholipid vesicles. However, the size distribution of the vesicles in these suspensions was different from that found for saturated symmetric-chain-length PC multilamellar vesicle suspensions. The latter PCs typically yield MLV suspensions with a heterogeneous distribution of vesicle sizes ranging from 0.1 to 5 μm in diameter [20]. The C(18)C(10)PC bilayer suspensions were composed of vesicles that ranged in size from 0.05 to 2 μm in diameter [17]. In addition, the size distribution was bimodal, with most of the vesicle sizes falling within the diameter ranges of 0.05–0.2 μm or 0.8–2.0 μm. The significance of these observations to the thermal history dependence of C(18)C(10)PC bilayer suspensions will become evident in the following sections.

3.3. Thermotropic behavior and morphology of bilayer suspensions derived from C(18)C(10)PC sonicated vesicles

A preparation of C(18)C(10)PC sonicated vesicles was incubated at 2° C for 72 h and examined by DSC. The initial heating scan was identical to the thermogram of Fig. 1C. The first cooling scan is shown in Fig. 2. This profile consisted of a prominent transition exotherm at 17.9° C (ΔH = 8.2 kcal/mol) and a much smaller transition exotherm at 14.6° C (ΔH = 0.9 kcal/mol). Thus, the incubated C(18)C(10)PC sonicated vesicle suspension consisted, predominately, of the phospholipid assemblies that give rise to the transition exotherm at 17.9° C.

An unstained drop of the above bilayer suspension was placed on an ice-cold microscope grid and examined by light microscopy. The suspension consisted of several aggregates of large sheet-like structures similar to those seen in suspensions of 1-stearoyl-2-lyso-*sn*-glycero-3-phosphocholine, a lysophospholipid shown to form extended lamellar sheets in the gel phase [21]. These structures were further examined by negative-stain EM. A typical electron micrograph is shown in Fig. 3. The sample consisted predominately of planar lamellar sheets that ranged in size from 0.2 to 3.2 μm (longest dimension). A few liposomes (0.07–0.15 μm in diameter) and residual sonicated vesicles (0.01–0.02 μm in diameter) could also be detected. The lamellar sheets proved to be unstable on the EM grids and would break up into progressively smaller sheet fragments over time. The micrograph of Fig. 3 was taken 10 min after the preparation of the grid. The range of sizes of the lamellar sheets, determined from a series of micrographs, was 0.2–9.5 μm (longest dimensions).

The C(18)C(10)PC sonicated vesicle suspension was thermally cycled through the phase-transition region (5–25° C) 15 times with DSC cooling scans performed after every five cycles. These DSC profiles are shown in Fig. 2. This thermal cycling resulted in an increase in the transition enthalpy of the exotherm at 14.6° C at the expense of the exotherm at 17.9° C. EM studies (not shown) performed on these bilayer preparations revealed that thermal cycling led to a progressive decrease in the number and average size of the planar lamellar sheets in the preparations. Concomitantly, there was a progressive increase in the number of liposomes in the suspensions. After 15 cycles the bilayer suspension consisted predominately of unilamellar vesicles ranging in size from 0.05–1.2 μm in diameter along with a small number of sheet fragments.

On the basis of the above studies, it is concluded that the phase-transition exotherm at 17.9° C seen in cooling scans of incubated C(18)C(10)PC bilayer suspensions arises from the planar lamellar sheets in the suspension, whereas the phase-transition exotherm at 14.6° C arises from the liposomes in the suspension.

3.4. The effect of vesicle size on the stability of C(18)C(10)PC liposomes in the gel phase

The preceding studies demonstrated that C(18)C(10)PC sonicated vesicles are unstable in the mixed-interdigitated gel phase. This results in their fusion and subsequent conversion into planar lamellar sheets. The effect of vesicle size on the stability of C(18)C(10)PC liposomes in the gel phase was investigated by using liposomal preparations with defined size distributions produced by the polycarbonate membrane extrusion technique. The sized C(18)C(10)PC liposomal suspensions were incubated at 2° C for 72 h and examined by DSC. The initial cooling scans for these suspensions are shown in Fig. 4. These profiles revealed that an increase in vesicle size resulted in a reduction in the proportion of planar lamellar sheets in the suspensions, as reflected by a reduction in the phase-transition enthalpy of the exotherm at 17.9° C. This conclusion was confirmed by negative-stain EM examinations of the bilayer suspensions. Taken in conjunction with a previous study of the size distribution of C(18)C(10)PC

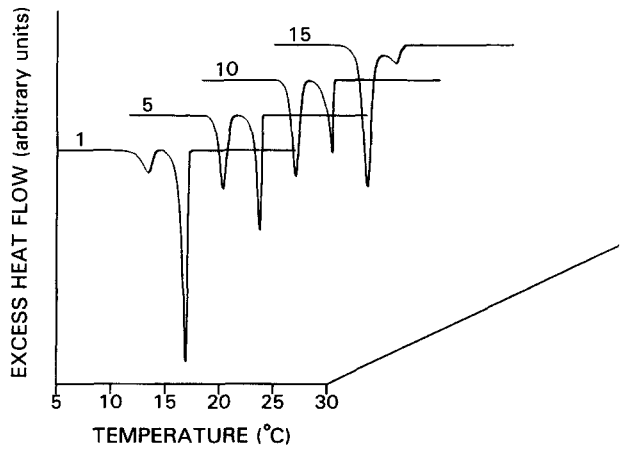

Fig. 2. DSC cooling scans of C(18)C(10)PC sonicated vesicle suspensions after 1, 5, 10, and 15 cycles between 5 and 25° C after an initial incubation at 2° C for 72 h.

extruded liposomes [17], the above results indicate that C(18)C(10)PC vesicles with diameters below 0.1–0.2 μm are unstable in the gel phase and will form planar lamellar sheets upon prolonged incubation at low temperatures.

From the combined studies described above, an explanation of the unusual thermotropic behavior exhibited by C(18)C(10)PC HS bilayer suspensions is possible. C(18)C(10)PC HS bilayer suspensions contain both small (0.05–0.2 μm diameter) and large (0.8–2.0 μm diameter) liposomes. The small liposomes are unstable in the mixed-interdigitated gel phase. However, as long as the suspension is not held at low temperatures for a prolonged period of time, the small liposomes remain intact. Such suspensions exhibit only the phase-transition exotherm at 14.6° C, which arises from the liposomes. Prolonged incubation of the suspension at low temperatures results in the fusion of the small liposomes and their subsequent conversion into planar lamellar sheets. The narrowing of the chain-melting phase-transition endotherm at 19.1° C observed in the initial heating scan after prolonged incubation of the suspension results from the replacement of the small liposomes, whose phase transitions are poorly cooperative [17], with the more thermally cooperative planar lamellar sheets. The corresponding initial cooling scan then contains a large exotherm at 17.9° C, which arises from the planar lamellar sheets in the suspension. However, the planar lamellar sheets are not stable in the liquid-crystalline phase and will slowly break up into liposomes with continued thermal cycling through the phase transition. Thus, the phase-transition exotherm at 14.6° C will progressively increase at the expense of the phase-transition exotherm at 17.9° C. It is proposed that this lamellar phase polymorphism is a

Fig. 3. Negative-stain electron micrograph of a C(18)C(10)PC sonicated vesicle suspension after incubation at 2° C for 72 h. Planar lamellar sheet fragments are clearly visible along with residual sonicated vesicles and a few larger liposomes. The scale bar represents 2.0 μm.

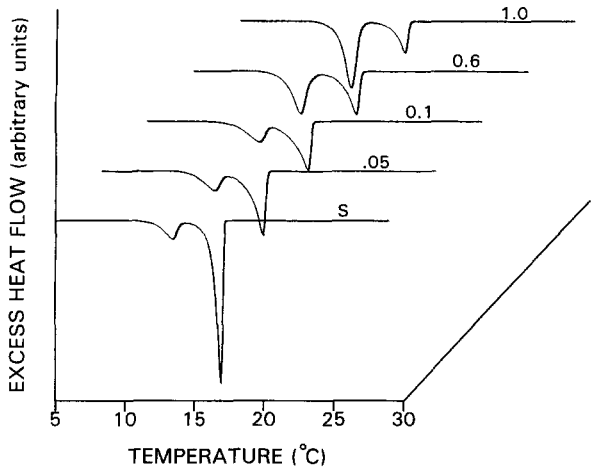

Fig. 4. DSC profiles showing the effect of vesicle size on the formation of planar lamellar sheets in C(18)C(10)PC bilayer suspensions. Sonicated vesicles (S) and HS bilayer suspensions sized through 0.05-, 0.1-, 0.6-, and 1.0-μm polycarbonate membranes were held at 2° C for 72 h and analyzed by DSC. The initial cooling scan of each suspension is shown.

general characteristic of phospholipids that form the mixed-interdigitated gel-phase packing.

3.5. The relationship of thermotropic behavior to lamellar phase polymorphism in ethanol-induced fully interdigitated bilayers

Boni et al. [22] demonstrated that C(16)C(16)PC vesicles below ~0.2 μm in diameter fuse to form extended lamellar sheets in the presence of ethanol concentrations sufficient to induce a fully interdigitated gel-phase packing. We examined such preparations to see if their thermotropic behavior was similar to that of the C(18)C(10)PC bilayer suspensions. A preparation of C(16)C(16)PC sonicated vesicles in 2.6 M ethanol was held at 2° C for 24 h. A sample of this preparation was examined by negative-stain EM and found to consist of extended bilayer sheets, analogous to those seen in the C(18)C(10)PC preparation of Fig. 3 and as reported by Boni et al. [22]. Fig. 5 shows the DSC heating (Fig. 5A) and cooling (Fig. 5B) scans of this preparation after three cycles between 5 and 55° C. The DSC cooling profile is almost identical to the corresponding profile for C(18)C(10)PC as shown in Fig. 1E. Negative-stain electron micrographs of this preparation revealed the presence of both lamellar sheets and liposomes. The transition exotherm at 36.2° C in Fig. 5B corresponds to the single transition exotherm seen in cooling scans of C(16)C(16)PC MLVs in 2.6 M ethanol (not shown). The transition exotherm at 40.1° C is thus proposed to arise from the lamellar sheets in the bilayer suspension, in analogy to the C(18)C(10)PC bilayer suspensions.

In the saturated mixed-chain-length phospholipid bilayer suspensions, lamellar-phase polymorphism arises spontaneously due to the presence of small vesicles in these preparations, which are unstable in the mixed-interdigitated gel phase. For the alcohol-induced fully interdigitated phase of saturated symmetric-chain-length PCs, lamellar-phase polymorphism is dependent upon the method used to hydrate the phospholipid. This was demonstrated with the following experiments. A dried film of C(18)C(18)PC, prepared by evaporation from chloroform, was hydrated with 2 M aqueous ethanol with vortexing at 60° C. After cycling the MLV suspension from 2 to 65° C three times, the sample was analyzed by DSC. The cooling scan (Fig. 5C) reveals a single transition exotherm at 48.9° C. Only liposomes were detected in this preparation by negative-stain EM. A second bilayer suspension was prepared by swelling dry C(18)C(18)PC powder with 2 M aqueous ethanol in the calorimeter vial at 60° C without physical agitation. After cycling the suspension between 2 and 65° C three times, the DSC cooling scan shown in Fig. 5D was obtained. In addition to the transition exotherm at 48.9° C, a second transition exotherm is seen at 53.5° C. Negative-stain electron micrographs of this preparation revealed the presence of both liposomes and lamellar sheets. The transition exotherm at 53.5° C is thus assigned to the lamellar sheets.

From the above studies, it is concluded that vortexing the phospholipid suspension produces a population of liposomes that are sufficiently large that they do not deform

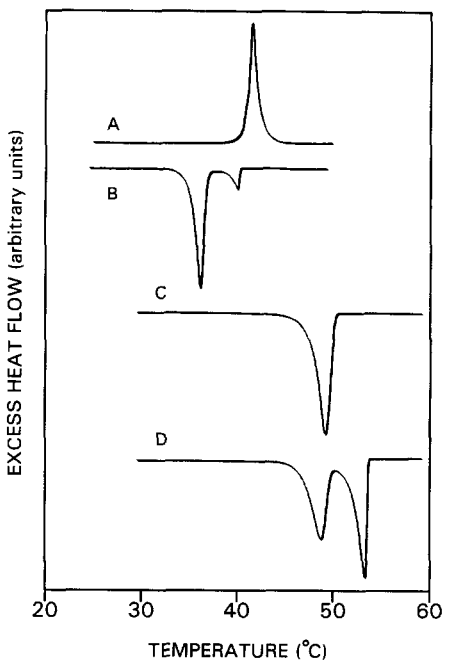

Fig. 5. Lamellar-phase polymorphism in ethanol-induced fully interdigitated C(16)C(16)PC and C(18)C(18)PC bilayers. Heating (A) and cooling (B) DSC scans of C(16)C(16)PC sonicated vesicles in 2.6 M ethanol after three cycles between 5 and 55° C after an initial incubation at 2° C for 24 h are shown. DSC cooling scans of C(18)C(18)PC bilayer suspensions after three cycles between 2 and 65° C after preparation by vortexing a dried lipid film in 2 M ethanol at 60° C (C) or by swelling of a dry lipid powder in 2 M ethanol at 60° C without mechanical agitation (D) are shown.

when in the fully interdigitated gel phase [22,23]. Thus, vesicle fusion and lamellar sheet formation does not occur in these preparations. Apparently, swelling of the C(18)C(18)PC powder either promotes the direct formation of planar lamellar sheets or the formation of small vesicles that subsequently fuse to form planar lamellar sheets.

3.6. Hysteresis of interdigitated gel to liquid-crystalline phase transitions

Results from DSC studies of bilayer suspensions that exhibit single interdigitated gel ↔ liquid-crystalline phase transitions like those of Fig. 1A and 1B have been used to argue that such interdigitated gel ↔ liquid-crystalline phase transitions are thermodynamically irreversible [10,24]. It has been proposed that these phase transitions proceed by way of metastable intermediates, such as the L_β phase in ethanol-induced fully interdigitated bilayers [24] or the partially-interdigitated L_β^P phase in mixed-interdigitated bilayers [10,14]. However, the results obtained here suggest that this hysteresis is caused by constraints imposed by the vesicle morphology and does not originate from true thermodynamic irreversibility. If these morphological constraints are released by forming planar lamellar sheets, the hysteresis is eliminated. If one uses the more thermodynamically appropriate phase-transition 'onset' temperature as a measure of T_m [25], the phase transitions of Fig. 1C and D and Fig. 5A and B are reversible to within $\pm 0.15°$ C if the transition exotherm arising from the lamellar sheets is employed. Thus, both types of interdigitated gel ↔ liquid-crystalline phase transitions are thermodynamically reversible two-state transitions that do not involve intermediate states.

3.7. Role of molecular geometry in the formation of interdigitated bilayers

The lamellar phase polymorphism exhibited by mixed-chain-length phospholipids that adopt a mixed-interdigitated gel phase packing can be understood by considering the effect of phospholipid molecular geometry on the packing constraints within the bilayer. Israelachivili derived a 'shape factor' that predicts the morphology adopted by amphiphiles in aqueous solution [26]. This shape factor is calculated from the expression $v/a_o l_c$, where a_o is the optimal area per amphiphile at the interface, v is its hydrocarbon chain volume, and l_c is the critical chain length of its hydrocarbon region. This shape factor predicts whether an amphiphile will form spherical micelles ($v/a_o l_c < 1/3$), nonspherical micelles ($1/3 < v/a_o l_c < 1/2$), flexible bilayers (vesicles) ($1/2 < v/a_o l_c < 1$), or planar bilayers ($v/a_o l_c \sim 1$) in aqueous solution.

For C(18)C(18)PC, the value of a_o is close to 0.6 nm^2 and l_c is 2.43 nm [27], which corresponds to the extended length of the stearic acid chain. The value of v can be estimated from the equation [26]

$$v \sim (0.0274 + 0.0269 n_c)\ \text{nm}^3$$

where n_c is the number of carbon atoms in the hydrocarbon region. For C(18)C(18)PC, $n_c = 36$ and the shape factor is 0.68, a value compatible with a vesicle morphology.

In the liquid-crystalline bilayer phase, the C(18)C(10)PC molecules are weakly coupled across the bilayer center [14]. Subsequently, the fundamental packing unit is a single C(18)C(10)PC molecule. Using values of $a_o = 0.6$ nm^2, $l_c = 2.43$ nm (for the longer stearic acid chain), and $n_c = 28$, a value of 0.53 is obtained for the shape factor. This value is just above the transition from a nonspherical micellar to a vesicular morphology. Thus, in the liquid-crystalline bilayer phase the molecular geometry of C(18)C(10)PC is consistent with the formation of small vesicles with a large radius-of-curvature. This explains the overall smaller size of the vesicles formed in C(18)C(10)PC HS bilayer suspensions relative to those formed in suspensions of saturated symmetric-chain-length PCs. The bimodal distribution of vesicle sizes in the C(18)C(10)PC HS bilayer suspensions may arise from a packing instability in the larger MLVs that initially form in the suspension. These larger MLVs may subsequently break down into smaller vesicles with more stable packing as the result of mechanical agitation or thermal cycling of the suspension through the phase transition.

In the mixed-interdigitated gel phase, the PC molecules are strongly coupled across the bilayer center. In this case, the fundamental packing unit is a dimer formed by two C(18)C(10)PC molecules on opposite sides of the bilayer. Using values of $a_o = 0.63$ nm^2 [8], $l_c = 2.43$ nm (the hydrocarbon bilayer thickness), and $n_c = 56$ (for two PC molecules), a value of 1.002 is obtained for the shape factor. This value is consistent with the formation of planar bilayers. Thus, the planar packing geometry required for the mixed-interdigitated gel phase is incompatible with the highly curved vesicles formed in the liquid-crystalline bilayer phase during the initial hydration of the phospholipid. Consequently, low-temperature incubation of these small vesicles results in a distortion of their shape as they attempt to assume a mixed-interdigitated packing. This, in turn, promotes the aggregation and fusion of the vesicles and their subsequent conversion into planar lamellar sheets [22]. In the liquid-crystalline phase, the loss of *trans*-bilayer coupling of the C(18)C(10)PC molecules renders the bilayer sheets unstable. Local structural fluctuations in these bilayer sheets would be predicted to promote the formation and 'bleebing off' of small vesicles, which allow for a more favorable lipid packing geometry. The above predictions, based on molecular geometry arguments, are borne out by the calorimetric and EM observations of the C(18)C(10)PC bilayer suspensions.

The geometric packing requirements of the mixed-inter-

digitated gel phase also suggest an explanation for the hysteresis that was noted in section 3.6. Upon cooling from the liquid-crystalline phase, the C(18)C(10)PC liposomes may adopt a packing motif characterized by large planar domains linked together by thin ribbons of boundary lipid. This closed-shell faceted arrangement may require considerable supercooling for its formation due to the long-range packing interactions that are involved. In contrast, this faceted arrangement can easily re-form a spherical geometry upon melting; thus, the liposomes have the same T_m upon heating as the planar lamellar sheets.

For the past 25 years liposomes have served as the preeminent model for the study of the biophysical properties of bilayer-forming phospholipids. This study reveals that the closed vesicle morphology may not be a suitable model for the study of phospholipids that form certain types of interdigitated bilayers. Furthermore, the lipid morphology most compatible with the packing geometry requirements of these phospholipids may change with the thermal phase of the lipids.

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