Thermotropic Properties of Model Membranes Composed of Polymerizable Lipids. 1. Phosphatidylcholines Containing Terminal Acryloyl, Methacryloyl, and Sorbyl Groups

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Abstract: The thermotropic phase behavior of hydrated bilayers of mono- and bis-substituted phosphatidylcholines (PC) containing either acryloyl, methacryloyl, or sorbyl ester groups at the chain terminus was studied by differential scanning calorimetry. Each of these compounds exhibits a single endotherm which occurs at a temperature lower than that of the main phase transition T_m of the corresponding linear saturated chain PC. Variation of the chain length of the sorbylPCs results in a pronounced odd/even alternation of the T_m. Consideration of the preferred conformation of glycerol ester lipids suggested by the crystal structure of dimyristoylPC dihydrate provides a basis for understanding the odd/even effect reported here. The interaction of the *sn*-2 chain sorbyl ester carbonyl with neighboring methylene chains appears to be predominantly intermolecular or intramolecular depending on whether the chain length is even or odd, respectively. Intermolecular interaction is expected to decrease the T_m to a greater extent than intramolecular interaction. The magnitude of the odd/even effect diminished with longer chain length as the free energy of stabilization contributed by van der Waals interchain interactions increased. A comparison of the T_m of a sorbyl ether PC and a sorbyl ester PC revealed an unexpectedly low T_m for the ether lipid. Analysis of this effect suggests previously undetected differences in the probable lipid chain conformations of ether and ester PCs. The T_m values of acryloyl-substituted PCs were somewhat higher than those of comparable chain-length sorbyl-substituted PCs. The addition of an isomethyl to the acryloyl group, i.e., methacryloyl, significantly depresses the T_m values. These systematic thermotropic studies of polymerizable lipids provide new insights into the relationship of lipid phase behavior and lipid chain substitution patterns, which is crucial to the design of novel molecules and the supramolecular assemblies formed from them.

Introduction

Hydrated supramolecular assemblies of lipids possess a variety of interesting properties, among them the ability to undergo important structural changes with temperature. These include the well known gel to liquid-crystalline phase transition of lipid bilayers as well as the transition(s) from bilayer to nonbilayer (nonlamellar) phases, e.g., inverted hexagonal (H_{II}) or inverted cubic phases (Q_{II}). These processes are highly cooperative in nature and therefore occur as well-defined temperatures. The observed thermal events result from changes in molecular packing in the nonpolar lipid chains and the polar head-group region.[1-5]

Differential scanning calorimetry (DSC) is a primary tool for the characterization of lipid bilayers.[6-9] The nonperturbing nature of DSC provides important advantages over other probe-based methods, especially for the study of lipid mixtures. The advent of high sensitivity DSC permits the direct study of lipids at sample concentrations that are comparable to those used in many liposome studies. The thermotropic transitions are observed as a maxima in the excess heat capacity vs temperature plot. The main phase transition, T_m, for the gel to liquid-crystalline transition is readily detected in addition to the total enthalpy associated with the transition ΔH_{cal}, the van't Hoff enthalpy ΔH_{vH}, and the cooperativity unit (CU) which is the ratio $\Delta H_{vH}/\Delta H_{cal}$. Small changes in lipid structure can dramatically alter these characteristics. Menger et al. reported a systematic study of bilayer perturbation by the substitution of methyl and carbonyl groups in various positions on the α- and β-chain (*sn*-1 and *sn*-2) of distearoylphosphatidylcholine (DSPC).[10-12] Both T_m and the ΔH_{cal} are diminished by chain methyl substitution, with the largest decrease occurring when the methyl group is positioned near the midpoint of the hydrocarbon chain. Lewis and McElhaney examined the effect of acyl chain length on the T_m for a variety of terminally alkyl-branched phospholipids.[13-17] The main phase transitions for methyl iso- and anteiso-branched as well as ω-*tert*-butyl- and ω-cyclohexyl-containing phosphatidylcholines (PCs) are depressed to differing extents relative to those of their unbranched analogs. Furthermore, short chain methyl iso-branched, ω-cyclohexyl-branched, and *tert*-butyl-branched PCs exhibit odd/even alternation of the T_m as the hydrocarbon chain length is sequentially increased by one carbon.[13,16,17] PCs with unbranched acyl chains do not show an odd/even alternation of the T_m.

The early 1980s saw the advent of a new class of lipid/liposome chemistry with the first reports of polymerizable lipids.[18-21] Reactive moieties—acryloyl, methacryloyl, itaconyl, dienoyl,

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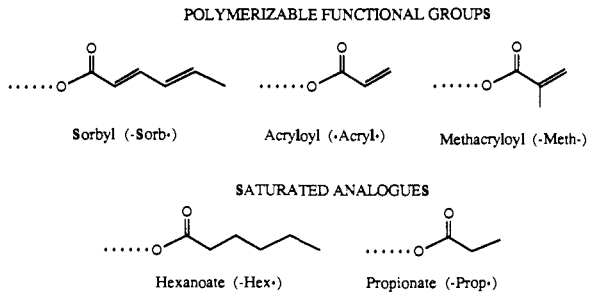


Figure 1. Structure of polymerizable and saturated esters incorporated into the ω-terminus of the acyl chain(s) of phosphatidylcholines.

muconyl, sorbyl, styryl, lipoyl, and diacetylenic—have been incorporated into lipid structures to render them polymerizable.[22,23] The reactive moiety can be positioned near the top, middle, or end of the lipid chain(s) as well as in the polar head group. The polymerization of reactive lipids in liposomes can result in significant enhancements of the liposomal chemical and colloidal stability.[22-25] Alternatively, photopolymerization of appropriately designed liposomes can be used to destabilize liposomes with leakage of aqueous contents.[26-28] An understanding of the phase behavior of bilayers composed of polymerizable lipids is crucial to the design of new molecules and the supramolecular assemblies formed from these molecules. Although most studies of polymerizable lipids include calorimetric data, which has recently been summarized,[29] there is little systematic information on the effect of the polymerizable groups on the thermotropic properties of the hydrated bilayers. In the present work, we examine the thermotropic effect of the incorporation of acryloyl, methacryloyl, and sorbyl polymerizable groups into the terminal end of the lipid hydrocarbon chain(s) of glycerol ester and ether PCs. This direct comparison of lipids synthesized and purified by the same methods and analyzed with the same instrumentation now permits new insights into the behavior of these interesting new bilayer assemblies.

The lipids characterized in this study include mono-substituted PCs, i.e., PCs with a saturated hydrocarbon *sn*-1 chain and the polymerizable group in the *sn*-2 chain, and bis-substituted PCs, which have polymerizable groups in both the *sn*-1 and *sn*-2 chains. The shorthand lipid notation is as follows: acryloyl, methacryloyl, and sorbyl functionalities are abbreviated Acryl, Meth, and Sorb, respectively, followed by the designation PC for phosphatidylcholine (Figure 1). The abbreviated name is preceded by the appropriate prefix mono or bis. The total number of atoms in each main chain minus the hydrogens is represented by a subscript at the end of the abbreviated name (note: each carbonyl is counted as one atom). The two chains may differ in length, and the subscripts represent the *sn*-1 and *sn*-2 chain lengths, respectively. Saturated (reduced) analogues of polymerizable PCs are propionate for the acryloyl esters and hexanoate for the sorbyl esters,

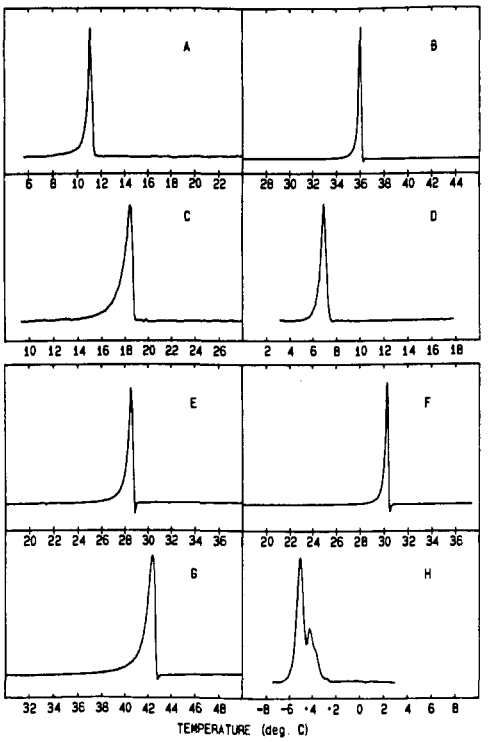
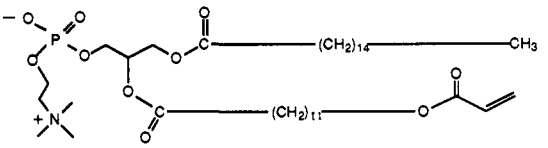


Figure 2. High-sensitivity DSC heating thermograms of aqueous dispersions of mono- and bis-SorbPC having acyl chain lengths ranging from 15 to 19 atoms as well as the saturated analog bis-HexPC$_{17,17}$. Thermograms were obtained at a scan rate between 10 and 12°/h: (A) mono-SorbPC$_{14,15}$, (B) mono-SorbPC$_{16,17}$, (C) bis-SorbPC$_{15,15}$, (D) bis-SorbPC$_{16,16}$, (E) bis-SorbPC$_{17,17}$, (F) bis-SorbPC$_{18,18}$, (G) bis-SorbPC$_{19,19}$, and (H) bis-HexPC$_{17,17}$.

which are abbreviated Prop and Hex, respectively. The structure below illustrates the notation.



mono-AcrylPC$_{16,16}$

Experimental Section

Methods. Compounds containing a UV-sensitive group were handled under yellow light. Reactions which require conditions were run under a nitrogen atmosphere. TLC was used to monitor each reaction and check the purity of the products along with ^{1}H-NMR spectra, which were obtained on a 250-MHz Bruker WM 250 spectrometer. Infrared spectra were taken on a Perkin-Elmer 983 spectrometer. UV–visible absorption spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer. FAB mass spectra were obtained using an AMD modified 311A-equipped mass spectrometer with a cesium gun. The purity of the lipids was assayed by TLC both before and after storage as an amorphous ice at −10 °C in benzene. Rapid silica gel chromatography was used to purify samples prior to calorimetry.

Synthesis. Ester Lipids. A summary of the procedures described by Lamparski et al.[27] follows. Fatty acids containing polymerizable groups were prepared by monoesterification of a long chain diol followed by oxidation of the hydroxy monoester. Mono- and bis-substituted PCs containing polymerizable fatty acids were synthesized by acylation of L-α-glycerophosphatidylcholine (GPC/$CdCl_2$) or lysopalmitoylPC (lyso PC), respectively, as previously reported (Figure 2).[11,13,27,30] The crude product was purified by flash silica gel chromatography using silicic acid (Biosil-A 200–400 mesh) as the solid support with various mixtures of CH_2Cl_2/MeOH. The dried lipid powder was dissolved in benzene to a concentration between 10 and 20 mg/mL, filtered through 0.42-μm organic filters to remove trace amounts of silicic acid, bubbled with argon

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Table I. Thermodynamic Characteristics of the Heating Endotherms of the *Mono*- and *Bis*-Sorbylphosphatidylcholines and the *Bis*-Hexanoate Analogs

PC	T_m (°C)[a]	ΔH (kcal mol^{-1})[b]	CU[c]
bis-SorbPC$_{15,15}$	18.5	9.98 ± 0.85	63 ± 2
bis-SorbPC$_{16,16}$	6.9	6.77 ± 0.14	140 ± 14
bis-SorbPC$_{17,17}$	28.8	7.48 ± 0.16	167 ± 9
bis-SorbPC$_{18,18}$	30.3	10.83 ± 0.14	210 ± 6
bis-SorbPC$_{19,19}$	42.5	10.83 ± 0.14	75 ± 7
mono-SorbPC$_{14,15}$	11.0	6.99 ± 0.62	166 ± 32
mono-SorbPC$_{16,17}$	36.1	10.5 ± 0.29	202 ± 22
bis-HexPC$_{17,17}$[d]	−5.0 (−7.5)[e]	10.7 ± 0.2[e]	nd[f]
bis-Sorb ether PC$_{17,17}$	11.4	8.5 ± 0.1	nd
bis-Hex ether PC$_{17,17}$[d]	−15.4 ± 1.1[e]	10.7 ± 0.2[e]	nd

[a] The T_m values varied by less than ±0.1° between successive runs, except for *bis*-Hex ether PC$_{17,17}$. [b] The ΔH values quoted are the average of the total enthalpy for succesive runs. [c] The CU values are the average for successive runs. [d] Samples exhibiting a T_m below 0 °C were hydrated in aqueous ethylene glycol: 35% ethylene glycol/aqueous buffer for Microcal MC-2 DSC and 44% ethylene glycol/aqueous buffer for Perkin-Elmer DSC-7. [e] Measured by Perkin-Elmer DSC-7. [f] nd = not determined.

for 10 min, and then stored as an amorphous ice at −10 °C. Overall yields ranged from 50 to 85% on the basis GPC-$CdCl_2$ or lysoPC.

Sorbyl Ether Glycerophosphocholine. The synthesis of the ether lipids were described by Lee and O'Brien.[31]

UV **Polymerization of Mono-SorbPC.** Extended bilayers of mono-SorbPC$_{14,15}$ were prepared in a manner identical to that described for DSC samples. The lipid suspension was placed in a 3-mL quartz cuvette and photopolymerized for 75 min by exposure to 254-nm light from a low-pressure Hg lamp located 1 cm away. The sample was agitated by bubbling argon. The loss of monomeric mono-SorbPC$_{14,15}$ was monitored by the decrease in the sorbyl chromophore at 256 nm.[27,32]

Differential Scanning Calorimetry. The lipids were lyophilized from benzene to a floculent powder and hydrated to a concentration of either 1 or 1.5 mg/mL with degassed buffer (10 mM Na_2HPO_4/150 mM NaCl at pH 7.4). Concentrations were determined by accurately weighing out known amounts of lipid (typically 5–10 mg) and adding the appropriate volume of buffer. Lipid suspensions were vortexed at temperatures greater than the T_m for 3–5 min until uniformity was observed followed by 10 freeze-thaw cycles (isopropyl alcohol/dry ice temperature to room temperature). High-sensitivity calorimetric thermograms were obtained on aqueous buffer aliquots (1.2631-mL cell volume) of lipids having endotherms at temperatures above 0 °C with a Microcal Model MC-2 differential scanning calorimeter. Thermograms were obtained at temperatures down to −7.0 °C from samples dispersed in a 35% ethylene glycol/aqueous buffer mixture. A scan rate between 10° and 15°/h was employed for all high-sensitivity measurements. A Perkin-Elmer DSC-7 differential scanning calorimeter was used to characterize the behavior of hydrated lipids (44% ethylene glycol/water) with endotherms below −6.0 °C.

Results

DSC heating curves were obtained on each of the polymerizable PCs and their saturated analogues. The thermodynamic parameters reported are the average of two different samples, each of which was scanned three times. The main phase transition temperature T_m, calorimetric enthalpy ΔH, and cooperative unit (number of lipids undergoing the phase transition at the same time; determined by dividing the van't Hoff enthalpy by the calorimetric enthalpy) are reported in Tables I and II.

SorbylPCs. Aqueous dispersions of five bis-substituted and two mono-substituted SorbPCs of different chain lengths were studied (Table I). Representative endotherms are shown in Figure 2. Each SorbPC lipid exhibited a single sharp transition. Pretransitions were not observed in any of these samples. In some cases, repetitive calorimetric scans produced thermograms, which were progressively broader with slightly depressed T_ms and smaller lipid cooperativity. This unusual behavior could possibly be due to low conversions of lipid to polymer or lipid hydrolysis to form small amounts of fatty acids during the heating scans to 65 °C.[13] However, in our experience, the SorbPCs do not polymerize on heating in the absence of initiator. Low levels of impurities (<1%) which are not readily detected by TLC or ^{1}H NMR will cause broadening of the main transition with a decrease in the lipid cooperative unit.[13] Samples of both bis-SorbPC$_{15,15}$ and bis-SorbPC$_{19,19}$ exhibited broad main transition endotherms indicating poor lipid cooperativity. This broadening of the endotherm could be either a function of the lipid-phase behavior or due to the presence of undetected impurities. Greater lipid cooperativity was observed for the hydrated bilayers of bis-SorbPC$_{16,16}$, bis-SorbPC$_{17,17}$, and bis-SorbPC$_{18,18}$.

The T_m at 28.8 °C observed by DSC for bis-SorbPC$_{17,17}$ was substantiated by X-ray diffraction and ^{31}P-NMR spectrometry. The X-ray diffraction revealed a transition from a well-ordered gel state to a disordered liquid-crystalline state as the sample was thermally cycled from temperatures below to above 29 °C.[33] The ^{31}P-NMR spectrum of the sample in the same temperature region showed a decrease in the basal line width above the T_m, as expected for the increased motion and the axial symmetry of the phosphate head group on the ^{31}P-NMR time scale.[34]

The melting temperatures of the sorbyl alcohols and fatty acids as well as the T_ms of the bis-SorbPCs are plotted as a function of acyl chain length in Figures 3 and 4, respectively. The sorbyl alcohol shows a distinctive odd/even alternation of the melting point with increasing hydrocarbon chain length. This means that the melting point of alcohols with an even number chain length

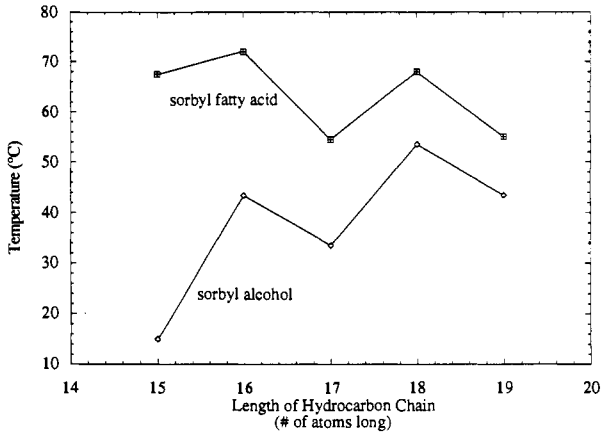

Figure 3. Chain-length dependence of the melting point of long chain sorbyl alcohols and fatty acids.

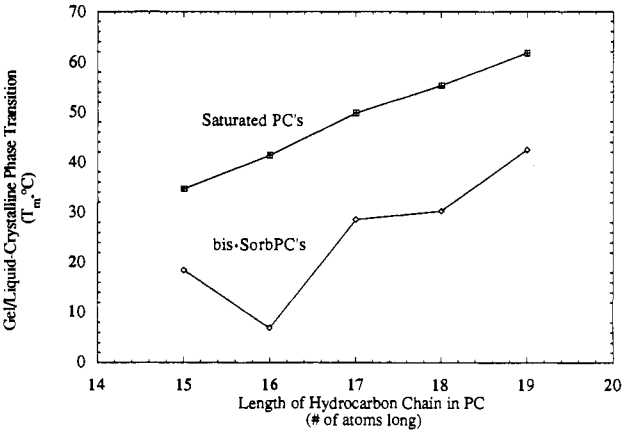

Figure 4. Chain-length dependence of the gel/liquid-crystalline phase transition temperature T_m of bis-SorbPCs.

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is greater than the average of the melting points of the two nearest odd number chain-length alcohols. The melting-point behavior of the sorbyl fatty acids shows a similar odd/even alternation. Odd/even melting-point behavior is characteristic of saturated alkanes[35] as well as some alkyl-substituted fatty acids.[13,14,16,17] The T_ms of the bis-SorbPCs are also dependent on the lipid chain length in an alternating even/odd manner. Figure 4 shows that the T_m of lipids with even-numbered chains is lower than the average T_m of the two adjacent odd-numbered chain lipids in the series. In the case of the three lipids with chain lengths of 15, 16, and 17, bis-SorbPC$_{16,16}$ has a lower T_m than each of the other two. Comparison of lipids with chain lengths of 17, 18, and 19 shows that the T_m for bis-SorbPC$_{18,18}$ is lower than the average of the other two but still greater than that of bis-SorbPC$_{17,17}$. These data indicate that the apparent odd/even effect is attenuated as the chain length is increased.

A comparison of T_m values for bis-SorbPCs and the corresponding saturated chain acylPCs of the same chain length reveals a notable depression in T_ms for all of the SorbPCs (Figure 4). This effect is especially pronounced when lipids of even number chain length are compared, e.g., bis-SorbPC$_{16,16}$ has a T_m of 6.9 °C compared to 41.4 °C for dipalmitoylphosphatidylcholine (DPPC, PC$_{16,16}$), because the unbranched saturated PCs do not exhibit an odd/even alternation in T_m values.

Two mono-substituted SorbPCs were examined, and each showed a single endotherm (Table I), as was the case for the bis-SorbPCs, which presumably corresponds to the gel/liquid-crystalline phase transition. The longer chain mono-SorbPC$_{16,17}$ (*sn*-1, palmitoyl chain; *sn*-2, sorbyl fatty acid, 17 atoms in length) showed a higher T_m, enthalpy, and cooperative unit than the shorter mono-SorbPC$_{14,15}$. Comparison of the T_m for the mono-SorbPCs to that of unbranched saturated PCs is complicated by the unequal length of the *sn*-1 and *sn*-2 chains in the mono-SorbPCs. An estimate of the expected T_m for a PC with a 14-carbon *sn*-1 chain and a 15-carbon *sn*-2 chain can be obtained by averaging the values for PC$_{14,14}$ (DMPC) and PC$_{14,16}$, which are 23.6 and 35 °C,[36] respectively. This suggests that the T_m of PC$_{14,15}$ should be about 29 °C. A similar analysis for PC$_{16,17}$ predicts the T_m value to be near 45 °C (based on the reported T_m of 41.4 °C for PC$_{16,16}$ and 49 °C for PC$_{16,18}$[36]). These estimated values indicate that the T_m of the shorter chain mono-SorbPC$_{14,15}$ is depressed by 18° whereas the incorporation of the reactive sorbyl ester into the longer chain mono-SorbPC$_{16,17}$ reduces its T_m by 9° from that expected for a saturated PC$_{16,17}$. The apparent effect of chain length on the magnitude of the perturbation of T_m will be considered further in the Discussion.

The T_m of extended bilayers of mono-SorbPC$_{14,15}$ was lost upon photopolymerization of the lipid. The loss of this thermotropic transition suggests entanglement and/or a steric arrangement of the linear polymer chains that are formed during the photopolymerization. Further consideration of the effect of polymerization on the phase behavior of the hydrated assemblies will be addressed in a subsequent study.

Acryloyl- and MethacryloylPCs. DSC thermograms of mono- and bis-acryloyl- and methacryloylPCs are depicted in Figure 5 (Table II). Both the mono- and bis-substituted PCs have the same chain length as PC$_{16,16}$ (T_m 41.4 °C), i.e., acryloyl and methacryloyl fatty acids are 16 atoms long. The incorporation of a single acryloyl group into the terminus of the *sn*-2 chain of mono-AcrylPC$_{16,16}$ decreases the T_m to 31.8 °C. The substitution of a second acryloyl group into the other chain, bis-AcrylPC$_{16,16}$, caused a further decrease of the T_m by only another 2 °C. Thus, the effects of the first and second acryloyl groups are not additive. Methacryloyl substitution caused an even greater perturbation of the bilayer, e.g., the T_m of mono-MethPC$_{16,16}$, is almost 30° lower than that of PC$_{16,16}$. The lipid with two methacryloyl groups, bis-MethPC$_{16,16}$, has a 15° lower T_m. Again, the second polymerizable ester group does not have as great an effect on the T_m as the first substitution.

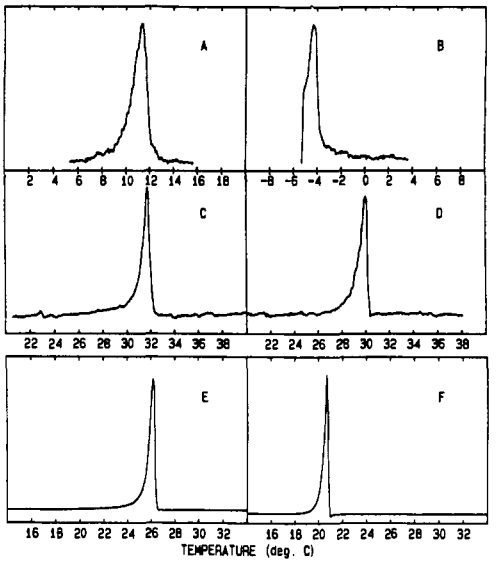

Figure 5. High-sensitivity DSC heating thermograms of aqueous dispersions of mono- and bis-acryloyl- and methacryloylPC's and the saturated analogs mono- and bis-propionate PC. The total chain length of the PCs is 16 atoms long. Thermograms were obtained at a scan rate between 10 and 12°/h: (A) mono-MethPC$_{16,16}$, (B) bis-MethPC$_{16,16}$, (C) mono-AcrylPC$_{16,16}$, (D) bis-AcrylPC$_{16,16}$, (E) mono-PropPC$_{16,16}$, and (F) bis-PropPC$_{16,16}$.

Table II. Thermodynamic Characteristics of the Heating Endotherms of the *Mono-* and *Bis*-Acryloyl- and Methacryloylphosphatidylcholines and the Saturated *Mono-* and *Bis*-Propionate Analogs

PC	T_m (°C)[a]	ΔH (kcal mol^{-1})[b]	CU[c]
mono-AcrylPC$_{16,16}$	31.8	8.90 ± 0.42	72 ± 10
bis-AcrylPC$_{16,16}$	30.0	6.90 ± 0.59	118 ± 20
mono-MethPC$_{16,16}$	11.4	10.50 ± 0.25	38 ± 0
bis-MethPC$_{16,16}$[d]	−4.4	5.30 ± 0.26	96 ± 6
mono-PropPC$_{16,16}$	26.1	12.77 ± 0.85	71 ± 4
bis-PropPC$_{16,16}$	20.7	12.31 ± 1.01	115 ± 14

[a] The T_m values varied by less than ±0.1° between successive runs. [b] The ΔH values quoted are the average of the total enthalpy for successive runs. [c] The CU values are the average for successive runs. [d] Sample was hydrated in 35% ethylene glycol/aqueous buffer.

Both the calorimetric enthalpy and cooperativity of the phase transition are affected by the incorporation of acryloyl and methacryloyl groups into the PC. The addition of a second acryloyl group or methacryloyl group lowers the transition enthalpy by 2 and 5 kcal/mol, respectively. The lipid cooperativity was increased slightly by the acryloyl substitution and was doubled for the methacryloyl lipids. Clearly, both the carbonyl and methyl groups cause significant perturbations of the lipid bilayer packing.

Saturated Ester Models for Acryloyl- and SorbylPCs. Saturated ester-substituted lipids, which are identical in acyl chain length and carbonyl location as mono- and bis-AcrylPC$_{16,16}$ as well as bis-SorbPC$_{17,17}$, were prepared as model lipids to distinguish between the effect of the ester chain substitution and the presence of double bond(s) in the lipid tail on the lipid phase behavior (Figures 2 and 5). Each of these saturated ester PCs exhibited a single main transition (Tables I and II) with even lower T_m values than the corresponding AcrylPC and SorbPC lipids. The T_m of mono-PropPC$_{16,16}$ at 26.1 °C is 5.7° lower than that of mono-AcrylPC$_{16,16}$, and the T_m of bis-PropPC$_{16,16}$ is 20.7 °C, which is 9.3° below that of bis-AcrylPC$_{16,16}$. The effects of the saturated ester groups are nearly additive. A greater effect was observed for bis-HexPC$_{17,17}$, which showed a T_m at −5.0 °C (−7.5 °C when measured by low-sensitivity DSC). This was a 32°

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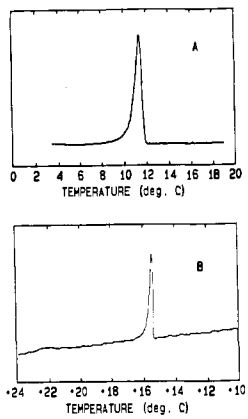
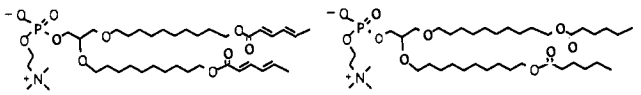


Figure 6. DSC heating thermograms of bis-ether PCs: (A) high-sensitivity DSC of bis-Sorb ether $PC_{17,17}$, scan rate of 10–12°/h; (B) low-sensitivity DSC of bis-Hex ether $PC_{17,17}$ (14.9 wt % in 44% ethylene glycol/aqueous buffer), scan rate of 1°/min.

decrease in the T_m compared to that of parent bis-Sorb$PC_{17,17}$ and a 54° decrease compared to that of the completely saturated dimargonylPC ($PC_{17,17}$, T_m = 49 °C). The difference in the T_m between bis-Prop$PC_{16,16}$ and $PC_{16,16}$ was 21°. In the latter case, the carbonyl group is closer to the lipid chain terminus.

Ether PCs. The two ether lipids shown were studied in order to compare the thermotropic behavior of chain-substituted ether and ester PCs (Table I, Figure 6). Both bis-Sorb ether $PC_{17,17}$ and its saturated analog, bis-Hex ether $PC_{17,17}$, were prepared as described previously.[31] The T_m of bis-Sorb ether $PC_{17,17}$ was



observed at 11.4 °C, which can be compared to the value of 28.8 °C for the corresponding ester PC. The significantly lower phase-transition temperature for the ether lipid was unexpected because ether PCs generally exhibit a somewhat higher T_m than the corresponding ester PC of the same chain length, e.g., the T_ms of $PC_{16,16}$ (DPPC) and 1,2-dihexadecyl-*sn*-glycero-3-phosphocholine (DHPC) are 41.4 and 43.7 °C, respectively. The ability of ether lipids to pack more closely together than ester lipids, due to the absence of the carbonyl groups, has been proposed to account for the somewhat higher T_m.[37,38] Possible reasons for the exceptional departure of bis-Sorb ether $PC_{17,17}$ from the usual behavior will be discussed below. Bis-Hex ether $PC_{17,17}$ showed a T_m at −16.2 °C, which is about 27° below that of the polymerizable bis-Sorb ether $PC_{17,17}$. The magnitude of the effect is similar to that found for bis-Hex$PC_{17,17}$ (see above).

Discussion

The thermotropic effects caused by the incorporation of polymerizable substituents into the terminal ends of the lipid chains are consistent with previous studies of branched chain lipids and reveal important aspects of these hydrated lipid assemblies. In all cases, the incorporation of an ester functionality, whether containing a polymerizable moiety or its saturated analog, into the hydrocarbon chain resulted in a depression of the main phase transition, T_m, compared to that of saturated unbranched PCs of similar chain length. These results are consistent with the previous studies of Lewis and McElhaney on the methyl iso- and anteiso-branched and ω-cyclohexyl- and ω-*tert*-butyl-substituted PCs.[13-17] Methyl substituents at various positions on the *sn*-2 chain of the PCs decrease the T_m to different extents depending upon the location of the methyl group on the chain.[10] In order to account for the decreases in both the T_m and the enthalpy, it is necessary to examine the energetics of the transition process. The gel phase is characterized by fully extended hydrocarbon chains in the all-trans conformation. At the phase transition, there is a reduction in van der Waals interchain interactions and an increase in the number of gauche C–C linkages. Schindler and Seelig calculated a value of 4.3 gauche conformers/chain on the basis of ^{2}H-NMR order parameters.[39] Recent FT–IR studies reported that there are between 3 and 4.2 gauche conformers/chain in deuterated-DPPC analogs[40] and 3.7 gauche conformers/chain in DPPC.[41] This results in an increase in the molecular area by 6 Å^2/chain and a decrease in the bilayer thickness by 20%.[42] Nagle has shown that van der Waals interchain interactions contribute 4.1, 5.5, and 7.3 kcal/mol to the transition enthalpy for $PC_{14,14}$ (DMPC), $PC_{16,16}$ (DPPC), and $PC_{18,18}$ (DSPC), respectively.[42,43] The introduction of a methyl group or other branching substituent reduces the attractive van der Waals interactions and significantly decreases the T_m and enthalpy of transition. McFarland and McConnell suggested that the lipid chains are endowed with a critical bend near their center which divides the chains into two segments of comparable length.[44] The segment associated with the head group lies at a 30° angle from the membrane surface, while the lower segment lies perpendicular to the surface. Substituents near the center of the chain stabilize the critical bend that develops when the gel phase reorganizes into a liquid-crystalline phase, thereby appreciably lowering the T_m and the ΔH_{cal} relative to those of an unbranched lipid. Since the chain segment between the central bend and the terminal methyl permits the segments to rotate along the locus of a cone with the bend at its apex, there is more interaction space available near the chain terminus to accommodate branching or kinking. Therefore, a substituent near the end of the chain causes far less packing distortion than a midchain substitution. The effects observed in this study of polymerizable PCs will be considered in light of these previously reported trends.

One of the main features of the present results is the pronounced odd/even alternation in the T_m observed for the bis-SorbPCs (Figure 4). Somewhat similar behavior was reported for ω-cyclohexyl, ω-*tert*-butyl, and short chain methyl iso-branched PCs.[13,16,17] Lewis and McElhaney reported that the odd/even effect is enhanced in compounds that contain bulky substituents such as a *tert*-butyl or a cyclohexyl at the ω-position and a polar component at the α-position.[16,17] While the sorbyl moiety is not as bulky as a cyclohexane ring, the protruding ester carbonyl and the relative stiffness of the trans diene appear to be sufficient to alter the packing in the crystal lattice between odd- and even-length chains. Odd/even discontinuities are also often observed in the solid-state behavior of several long chain paraffins as well as in the melting points of a variety of fatty acids.[13,17,35,45,46] The odd and even chain-length sorbyl alcohols and fatty acids also exhibit alternating melting points with chain length (Figure 4). The observed solid-state phenomenon results from different end-group interactions which, in turn, effect the tilting of odd- and even-length chains during formation of the crystalline lattice.

Consideration of the preferred conformation of glycerol ester lipids suggested by the crystal structure of dimyristoylPC ($PC_{14,14}$) dihydrate[47] provides a basis for understanding the odd/even alternation observed in certain substituted PCs. Groups attached near the ω-terminus of the *sn*-1 and/or *sn*-2 acyl chain of a PC

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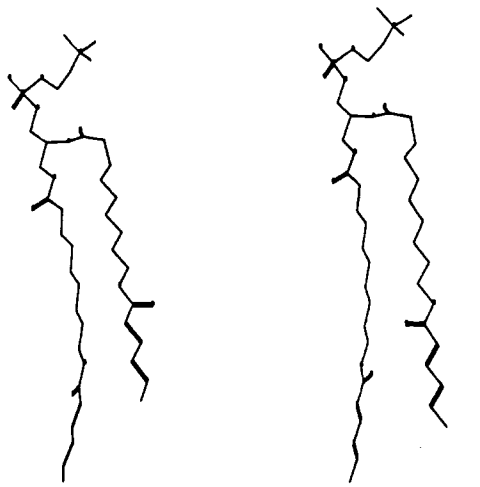

bis-SorbPC$_{16,16}$ *bis*-SorbPC$_{17,17}$

Figure 7. Drawings of the all-trans extended conformation of bis-SorbPC$_{16,16}$ (left) and bis-SorbPC$_{17,17}$ (right).

are inequivalent to one another since the *sn*-1 chain penetrates further into the bilayer. If the SorbPC acyl chains have a similar orientation as those of PC$_{14,14}$, then the mode of interaction between substituents on the *sn*-1 and *sn*-2 chains will depend on whether there are an odd or even number of atoms in the chain. Figure 7 shows the conformation of bis-SorbPC$_{16,16}$ and bis-SorbPC$_{17,17}$, respectively, which were derived from the structure of PC$_{14,14}$[47,48] by extending and modifying the two acyl chains to incorporate the terminal sorbyl ester groups. In each drawing, the sorbyl ester carbonyl on the *sn*-1 chain points toward a neighboring lipid which would be located either in front of the plane of the page or behind the plane of the page. The energetic contribution of this carbonyl should be similar in each compound. The sorbyl ester carbonyl on the *sn*-2 chain of these lipids lies in the plane of the page. In the case of the even-numbered chain bis-SorbPC$_{16,16}$, the carbonyl oxygen points toward a neighboring lipid chain, whereas in the example of an odd-numbered chain lipid (bis-SorbPC$_{17,17}$), the carbonyl oxygen points toward the second chain of the same lipid. The carbonyl–methylene interaction of the *sn*-2 chain sorbyl ester carbonyl appears to be predominantly intermolecular or intramolecular depending on whether the chain length is even or odd, respectively. Energetic differences between intramolecular and intermolecular interactions are expected to exist regardless of the lipid chain tilt to the bilayer normal. Therefore, PCs with either even- or odd-numbered chains may have similar crystallographic structures yet differ in their energetics. A similar explanation was proposed for the observed odd/even alternation exhibited by ω-*tert*-butylPCs,[17] whereas differences in acyl chain tilt were proposed to account for the odd/even behavior in ω-cyclohexyl[17] and short chain methyl iso-branched PCs.[13] Note that the odd/even effects observed for short chain methyl iso-branched PCs can also be explained by intra- or intermolecular interactions between methyl groups and nearest neighbor methylenes, e.g., methyl iso-branched PCs of odd-numbered chain lengths have their methyl groups directed inward in the same manner as the carbonyl of odd-length SorbPCs. Both types of substituents influence the T_m in a similar fashion. In other words, PCs with either alkyl or carbonyl substituents oriented in a manner that gives preferential intramolecular interaction have higher T_m values than PCs where the orientation of the substituents favors intermolecular interaction. In the latter case, the substituents perturb the bilayer to a greater extent with a consequent destabilization of the gel phase and stabilization of the liquid-crystalline phase. The increased molecular area per molecule due to the formation of kinks in the lipid chain at temperatures above the T_m will be stabilized by substituents which point away from the lipid. On the other hand, substituents directed toward the other chain of the same lipid will have a smaller stabilizing effect on the liquid-crystalline phase.

The significant difference in thermotropic behavior between bis-SorbPC$_{15,15}$ and bis-SorbPC$_{16,16}$ also signals the presence of a more stable gel phase in the former lipid. The increase in gel-state stability is indicated by the transition entropy ΔS, which was 34 cal mol^{-1} K^{-1} for bis-SorbPC$_{15,15}$ and 24 cal mol^{-1} K^{-1} for bis-SorbPC$_{16,16}$. The studies of McElhaney and co-workers[13,16,17] indicate that PCs with branched terminal groups can form different types of gel phases depending on whether the chain has an even or odd number of atoms. Although we do not presently have spectroscopic evidence for the existence of different types of gel phases for the SorbPCs, this possibility will be explored in the future.

It can be seen in Figure 4 and Table I that the decrease in T_m for the longer even chain-length bis-SorbPC$_{18,18}$ is less than that of the shorter chain bis-SorbPC$_{16,16}$. If the phase transition temperature of all bis-SorbPCs where linearly dependent on acyl chain length over the range investigated, the even-length PCs of 16 and 18 atoms would have predicted T_ms of 23.2 and 35.7 °C (computed from the midpoint of adjacent odd-length SorbPCs). However, the difference between the predicted and experimental T_ms were 16.3° and 5.4°, respectively. This is probably a consequence of the free energy stabilization contributed by van der Waals interchain interactions, which increase with the acyl chain length. Eventually, the van der Waals interactions will be sufficiently greater than the energy associated with the steric and dipolar effects of the carbonyl oxygen that the van der Waals interactions will dominate bilayer stabilization. The odd/even discontinuities in T_m observed in Figure 4 should eventually disappear for longer acyl chains.

The phase behavior observed for both mono-substituted SorbPCs, mono-SorbPC$_{14,15}$ and mono-SorbPC$_{16,17}$, resembles that of the corresponding odd chain-length bis-substituted PCs because the sorbyl ester carbonyl on the *sn*-2 chain is oriented to primarily favor intramolecular interactions. Comparison of the T_m values for these two lipids to those of the corresponding unbranched saturated chain PCs indicates that the ester carbonyl in mono-SorbPC$_{14,15}$ reduces the T_m by 18° and in the mono-SorbPC$_{16,17}$, the T_m is reduced by only 9°. These data may be interpreted in the same manner as proposed for the bis-SorbPCs, that is, the van der Waals chain interactions become relatively more important as the chain length increases and the ester carbonyl is displaced further from the lipid backbone.

The second focus of this study involves the mono- and bis-substituted PCs which contain either acryloyl or methacryloyl substituents (Table II) in the hydrocarbon chain terminus. It is helpful to recall the effect of simple methyl substitution at various positions of the *sn*-2 chain of distearoylPC (PC$_{18,18}$).[10] Methyl substitution at carbons 4, 10, or 16 of PC$_{18,18}$ caused a decrease in the T_m from 54.8 to 41.5, 5.6, and 38.5 °C, respectively. A parallel decrease in transition enthalpy was observed as the methyl substituent position was varied along the hydrocarbon chain. Note that in each of these cases, an even-numbered carbon was the site of methyl substitution, which favors intramolecular interaction. The ester carbonyl of both acrylyol and methacryloyl PCs is located in the same position on the acyl chain as the methyl group of methyl anteisoDPPCs described by Lewis and McElhaney.[14] The ester carbonyl of mono-AcrylPC$_{16,16}$ decreases the T_m by about 11 °C from that of PC$_{16,16}$, whereas the methyl in the equivalent-length methyl anteisoPC decreases the T_m by 32 °C. This appears to be a consequence of the smaller volume occupied by the carbonyl compared to a methyl substituent which rotates about the C–C bond. The T_m of mono-MethPC is much

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lower than that of mono-AcrylPC, since the methacrylyol group contains both a carbonyl in the anteiso position and a methyl group in the iso position, thereby occupying an even greater volume in the chain terminus which decreases the van der Waals attraction forces.

Comparison of the T_ms of bis-AcrylPC$_{16,16}$ (30.0 °C) and bis-SorbPC$_{16,16}$ (6.9 °C) illustrates the combined effect of the location of the ester group along the 16-atom chain and the orientation of the terminal ester carbonyl in a manner that favors either intermolecular or intramolecular interactions. In bis-AcrylPC$_{16,16}$, the ester carbonyl is located at the C-14 position of the acyl chain, which, as noted earlier, causes a 11° decrease in the T_m from that of the reference PC$_{16,16}$ (DPPC). The *sn*-2 ester carbonyl at this chain position is expected to primarily interact intramolecularly with the neighboring acyl chain of the same lipid. In contrast, the bis-SorbPC$_{16,16}$ ester carbonyl is located at the C-11 position of the acyl chain where it is closer to the critical bend at the chain midpoint. The decrease in T_m is consistent with the trends expected from the previous methyl substitution studies. However, the effect is magnified by the orientation of the *sn*-2 sorbyl ester carbonyl at the odd-atom chain position, which favors intermolecular carbonyl–methylene interaction leading to a greater decrease in T_m.

In a preliminary manner, we have examined how the lipid T_m is influenced by the unsaturation of the polymerizable moieties located in the acyl terminus. The saturated analogs of mono- and bis-AcrylPC$_{16,16}$ and bis-SorbPC$_{17,17}$ exhibit significantly lower T_ms than the parent polymerizable lipids. This effect appears unusual when considering that unsaturation in the acyl chain reduces the van der Waals attraction forces due to poorer bilayer packing and effectively lowers the enthalpic parameters for melting. However, trans olefins and dienes cause less disruption of the bilayer packing than the corresponding cis double bonds. Furthermore, the effect of unsaturation on chain packing is decreased as the unsaturation is moved down the chain away from the critical bend in the same manner as observed for methyl-branched PCs.[10] The observed T_ms indicate that whereas the ester carbonyls in these lipids destabilize the gel phase, the "ene" in AcrylPC and the "diene" in SorbPC may provide some stabilization of the gel phase, perhaps by π–π-overlap of the double bond(s) with adjacent PCs. Saturation of these groups increases the number of C–C gauche conformations, allows greater freedom of rotation of the C–C σ-bonds compared to C–C π-bonds, and eliminates any π–π-overlap. Each of these effects serves to destablize the gel phase and depress the T_m. The observed decrease in T_m for saturated PropPC$_{16,16}$ compared to AcrylPC$_{16,16}$ is probably due to the greater motion of the C–C σ-bond as well as to the loss of the π–π-overlap of orbitals. An increase in the number of gauche conformers is unlikely since only one C–C σ-bond is added to each acyl chain. The even larger decrease in T_m exhibited by HexPC$_{17,17}$ compared to SorbPC$_{17,17}$ could be due to a combination of all three factors affecting the packing of the chain.

The distinctive thermotropic characteristics of bis-Sorb ether PC$_{17,17}$ was initially puzzling. Ether PCs usually exhibit a slightly higher T_m than that of the same chain-length ester PC. However, in the odd-atom chain-length bis-Sorb ether PC$_{17,17}$, the T_m was more than 17° lower than that of bis-SorbPC$_{17,17}$. An interesting hypothesis to account for this seeming anomaly is an extension of the previous consideration of the orientation of the carbonyl oxygen in the all-trans extended conformation of the chain. The sorbyl ester carbonyl in the *sn*-2 chain of bis-SorbPC$_{17,17}$ is expected to primarily interact in an intramolecular manner as discussed earlier. The conformations of the ester groups which connect the acyl chain to the glycerol backbone are shown in Figure 7. The carbonyl of the *sn*-2 chain in ester PCs is directed toward the water-bilayer interface,[47] where it may hydrogen bond with water.[49] The structure of fluid PC bilayers determined by the joint refinement of X-ray and neutron diffraction data indicates that water permeates into and beyond the region of the glycerol backbone in dioleolylPC.[50] Therefore, H-bonding is likely to contribute to the overall energetics which control the preferred conformation of the *sn*-2 PC chain. When the lipid structure is changed from an ester PC (bis-SorbPC$_{17,17}$) to an ether PC (bis-Sorb ether PC$_{17,17}$), the ester carbonyl at the glycerol backbone becomes a methylene group which no longer hydrogen bonds. We suggest that the absence of H-bonding permits the *sn*-2 ether PC chain to adopt a different conformation than that shown in Figure 7 for the ester PC. Any change in the orientation of the chain near the glycerol backbone will alter the orientation of the carbonyl at the 12-position and significantly increase the probability of sorbyl ester carbonyl interaction with neighboring lipids. To the extent that intermolecular interaction is increased, the T_m will be depressed. The absence of comparative crystallographic or modeling data for the glycero ether and glycero ester lipids limits further analysis of the lipid chain conformations. The unusual nature of the comparative calorimetric data reported here is so striking that future crystallographic and modeling studies of ether and ester lipids should be pursued to more fully characterize the differences between these lipid classes, e.g., these calorimetric differences may be due to the presence of different gel phases in the ester and ether PCs.

This report describes the first systematic study of the thermotropic behavior of chain-substituted polymerizable lipids. These results provide new insights into the effect of molecular substitution patterns on supramolecular-phase behavior. The lipid gel to liquid-crystalline phase transition T_m is sensitive to both the location of the functional group along the chain as well as to the orientation of branching substituents, e.g., terminal ester carbonyls. The observed odd/even effect highlights the probable consequence of chain orientation in ester lipids. Furthermore, the usual behavior of Sorb ether PC$_{17,17}$ suggests previously unsuspected differences in the chain packing of ether and ester PCs. These current and future studies will facilitate the design of novel lipids and materials on the basis of supramolecular assemblies of those lipids.

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