Gel to Liquid-Crystalline Phase Transitions of Aqueous Dispersions of Polyunsaturated Mixed-Acid Phosphatidylcholines†

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ABSTRACT: The thermotropic properties of aqueous dispersions of synthetic mixed-acid polyunsaturated 1,2-diacyl-3-*sn*-phosphatidylcholines (PC) have been studied by differential scanning calorimetry. The gel to liquid-crystalline phase transition temperature (T_c) of 1-stearoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (SLPC) was −16.2 ± 1.6 °C ($\bar{x}$ ± SD, nine dispersions, three separate synthetic preparations); that for a preparation of 1-stearoyl-2-linolenoyl-PC (SLnPC) was −13 °C and for a preparation of 1-stearoyl-2-arachidonoyl-PC (SAPC) was −12.6 ± 1.0 °C (three dispersions, one preparation). The temperatures of maximum heat flow (T_{max}) for the lipid transitions were −14.4 ± 1.3, −11, and −10.7 ± 0.9 °C and the enthalpies were 3.3 ± 1.0, 6.6, and 5.3 ± 1.8 kcal·mol^{-1} for SLPC, SLnPC, and SAPC, respectively. The transition temperatures and enthalpies are rationalized on the basis of existing data on the effect of double-bond position on T_c and are interpreted with the use of a statistical mechanical model. The trend of the transition temperatures with the introduction of multiple double bonds reflects opposing effects due to increased molecular area and decreasing degrees of freedom of rotation about carbon–carbon bonds.

The lipid bilayer, as a simple model for natural membranes, has received extensive investigation over the past decade [for reviews, see Chapman (1975) and Lee (1977a,b)]. Much attention has been focused on the thermotropic characteristics and phase behavior of bilayers composed of various classes and molecular species of phospholipids. It is understood that the physical properties of lipids are, in general, dependent upon head group structure and length and degree of unsaturation of the acyl chains. Most detailed physical–chemical studies have been concerned with single-acid saturated phosphatidylcholines and their interactions with other membrane components. Recently, several investigations have involved model membranes made with mixed-acid lecithins containing in some cases unsaturated acyl moieties (Phillips et al., 1972; Barton & Gunstone, 1975; Seelig & Waespe-Šarčevič, 1978; Keough & Davis, 1979; Chen & Sturtevant, 1981; Davis et al., 1981; Stümpel et al., 1981; Mason et al., 1981). These systems pertain more to biological membranes where, among the lecithins, the mixed-acid unsaturated species predominate. Relatively little work has been done, however, on lecithins containing polyunsaturated fatty acids. This may be a reflection of the difficulties inherent in the synthesis of these compounds. Although there are data available on the monolayer interactions (Tinoco & McKintosh, 1970; Ghosh et al., 1971; Demel et al., 1972; Ghosh & Tinoco, 1972; Evans & Tinoco, 1978) and the permeability of liposomes of such lecithins (deGier et al., 1968), it is remarkable that there is little information on their thermotropic or other physical–chemical properties.

It has been shown that small structural modifications within a given molecule can influence the gel to liquid-crystalline (lc)[1] transition temperature of aqueous phospholipid dispersions, i.e., the position of the double bond (Barton & Gunstone, 1975), the glycerol to chain linkage and the methylation of the head group nitrogen (Vaughan & Keough, 1974), and the distribution of the acyl groups at either the *sn*-1 or *sn*-2 positions on the glycerol backbone (Keough & Davis, 1979; Chen & Sturtevant, 1981; Davis et al., 1981; Stümpel et al., 1981; Mason et al., 1981). Throughout the literature it is often tacitly assumed that the degree of unsaturation of the acyl chain also has a significant effect on the phase behavior of lecithins. It was important therefore to study the thermal properties of a homologous series of mixed-acid lecithins containing a different number of cis double bonds as a means of assessing the relationship between the degree of unsaturation and phase behavior. While this paper was in preparation, a paper on the fluorescence of diphenylhexatriene in liposomes made with lipids containing different numbers of double bonds in one chain was published (Stubbs et al., 1981). These workers found that there were only small or no differences in the wobbling diffusion constant, the cone angle, and the steady-state anisotropy in the range from 10 to 50 °C for liposomes of lipids containing different numbers of double bonds per chain. This indicated that there were only small differences in the order and rate of motion of the hydrocarbon chains as seen by the probe over that temperature range for the different liposomes. The work presented here describes differential scanning calorimetric studies of aqueous dispersions of the lecithins 1-stearoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (SLPC), 1-stearoyl-2-α-linolenoyl-*sn*-glycero-3-phosphocholine (SLnPC), and 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (SAPC). A preliminary account of some of this work has been presented (Coolbear & Keough, 1980).

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[1] Abbreviations: DSC, differential scanning calorimeter (calorimetry); DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; GLC, gas–liquid chromatography; lc, liquid crystal (crystalline); PC, phosphatidylcholine; SAPC, 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine; SLPC, 1-stearoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine; SLnPC, 1-stearoyl-2-α-linolenoyl-*sn*-glycero-3-phosphocholine; SOPC, 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; T_c, gel to lc transition temperature; TLC, thin-layer chromatography; T_{max}, temperature of maximum heat flow into or out of a sample; UV, ultraviolet; $\Delta T_{1/2}$, width at half-maximum excess heat capacity.

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Materials and Methods

Materials. Some DSPC (99% pure), stearic acid, *N,N*-dicyclohexylcarbodiimide, and carbonyldiimidazole were obtained from Sigma Chemical Co., St. Louis, MO. A preparation of SLPC (SLPC III) and SAPC was obtained from Avanti Polar Lipids, Birmingham, AL. Linoleic and α-linolenic ($18:3^{\Delta 9,12,15}$) acids (99% pure) were purchased from Nu Chek Prep, Elysian, WI. Sources of other reagents and solvents have been described before (Davis et al., 1980, 1981).

Lipid Syntheses. (*A*) *DSPC.* Some DSPC was synthesized by the method of Gupta et al. (1977) or that of Roseman et al. (1978) from glycerophosphocholine that had been prepared from egg yolk lecithin (Brockerhoff & Yurkowski, 1965). Purification was as described previously (Keough & Davis, 1979).

(*B*) *SLPC.* Preparations of SLPC were synthesized by the reacylation of 1-stearoyl-*sn*-glycero-3-phosphocholine, prepared from DSPC by phospholipase α_2 digestion according to Keough & Davis (1979), with linoleoyl anhydride made as described by Selinger & Lapidot (1966). Recrystallization of the anhydride was omitted. Instead, the parent liquor (CCl_4) was removed by rotary evaporation and the anhydride residue added as a viscous suspension to the reacylation vessel. The reacylation technique of Roseman et al. (1978) for mixed-acid lecithins was employed. This procedure, an adaptation of that described by Cubero-Robles & van den Berg (1969), was initially chosen as it was thought that the relatively mild, nonalkaline conditions of reacylation might help to preserve the integrity of the polyunsaturated chains. One preparation of SLPC (II) was made under the reacylating conditions of Gupta et al. (1977) on the $CdCl_2$ adduct of lysostearoyllecithin (Chakrabarti & Khorana, 1975).

In preliminary syntheses of SLPC, it was found that although the lipid products gave a unique lecithin spot on TLC, spectra analyses and GLC analyses of fatty acids of the lecithins indicated that limited oxidation of the unsaturated moiety had occurred. To minimize this autoxidation during reacylation and purification, all further procedures were carried out as far as possible in a special chamber in an oxygen-free, N_2 atmosphere protected from laboratory light and heat. An antioxidant, either hydroquinone or *N'*-tetramethylphenylenediamine dihydrochloride, was included in the reacylation mixture. In this way, lipids with a 1:1 mole ratio of acyl moieties and with no apparent oxidation, as seen by UV and fatty acid analysis, were produced. Although precautions were taken to eliminate oxidation, and newly synthesized lipids were stored at −20 °C either dry in vacuo or in $CHCl_3$ under N_2, with time (in one case days) oxidation products were detected in these samples. Consequently, the lipids prepared in this laboratory were used for study immediately following purification, when essentially no absorbance at ~230 nm was detected.

(*C*) *SLnPC.* SLnPC was prepared according to the technique of Gupta et al. (1977). The appropriate lysolecithin was formed by hydrolysis of DSPC with phospholipase A_2 in borate buffer (Chakrabarti & Khorana, 1975). A $CdCl_2$ adduct of this lysolecithin (Chakrabarti & Khorana, 1975) was then used for reacylation with linolenoyl anhydride as described above. The crude reaction mixture was subjected to chromatography on Rexyn-I300 (Fisher Chemical Co.) as described by Gupta et al. (1977) before purification on silicic acid (Keough & Davis, 1979).

Purification and Analysis of Lipids. The crude reacylation products were purified essentially as described by Keough & Davis (1979). However, the acetone–chloroform precipitation steps were omitted due to the higher solubility of the unsaturated lipids in acetone–chloroform solutions. Purity of the final products was determined by TLC, by GLC, and by positional analysis for the presence of reverse isomers in the mixed-acid lecithins. Ultraviolet spectral analysis for the presence of oxidation products was like that described by Klein (1970). Solutions in the range of 0.1–1 mg·mL^{-1} in either absolute or 95% ethanol were employed. In one analysis of SAPC, chloroform–methanol (1:1 v/v) was used. The presence of conjugated dienes and hydroperoxy groups was monitored by extinction at 230 nm (Klein, 1970; Chan et al., 1979) and of conjugated trienes by extinction at 265–270 nm (Huang, 1969).

The pure lipids, dissolved in chloroform, were dried under N_2 and evacuated for a further 16 h over P_2O_5. They were then dispersed in excess water (lipid–water, 1:2 or 1:3 w/w) and thermal analyses performed on a Perkin-Elmer DSC-2 as described previously (Keough & Davis, 1979). Generally, heating rates of 5 deg/min and sensitivities of 0.5–2.0 mcal/s full scale were employed. Samples were routinely taken through the ice–water melt to ensure the presence of excess water. Transition temperatures (T_c) were taken as the intersection of the sharpest tangent to the leading edge of the transitions and the extrapolated base lines. We shall refer to the temperature of maximum heat flow into or out of a transition as T_{max}. This value will be close to the thermodynamic value T_m for half-completion of the transition as long as the transition is relatively symmetric.

Results

Lipid Analysis. The samples used for these analyses were all pure by TLC. In one sample of SLPC III of the many used for DSC runs a trace of lyso-PC was observed after the DSC scans. SLPC I, SLPC II, and SLnPC had essentially equimolar fatty acid ratios on GLC. SLPC III was analyzed by GLC a large number of times either from the stock solution or from the lipid extracts of DSC runs. It usually gave between 0 and 3% excess stearate in these analyses when the lipid was transmethylated in 2% HCl in methanol at 70 °C for 2.5 h or in 6% H_2SO_4 in methanol at 65–80 °C for 4–16 h. The use of higher transmethylation temperatures (90–95 °C) resulted in a reduction of the linoleate content and the concomitant appearance of one or two peaks with retention times near those expected for C_{20} fatty acid methyl esters. Its expected that chains with oxidation intermediates may be more labile during transmethylation than unoxidized chains. The SAPC samples showed 56% stearate and 44% arachidonate and traces of two esters with shorter ($\sim C_{16}$) and longer ($\sim C_{24}$) retention times. SLPC III was analyzed a large number of times from stock solutions and in extracts from DSC dispersions over a period of 6 months, and little oxidation was observed in that time (E^{230}_{mol} = 50–300, E^{265}_{mol} = 6–38; palmitoyllecithin gave E^{230}_{mol} = 60 and E^{265}_{mol} = 5). The sample of SAPC had undergone some limited oxidation (E^{265} = 190–730) in DSC extracts. The samples of SLPC I and SLPC II had undergone acyl migration of 8% and SLPC III about 17%. The SLnPC was 10% acyl migrated. Acyl migration was not determined on the SAPC sample.

DSC Analysis. Lipid Transitions. Typical heating and cooling thermograms for a preparation of SLPC synthesized in this laboratory are illustrated in Figure 1a,b. The transitions of SLPC I and SLPC II were broad, although symmetrical. The half-height width of SLPC I was 3–4 °C for both endo- and exotherms. The same result was obtained over different scan rates (1.25–20 deg/min) and full-scale sensitivities. The T_c for the endotherms was −18.5 °C, the T_{max}

Table I: Gel to Liquid-Crystalline Transition Temperatures and Enthalpies for Aqueous Dispersions of Lecithins Containing Increasing Numbers of Double Bonds

lecithin	experiment		theory[a]		
	T_c (°C)	ΔH (kcal·mol^{-1})	T_c (°C)	ΔH (kcal·mol^{-1})	A_m/A_o
DSPC[b]	54.5, 58, 58.24, 54.9, 54.00, 55.1	9.8, 10.67, 10.84, 10.6, 7.89, 10.3	55.2[c]	14.7	1.00
SOPC[d]	6.3	5.4	6.3	8.1	1.31
SLPC[e]	−16.2 ± 1.6 ($\bar{x}$ ± SD, n = 9)	3.3 ± 1.0 (n = 8)	−16.2	6.2	1.55
SLnPC	−13.0	6.6	−13.0	5.6	1.64
SAPC	−12.6 ± 1.0 (n = 3)	5.3 (3.5, 7.1, n = 2)	−12.6	4.7	1.63
DOPC[f]	−17.6, −22, −21, −15.8	8.0, 7.6, 7.7	−19.1	5.1	1.59

[a] Calculation of T_c and ΔH using eq 1-9 from the Appendix. For all calculations γ_B = 0.36789 and δ = −6.4. [b] Data for DSPC taken from the following sources in the order of presentation: P. J. Davis and K. M. W. Keough, unpublished observations; Phillips et al., 1969; Hinz & Sturtevant, 1972; Mabrey & Sturtevant, 1976; Chen & Sturtevant, 1981. [c] Values from model S-1 of Berde et al. (1980). [d] Data from Davis et al. (1981). [e] Three separate synthetic preparations, eight to nine separate dispersions. [f] Data taken from the following sources in the order of presentation: P. J. Davis and K. M. W. Keough, unpublished observations; Phillips et al., 1969; Barton & Gunstone, 1975; Silvius & McElhaney, 1979.

was −16.5 °C, and the enthalpy was 2.2 kmol·mL^{-1}. The corresponding exotherms seen on the leading edge of the ice peaks on cooling gave an apparent onset of approximately −14.5 °C and T_{max} of −18 °C. The exotherm seen at T_{max} of −39 °C in Figure 1b is not related to SLPC but occurs in a large number of lipid–water dispersions that are cooled from a temperature greater than 0 °C (K. M. W. Keough, unpublished observations). A second preparation of SLPC (II) gave very similar thermograms and a T_c of −19 °C and T_{max} of −16 °C.

A third batch of SLPC (SLPC III) was available in relatively large quantity, and seven dispersions were analyzed over 6 months. Two of these aqueous dispersions were also analyzed after various times of storage at −20 °C. The limits of shapes that were observed in various fresh dispersions are shown in Figure 1c,d. The T_c varied from −14.2 to −17.1 °C ($\bar{x}$ ± SD = −15.1 ± 0.8 °C) and the T_{max} from −12.3 to −15.2 °C ($\bar{x}$ ± SD = −14.6 ± 0.9 °C). The enthalpies obtained for these various dispersions ranged from 2.2 to 4.9 kcal·mol^{-1} ($\bar{x}$ ± SD = 3.5 ± 1.0 kcal·mol^{-1}). None of the variability could be ascribed in a systematic way to any parameter associated with the analytical data on the SLPC (obtained from TLC, GLC, or UV analyses) or with various parameters associated with the sample preparation (evacuation time, temperature used to make the dispersion) or with the heating sequences employed. Some, but not all, heating thermograms of SLPC III showed a very low enthalpy endotherm around −25 °C. On cooling, exotherms were usually partly or completely masked by the freezing of supercooled water. The summary data given for SLPC in Table I are averages based upon all runs from samples showing similar analytical purity.

Figure 1e shows thermograms for the preparation of SLnPC that had undergone 10% acyl migration but was otherwise analytically pure. This material gave a narrower exotherm than those of SLPC with a T_c of −13 °C, T_{max} of −11 °C, a half-height width of 2.2 deg, and a transition enthalpy of 6.6 kcal·mol^{-1}. The exotherms showed a broader transition with an extrapolated T_c of −11 °C and a T_{max} of −13 °C being observed.

Figure 1f shows a thermogram obtained from the sample of SAPC. It had a T_c of −12.4 °C (range −11.3 to −13.1 °C in three dispersions) and a T_{max} of −10.7 °C (range −9.7 to −11.3 °C). The half-height width was 2.4 °C, and the enthalpy was 5.3 kcal·mol^{-1} (range 3.5–7.1 kcal·mol^{-1} in two dispersions). In this instance, there may have been some effect of oxidation on the enthalpy as the higher value was obtained when E^{265}_{mol} = 190 while the lower one was obtained when E^{265}_{mol} = 730. There was, within the limits of error, no effect of this small amount of oxidation on T_c, T_{max}, or $\Delta T_{1/2}$.

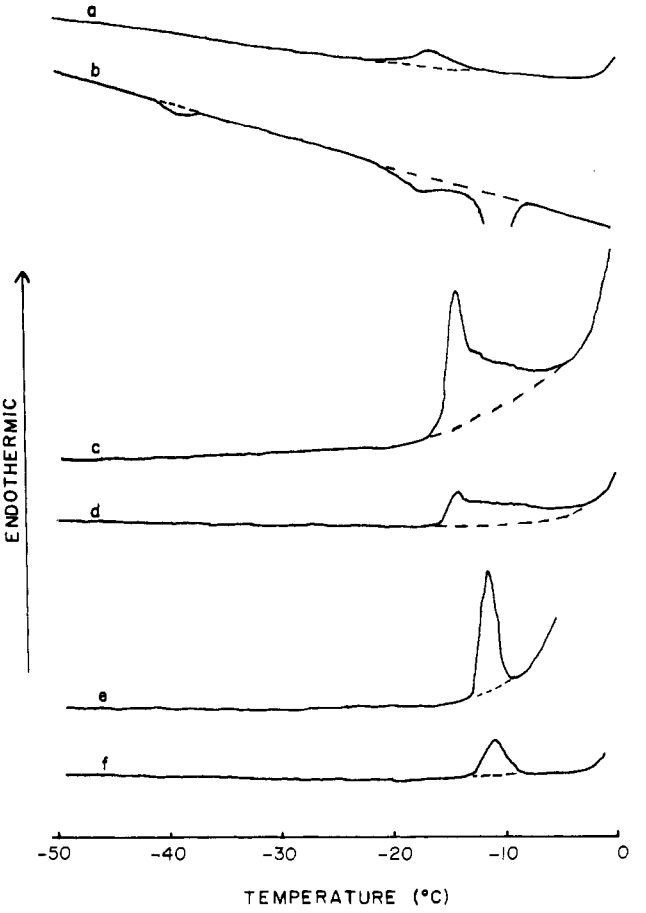

FIGURE 1: Differential scanning calorimetric thermograms of dispersions of a number of polyunsaturated lecithins: (a) heating run of SLPC I; (b) cooling run of SLPC I; (c) heating run of SLPC III; (d) heating run of SLPC III—no detectable analytical difference from (c); (e) heating run of SLnPC; (f) heating run of SAPC; (a–f) programming rate was 5 deg/min. Full-scale sensitivities varied from 0.5 to 5 mcal/s.

Some samples of SLPC and SLnPC were dispersed in 50% aqueous ethylene glycol to eliminate the water transition. The effects on the lipid transitions were highly variable. In general, most samples had broadened transitions with T_c and T_{max} being shifted a few degrees. The measured enthalpies were generally decreased. In two samples of SLPC, broad transitions that led to high enthalpies were seen, but they were not consistently

reproducible. We and others (van Echteld et al., 1980; Davis et al., 1981) have found that 50% aqueous ethylene glycol effects different lecithins in different ways. The variability with SLPC is the most extreme we have seen.

Discussion

Lipid Transitions. In dealing with polyunsaturated lipids there is a potential problem of oxidation and its effect upon phase behavior. It has been observed in the presence of limited oxidation of lipids that transition temperatures shift upward slightly and enthalpies are not affected within normal variability. When oxidation is extensive, there is a decrease in enthalpy and a disappearance of the gel to lc endotherms (K. P. Coolbear and K. M. W. Keough, unpublished observations). Nearly all the samples investigated here were essentially free of oxidation products. The UV analysis of SAPC suggested it had undergone limited oxidation by the time of the third sample preparation.

A compilation of data, obtained from this laboratory and elsewhere, on the gel to lc phase transition temperatures and enthalpies for a series of C_{18} lecithins and for SAPC is given in Table I. For the C_{18} lecithins, it can be seen that the introduction of the first cis double bond at carbon 9 in the acyl chain at the *sn*-2 position results in a dramatic decrease in transition temperature of ~50 deg. The second double bond at carbon 12 effects a further decrease of ~22 deg, whereas the third double bond at carbon 15 induces no further decrease on the T_c; in fact, the T_c is increased by 3 °C.

This interesting sequence of findings can be rationalized with the data of Barton & Gunstone (1975) and can be accommodated within the framework of an existing statistical mechanical model (Jacobs et al., 1977; Berde et al., 1980) for phase transitions in membranes. The theoretical interpretations will be given in the Appendix. In their studies on *cis*-octadecenoate isomers of phosphatidylcholines, Barton & Gunstone (1975) observed minimum values of transition temperatures and enthalpies when the double bond was at the middle of either one or both of the acyl chains. The reduction in T_c from that of DSPC induced by the double bond was smaller as the double-bond position moved toward either end of the hydrocarbon chain. Spin-label and nuclear magnetic resonance studies (Hubbell & McConnell, 1971; Levine et al., 1972; Seelig & Waespe-Šarčevič, 1978) have also shown that the molecular motion of the hydrocarbon chain increases toward the methyl end. In SAPC, some increase in T_c might be anticipated because of the increase of two carbons in the *sn*-2 chain. There are, however, four double bonds in the 2 chain at C-5, -8, -11, and -14, but these are not disruptive enough of packing to reduce the transition temperature below that of the other lecithins studied; indeed, the T_c is marginally higher. As will be discussed below, these results suggest that at least in mixed-acid lecithins, there may be some effective ordering induced in the unsaturated chains by the presence of multiple double bonds. ^2H NMR studies have indicated that the region of the double bond has greater order than regions of single-bonded carbons that are remote from the double bond (Seelig & Waespe-Šarčevič, 1978). These results are consistent with some other findings on the physical properties of lipids with multiple double bonds. Ghosh & Tinoco (1972) and Evans & Tinoco (1978) observed in monolayer studies of mixed-acid lecithins that the first double bond to be introduced into a chain had the greatest effect on molecular area. Stubbs et al. (1981) found that the cone angle, wobbling diffusion coefficient, and steady-state anisotropy of 1,6-diphenyl-1,3,5-hexatriene in a series of unsaturated mixed-acid lecithins above T_c were similar, although not identical, to one another. Also, during manipulations of fatty acid compositions of lymphocytes and leukemic murine T cells, it has been found that the behavior at 37 °C of intercalated fluorescent probes was not very much affected by changes in the fatty acid compositions of the phospholipids (Stubbs et al., 1980; McVey et al., 1981). Herring et al. (1980) have found that the fluidity of plasma membranes of *Dictyostelium discoideum* as measured by diphenylhexatriene fluorescence depolarization or by electron spin resonance of 5-doxylstearic acid probes was almost unaltered even when substantial amounts of 18:2 fatty acids were replaced by 18:3 and 20:4 fatty acids. These observations are consistent with the effects of double bonds on the T_c of saturated–unsaturated lecithins and with the observations of Stubbs et al. (1981) about DPH fluorescence in liposomes of mixed-acid PCs.

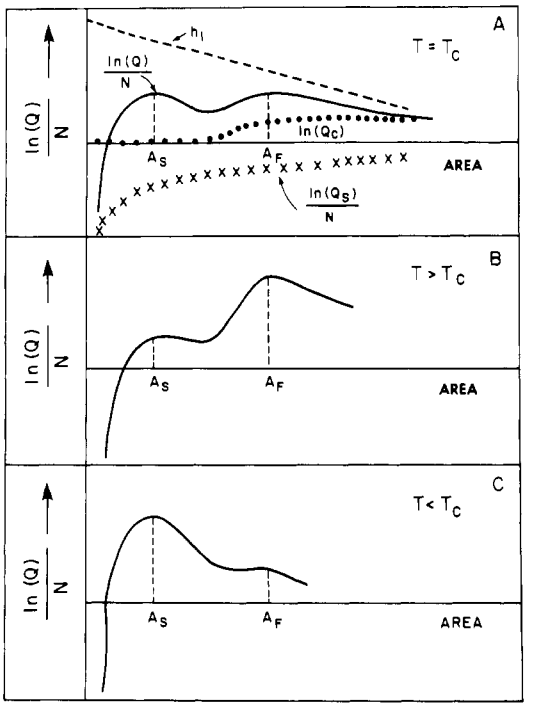

FIGURE 2: (A) A plot of $(\ln Q)/N$ (solid curve) and its components h_1 (dashes), $\ln Q_c$ (dots), and $(\ln Q_s)/N$ (crosses) vs. area (A). Systems with areas A_s and A_f (corresponding to solid and fluid, respectively) have the same temperature, pressure, and Gibbs free energy, and they can be coexisting phases at a first-order phase transition. (B) At temperature T above T_c, $\ln Q(A_s)$ is less than $\ln Q(A_f)$; i.e., the free energy of the fluid phase is less than that of the solid, and the fluid will be the stable phase. (C) At temperature T below T_c, $\ln Q(A_s)$ is greater than $\ln Q(A_f)$, and the solid phase will be stable.

The transition temperatures of DOPC, SLPC, SLnPC, and SAPC all fall in a relatively small range, but a fair variation in measured transition enthalpies has been observed. There is also variation between and within individual samples as was seen in the samples of SLPC III. In the case of SLPC III, there was difficulty in obtaining exact base lines on the rising water peaks, but this would likely account for only some of the variability. We have included in Table I some values of the enthalpy of DSPC found by various workers to show that such variability is not relegated to these unsaturated lipids. Others have found that the transition enthalpies for different dispersions of the same batches of dipalmitoyl-PC and *N*,*N*-dimethyldipalmitoylphosphatidylethanolamine were also variable (Vaughan & Keough, 1974; Chen et al., 1980; Chen & Sturtevant, 1981). At least some if not all of this variability is likely associated with differences in the physical form of the dispersions (Chen & Sturtevant, 1981). In the case of these polyunsaturated lipids, the presence of undetected oxidation

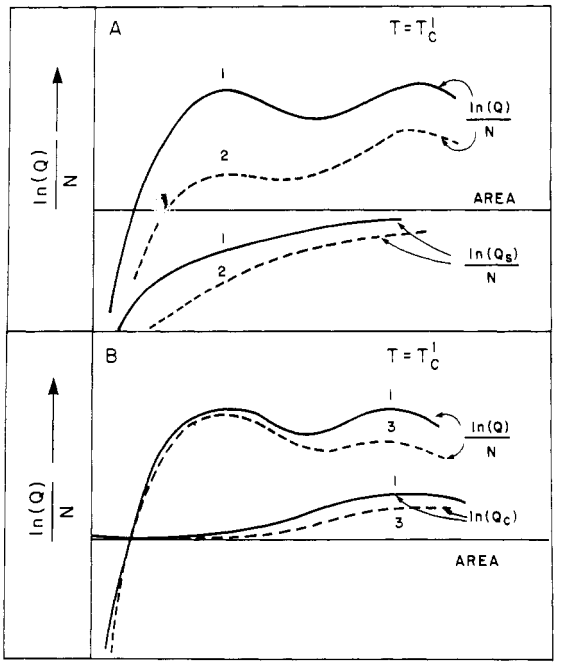

FIGURE 3: Effects of changes in terms of eq 4 on dependence of partition function on area, recorded at fixed temperature $T = T_c^1$. (A) For a system of molecules whose plots of $(\ln Q)/N$ and $(\ln Q_s)N$ vs. area are shown in solid curves labeled (1), we find a transition temperature T_c^1. Assume that the molecules are modified in some fashion that affects only the excluded area (ignore for discussion the effects on P_{HD} in the expression for q_c); this will result in shifts in the plot of $(\ln Q_s)/N$ and $(\ln Q)N$ to the dashed curves (2). This effects a destabilization of the solid relative to the fluid phase, and the transition temperature for system 2, T_c^2, would be less than T_c^1. (B) For system 1, we now envision a hypothetical change that only serves to reduce the configurations accessible to the chains in the fluid phase. This new system (3) shows a destabilization of the fluid phase relative to the solid phase, and T_c^3 would be greater than T_c^1.

products might also cause some variability in transition enthalpy. There was also some variability in the transition widths, especially of SLPC. The widths may be expected to be affected by the sample purity and even by instrumental variability, but observations from this laboratory (Davis et al., 1980, 1981) show that there is no single causative factor. For these samples of SLPC, the differences do not appear to be due to differences in sample purity. Even with the variability, however, the enthalpy of transition of SLPC was lower and the half-height width of the transition was greater than those of the other mixed-acid lipids.

Theoretical Aspects of Lipid Transitions. The effect on T_c of multiple double bonds in one chain of a mixed-acid lecithin can be interpreted by using an extension of previous theoretical models (Berde et al., 1980; Jacobs et al., 1975, 1977). In brief, the nonmonotonic decrease in transition temperature with the introduction of multiple double bonds occurs because of countervailing effects of factors that would normally lower the temperature such as increasing areas per molecule and those that raise the temperature such as decreasing freedom of rotation in the chains. The values of T_c and ΔH calculated from the model are given in Table I. A full description of these effects and of the calculation of values for T_c and ΔH is given in the Appendix.

Conclusions

The introduction of double bonds into membrane lipids has generally been equated with an increase in fluidity. The above data suggest that increasing the unsaturation of a single acyl chain in a phospholipid does not necessarily lead to a systematic decrease in the T_c of the lipid and may not result in increased fluidity at any given temperature above T_c. As discussed above, the overall effect of multiple double bonds on T_c will be a compromise between bond orientation and rotational freedom, chain interactions, and steric restrictions imposed by molecular packing. These results suggest that interpretations utilizing the number of double bonds or amounts of polyunsaturated acids as indices of lipid fluidity should be cautious.

Acknowledgments

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Appendix

Description of Model and Calculations for Phase Transition Temperature and Enthalpies. The trends in the transition-temperature data for glycerophosphocholines having polyunsaturated acyl chains in the *sn*-2 position can be interpreted by extension of previous models by one of us (Berde et al., 1980) and co-workers (Jacobs et al., 1975, 1977). In our models, the important interactions leading to the bilayer phase transition are the short-range repulsions between molecules, longer dispersion attractions between acyl chains, longer range repulsions or attractions between head groups (including dipolar effects, hydrogen bonding, interaction of the head groups with the aqueous phase, and other "end effects"), and gauche–trans isomerizations of the single bonds in the acyl chains.

The Hamiltonian $H(N,A,T)$ of a system of N molecules ($2N$ chains) at temperature T and area A is expressed as a sum of terms pertaining to each of these types of interactions. From a model with degrees of freedom, X and Y, as described before (Berde et al., 1980), we have the following specification of the Hamiltonian:

$$H(X^N,Y^{2N}) = H_s(X^N,Y^{(0)2N}) + Nh_1(a) + \sum_{j=1}^{2N} h_c(Y_j;a) \qquad (1)$$

where H_s contains the short-range repulsive interactions between molecules, $a = A/N$ is the area per molecule, $h_1(a)$ is a mean-field term describing the long-range dispersion attractions between chains and long-range attractions or repulsions between head groups, and h_c is the chain conformation Hamiltonian, which describes interactions associated with conformations of the chains that increase the chain's area beyond that associated with $Y^{(0)}$, the minimum-area conformation. For the species of interest here, the chains in the 1 and 2 positions are not identical, and the final term is separated into two terms:

$$\sum_{j=1}^{2N} h_c(Y_j;a) = \sum_{m=1}^{N} h_c^s(Y_{2m-1};a) = \sum_{m=1}^{N} h_c^u(Y_{2m};a) \qquad (2)$$

where h_c^s and h_c^u are the chain Hamiltonians for the saturated and unsaturated and unsaturated chain, respectively.

From this, we form the partition function $Q(N,A,T)$, which, following eq 7–11 and 32 of Berde et al. (1980), becomes

$$Q(N,A,T) = Q_s(N,A,T) \exp[-\beta N h_1(a)] q_c^s(a,T)^N q_c^u(a,T)^N \qquad (3)$$

where $\beta = 1/(kT)$ and k is the Boltzmann constant. Q_s is a partition function that includes only short-range repulsions

between molecules whose chains are in the conformation $Y^{(0)2N}$, and q_c^s and q_c^u are the partition functions for single saturated chains (under the influence of h_c^s) and single unsaturated chains (under the influence of h_c^u), respectively. Taking logarithms, we find

$$\ln Q(N,A,T) = \ln Q_s(N,A,T) - \beta N h_1(a) + N \ln q_c^s(a,T) + N \ln q_c^u(a,T) \quad (4)$$

Recall that the Helmolz free energy $F = kT \ln Q$. As described previously (Huang, 1967; Jacobs et al., 1975), if a plot of ln Q vs. area, evaluated at a particular temperature T, shows two maxima of equal height with areas A_1 and A_2, then systems at areas A_1 and A_2 have the same temperature, pressure, and F, and they can be coexisting phases at a first-order phase transition. Since ln Q as given by eq 4 is a sum of terms, we can understand qualitative features that raise or lower the transition temperature in this series of compounds by considering effects on each term in eq 4. Schematic plots of ln Q and its terms vs. area are given in Figure 2 to show the effects on the partition function at temperatures below, at, and above T_c. Also, as shown in Figure 3A, features that increase the area per molecule at closest packing lead to decreases in ln Q_s, and if this factor were acting in isolation, there would be a relative destabilization of the solid phase relative to the fluid phase and a lowering of the transition temperature. As shown in Figure 3B, features that lead to a reduction in the number of attainable gauche–trans isomerizations of the single bonds in a chain or that increase the intramolecular energy associated with these isomerizations would result in a lowering of the value of ln Q_c, and this reduction would be more pronounced at high areas of the system than at low areas. This leads to a destabilization of the fluid phase relative to the solid phase and, if acting in isolation, would produce an elevation in transition temperature.

For the series of diacyl-*sn*-glycero-3-phosphocholines DSPC, SOPC, and DOPC, previous calculations (Berde et al., 1980) ascribed the ordering of transition temperatures DSPC > SOPC > DOPC to an ordering of excluded areas (or areas per molecule at closest packing) DSPC < SOPC < DOPC. Detailed consideration of many alternative models indicated that considerations of molecular shape and excluded area were the most important features in this experimental trend.

Examination of the structure of oleic, linoleic, and linolenic acids and consideration of tilting and "back-filling" effects (Nagle, 1976; Jackson, 1976; Berde, 1978; Berde et al., 1980) suggest that for the series of lecithins studied here the area per molecule at closest packing will follow the sequence SOPC < SLPC < SLnPc, although the effect of introduction of a third double bond in the excluded area will be much less than the effect of introduction of the second double bond. Since the experimental transition temperatures for this series do not decrease monotonically as the number of double bonds in the chain is increased, features other than excluded area must also be of importance for these trends.

Our interpretation is that for this series, the effects of molecular excluded area are counterbalanced by the effects of the double bonds on the chain conformation partition function q_c^u. In the rotational isomeric state approximation [see Flory (1969)], each single bond in a saturated chain has three discrete states, trans, gauche$^+$, and gauche$^-$. Introduction of a gauche bond occurs with an intramolecular energy of ϵ = 0.5 kcal·mol^{-1} and also increases the area per molecule, generating increased repulsions from neighboring molecules; thus, energy considerations favor maintenance of the all-trans state. These repulsions are greatest at high densities and least at low densities. Maximization of entropy favors the occurrence of gauche conformations. The reader should refer to Jacobs et al. (1975) (pp 3994–3995) for insight into the principles underlying the construction of q_c^s.

In chains containing cis unsaturated double bonds, the considerations are similar, but there are two major differences. First, at the double bond (denoted j) there is only a single allowed conformation. Second, as described by Mark (1966) and by Flory (1969), the energies, bond angles, and projected areas associated with conformations at bonds $j \pm 1$ are different from those associated with other single bonds. In the model, introduction of a double bond eliminates two degrees of freedom associated with bond j, and at area A and temperature T this effect alone would lower the value of q_c^u relative to the corresponding q_c^s by the amount of 2 exp$[-\beta(\epsilon + P_{HD}\Delta A/N)]$ where P_{HD} is defined as in Jacobs et al. (1975).

This effect on q_c^u relative to q_c^s is greater at high areas than at low areas, as shown in Figure 2B, since at low areas the contribution of the two gauche states to q_c^s is very small. Stated in another way, the effect of introduction of a double bond on q_c^u is to produce a relatively greater destabilization of the fluid phase than the solid phase, and this effect, if acting in isolation, would serve to increase the transition temperature.

In the fluid phase, both experimentally and in our models, the probability of a single bond being in the gauche conformation increases for bonds closest to the methyl terminus; the gauche bonds closest to the methyl terminal make the largest contributions to q_c^s or q_c^u. For given values of A and T, removal of degrees of freedom by insertion of a double bond results in a greater lowering of $q_c^u(A,T)$ relative to $q_c^s(A,T)$ for double bonds closer to the methyl terminus than for double bonds closer to the carboxyl terminus. Qualitatively, the effect of the double bond in destabilizing the fluid phase relative to the solid phase is greatest for double bonds closest to the methyl terminus because this eliminates those gauche bonds most important to the stability of the fluid phase. Sample calculations using a modification of model CD-II from Berde et al. (1980), for a hypothetical case in which we assume that double-bond position has no effect on the excluded area, indicate that these effects on q_c^u lead to a monotonic increase in T_c with placement of the doulbe bond closer to the methyl terminus of the chain. The magnitude of this effect for various versions of the model is about a 15–25 °C difference for T_c between j = 9 and j = 15.

As the number of double bonds in the chain is increased, more potential gauche bonds are eliminated, and a further destabilization of the fluid phase relative to the solid phase occurs. Thus, in our interpretation, SLnPC melts at a higher temperature than SLPC, both because the former compound has more double bonds and because it has double bonds close to the methyl terminus.

A second feature of chains with polyunsaturation is the occurrence of many single bonds adjacent to the double bond whose bond angles, conformational energies, and projected areas are different from those of other single bonds (Mark, 1966; Flory, 1969; Berde et al., 1980).

We have performed calculations of transition temperatures and enthalpies changes for this series of compounds, using simplified expressions for q_c^u for oleic, linoleic, linolenic, and arachidonic chains. Several models with slightly different descriptions of packaging arrangements and area changes associated with chain states were used, but all gave similar qualitative agreement with experiment. We present model U-1, as it is the simplest and illustrates the basic principles.

The double bond, denoted j, has a single allowed conformation. Bonds $j \pm 1$ adjacent to the double bond have allowed

states at angles 0° and ±60° in the rotational isomeric state approximation (Flory, 1969; Mark, 1966). The energies associated with conformations at $j + 1$ and $j - 1$ are determined both by the torsional energy about a single bond and by the hydrogen–hydrogen repulsive energy of pendant hydrogens at carbons $j - 1$ and $j + 2$. Following Mark (1966) and Flory (1969), we assign energies of $\mu = 0$ kcal·mol^{-1}, $\lambda = 0.8$ kcal·mol^{-1}, and $\kappa = 1.6$ kcal·mol^{-1} to the pairs of bond angles at $j - 1$ and $j + 1$ of ±60°,±60°, 0°,±60° or ±60°,0°, and 0°,0°, respectively.

The minimum area A_m of a system of N molecules ($2N$ chains) is given by

$$A_m = A_0(1 + \gamma_c{}^u a^u) \tag{5a}$$

for the 1-stearoyl 2-unsaturated series and

$$A_m = A_0(1 + 2\gamma_c{}^u a^u) \tag{5b}$$

for the 1,2-diunsaturated series, where A_0 is the area of a system of $2N$ saturated chains at closest packing, a^u is the difference in projected area between a polyunsaturated chain and a saturated chain, and $\gamma_c{}^u$ is an area parameter, $0 < \gamma < 1$, to account for "back-filling" and related effects (Berde et al., 1980). The expressions for the hard-disk and mean-field terms are as in previous models.

As in previous models, we assume that gauche conformations of the single bonds increase the effective area of a chain and do so against an effective pressure that is proportional to the hard-disk pressure. In model U-1, as a simplest case, we assume that all combinations of angles 0° and ±60° at $j + 1$ give roughly the same projected area of a chain in the solid phase and that gauche bonds at other positions in the chain give area increases as described in previous models. The chain partition function $q_c{}^u$ for a linoleic chain then becomes $q_c{}^u = q_1 + q_2 + q_3$, where

$$q_1 = (4e^{-\beta\mu} + 4e^{-\beta\lambda} + e^{-\beta\kappa})^2$$

$$q_2 = \sum_{i=1}^{7} 2e^{-\beta\epsilon} q_1 (1 + 2e^{-\beta\epsilon})^{16-i-6} \times \exp\left[\frac{-\beta}{N} P_{HD} 0.14 \gamma_B (16 - i + 1)\frac{A_0}{A}\right]$$

$$q_3 = \sum_{i=14}^{16} 2e^{-\beta\epsilon} q_1 (1 + 2e^{-\beta\epsilon})^{16-i} \times \exp\left[\frac{\beta}{N} P_{HD} 0.14 \gamma_B (16 - i + 1)\frac{A_0}{A}\right] \tag{6}$$

q_1 includes all states for which bonds 1–7 and 14–16 are all-trans, q_2 includes all states for which the first gauche bond occurs at bonds 1–7, and q_3 includes all states for which the first gauche bond occurs at bonds 14–16.

In a similar fashion, there is a projected area increase associated with the linolenic chain, and the chain partition function $q_c{}^u = q_1 + q_2$, where

$$q_1 = (4e^{-\beta\mu} + 4e^{-\beta\lambda} + e^{-\beta\kappa})^3$$

$$q_2 = \sum_{i=1}^{7} 2e^{-\beta\epsilon} q_1 (1 + 2e^{-\beta\epsilon})^{16-i-9} \times \exp\left[\frac{-\beta}{N} P_{HD} 0.14 \gamma_B (16 - i + 1)\frac{A_0}{A}\right] \tag{7}$$

In q_1 all bonds 1–7 are trans, while in q_2 the first gauche bond occurs at 1–7.

By a similar argument, the chain partition function for an arachidonic chain is $q_c{}^u = q_1 + q_2 + q_3$, where

$$q_1 = (4e^{-\beta\mu} + 4e^{-\beta\lambda} + e^{-\beta\kappa})^4$$

$$q_2 = \sum_{i=1}^{3} 2e^{-\beta\epsilon} q_1 (1 + 2e^{-\beta\epsilon})^{18-i-12} \times \exp\left[\frac{-\beta}{N} P_{HD} 0.14 \gamma_B (18 - i + 1)\frac{A_0}{A}\right]$$

$$q_3 = \sum_{i=16}^{18} 2e^{-\beta\epsilon} q_1 (1 + 2e^{-\beta\epsilon})^{18-i} \times \exp\left[\frac{-\beta}{N} P_{HD} 0.14 \gamma_B (18 - i + 1)\frac{A_0}{A}\right] \tag{8}$$

$q_c{}^s$, the chain conformation partition function for saturated chains, is given by eq 20 of Berde et al. In order to permit fair comparison, $q_c{}^u$ for oleic acid chains was constructed as $q_c{}^u = q_1 + q_2 + q_3$, where

$$q_1 = 4e^{-\beta\mu} + 4e^{-\beta\lambda} + e^{-\beta\kappa}$$

$$q_2 = \sum_{i=1}^{7} 2e^{-\beta\epsilon} q_1 (1 + 2e^{-\beta\epsilon})^{16-i-3} \times \exp\left[\frac{-\beta}{N} P_{HD} 0.14 \gamma_B (16 - i + 1)\frac{A_0}{A}\right]$$

$$q_3 = \sum_{i=11}^{16} 2e^{-\beta\epsilon} q_1 (1 + 2e^{-\beta\epsilon})^{16-i} \times \exp\left[\frac{-\beta}{N} P_{HD} 0.14 \gamma_B (16 - i + 1)\frac{A_0}{A}\right] \tag{9}$$

Again, q_1 includes states for which bonds 1–7 and 11–16 are trans, q_2 includes states for which the first gauche bond occurs at bonds 1–7, and q_3 includes states for which the first gauche bond occurs at bonds 11–16.

This model, though simplistic particularly in its description of the effective areas of various conformations with different angles of single bonds in the vicinity of the double bonds, has the following virtues: (1) it is relatively simple, (2) it allows for comparison between members of the series of polyunsaturated compounds, (3) it is qualitatively correct in describing the reduction in degrees of freedom for chain bending associated with the introduction of multiple double bonds, and (4) there are no new adjustable parameters in $q_c{}^u$. We have examined models with more complicated treatments of the bonds near the double bonds and have found qualitatively similar results. As in previous models, transition temperatures were obtained by Maxwell constructions on plots of ln Q vs. area (eq 4), and enthalpies were derived from the partial derivatives of ln Q with respect to β.

In our previous treatment of phosphatidylcholines with monounsaturated chains, the model calculations proceeded as follows. The values of Δa_c for each member of the series were estimated from molecular models (Berde et al., 1980, eq 27–30). $\gamma_c{}^u$ was adjusted to obtain agreement with experiment for DOPC, the value of $\gamma_c{}^u$ was then fixed, and transition temperatures for the other members of the series were calculated and compared with experiment.

In the present case, we hypothesize that the observed nonmonotonic dependence of transition temperature on the number of double bonds in the 2-position chain is due to opposing influences of multiple double bonds on the excluded area A_m and on the accessible degrees of freedom in $q_c{}^u$ at higher areas.

A necessary condition for the validity of this hypothesis is that the experimental trend in transition temperature be simulated by models in which A_m/A_0 increases monotonically with the number of double bonds in the chain.

A series of calculations was performed with the equations described above. γ_B and δ were taken from model S-1 or Berde et al. (1980) and were held fixed for this series of calculations. As shown in Table I, agreement with experiment was found by assuming that the series of mixed-acid phosphatidylcholines with stearic acid in the 1 position and 18-carbon chains with variable numbers of double bonds in the 2 position has a monotonic increase in A_m as more double bonds are added. Examination of this series of calculations supports our assertion that increasing numbers of double bonds lowers transition temperatures via effects on excluded area and raises transition temperatures via effects on degrees of freedom for chain bending in q_c^u. If, for the sake of illustration, A_m for SLnPC were assumed to be equal to A_m for SLPC, then the effect of increasing from two to three double bonds in q_c^u would raise the transition temperature from −16.2 for SLPC to 6.0 °C for SLnPC. Extension of the model to SAPC shows slight discrepancy with this trend; agreement with experiment is achieved by assuming that SLnPc and SAPC have almost equal values of A_m. Values of ΔH are also shown in Table I. In experiment and in theory all of the compounds with unsaturated double bonds have lower transition enthalpies than DSPC. In a general sense, our model would predict smaller transition entropies with increasing numbers of double bonds. Since the temperature of transition depends on double-bond number and is nonmonotonic, the dependence of transition enthalpy (which equals $T\Delta S$ of transition) on the number of double bonds is not straightforward, and we hesitate to intepret the details of the dependence of ΔH of transition on the number of double bonds.

Registry No. 1-Stearoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine, 27098-24-4; 1-stearoyl-2-linolenoyl-*sn*-glycero-3-phosphocholine, 35418-57-6; 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine, 35418-59-8.

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