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Gel to Liquid-Crystalline Transition Temperatures of Water Dispersions of Two Pairs of Positional Isomers of Unsaturated Mixed-Acid Phosphatidylcholines†

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ABSTRACT: The gel to liquid-crystalline phase transition temperatures of dispersions of mixed-acid *sn*-1,2-lecithins which contain one unsaturated and one saturated fatty acid have been studied by differential scanning calorimetry. The temperature for 1-oleoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine (containing no reversed isomer) was −9.3 °C while that for 2-oleoyl-1-palmitoyl-*sn*-glycero-3-phosphocholine (containing 8% of the reversed isomer) was −2.6 °C. The temperature for 2-oleoyl-1-stearoyl-*sn*-glycero-3-phosphocholine (containing 6% of the reversed isomer) was 6.3 °C while that for 1-oleoyl-2-stearoyl-*sn*-glycero-3-phosphocholine (containing 18% of the reversed isomer) was 8.6 °C. The differences in transition temperatures for the isomers of a pair containing the same two acids were consistent with those observed for positional isomers of saturated mixed-acid lecithins in that the isomer of the pair which had the longer fatty acid in the *sn*-1 position had the lower temperature. The phase transition temperatures of pairs of isomers containing palmitate and oleate at the *sn*-1 and -2 positions were different by at least 6.7 °C, while those containing stearate and oleate were different by at least 2.3 °C. Differences in the chain lengths of the fatty acids at the two positions of the glycerol appear to predominate over differences in the depths of the double bonds in the bilayer in determining the transition temperatures.

It has been observed that, when they are dispersed in water, pairs of positional isomers of saturated mixed-acid *sn*-1,2-phosphatidylcholines containing either myristate plus palmitate or stearate plus palmitate exhibit different gel to liquid-crystalline transition temperatures and different enthalpies of transition (Keough & Davis, 1979). These observations have recently been confirmed and extended by Chen & Sturtevant (1981). In these studies, a consistent pattern emerged in that it was always the member of an isomeric pair which contained the longer acid at the *sn*-1 position which had the lower T_c and enthalpy. In most instances, lipids of biological origin contain at least one acid with at least one double bond. Lipids which have double bonds have lower transition temperatures than lipids with equivalent saturated chains. The ultimate T_c of an unsaturated lecithin is dependent, however, not only on the presence or number of double bonds but also on their position in the chain (Barton & Gunstone, 1975).

It is of interest to know if the disruptive effect of the double bond would be great enough to supersede packing differences in pairs of positional isomers caused by alternating the position of the chain on the glycerol (Keough & Davis, 1979). Here we report upon the transition temperatures of two pairs of isomeric lecithins each containing a saturated (either palmitate or stearate) and an unsaturated (oleate) chain—unsaturated mixed-acid lecithins.

Materials and Methods

Preparation and Analysis of Lipids. Dipalmitoylphosphatidylcholine, distearoylphosphatidylcholine, OPPC,[1] and various fatty acids were purchased from Sigma Chemical Co., St. Louis, MO. Dioleoylphosphatidylcholine was either made by a method described before (Cubero Rubles & van den Berg, 1969; Keough & Davis, 1979) or was purchased from Sigma Chemical Co. Two batches of SOPC and one

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[1] Abbreviations used: DSC, differential scanning calorimetry (ic); lc, liquid crystal(line); OPPC, 1-oleoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine; OSPC, 1-oleoyl-2-stearoyl-*sn*-glycero-3-phosphocholine; PC, phosphatidylcholine; POPC, 2-oleoyl-1-palmitoyl-*sn*-glycero-3-phosphocholine; SOPC, 2-oleoyl-1-stearoyl-*sn*-glycero-3-phosphocholine; T_c, gel to lc transition temperature; T_m, temperature of maximum heat flow into or out of a sample during a thermal event.

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Table I: Thermal Analysis Data for Unsaturated Mixed-Acid Phosphatidylcholines[a]

	OPPC	POPC	SOPC	SOPC	OSPC
acyl migration (%)	0	8	6	18	18
N (number of determinations)	3	5	5	5[b]	4[b]
			Heating Runs		
T_c (°C) (onset)	−9.3 ± 0.9	−2.6 ± 0.2	6.3 ± 0.4	5.9 ± 0.5	8.6 ± 0.3
T_m (°C)	−7.9 ± 0.9	−0.8 ± 0.5	8.3 ± 0.6	8.5 ± 1.0	10.9 ± 0.3
$\Delta T_{1/2}$ (°C)	2.3 ± 0.5	2.4 ± 0.3	2.1 ± 0.6	4.5 ± 0.5	4.7 ± 0.3
ΔH (kcal/mol)	4.6 ± 0.8	5.4 ± 0.3	5.4 ± 0.3	6.6 ± 0.6	6.7 ± 0.2
			Cooling Runs		
T (onset) (°C)	−7.4 ± 1.1	−0.4 ± 0.4	8.4 ± 0.9	10.3 ± 1.0	12.0 ± 1.7
T_m (°C)	−9.5 ± 0.7	−2.6 ± 0.4	6.3 ± 0.4	6.4 ± 0.8	8.0 ± 0.9
$\Delta T_{1/2}$ (°C)	2.5 ± 0.2	2.6 ± 0.4	2.2 ± 0.5	3.8 ± 0.5	4.4 ± 0.3
ΔH (kcal/mol)	4.3 ± 0.1	5.1 ± 0.1	5.4 ± 0.3	5.9 ± 0.7	6.4 ± 0.7

[a] Values are $\bar{X}$ = SD. [b] These represent determinations on two batches.

batch of OSPC were prepared as described previously (Cubero Robles & van den Berg, 1969; Keough & Davis, 1979). A batch of OSPC, one batch of SOPC, and the POPC were prepared by the method of Gupta et al. (1977); the OSPC was made by using the free lysooleoyl-PC, while $CdCl_2$ complexes of the appropriate lyso-PCs (Chakrabarti & Khorana, 1975) were employed in the synthesis of SOPC and POPC. The lipids were purified by chromatography on silicic acid (Keough & Davis, 1979). On thin-layer chromatography (Keough & Davis, 1979) with heavily loaded plates (0.5–0.75 mg of lipid/spot), the mixed-acid lecithins were found to be essentially pure with the exception of a trace of the 1,3-diacyllecithin. Positional analyses were carried out by utilizing either of two procedures for hydrolysis with phospholipase A_2 (Gupta et al., 1977; Keough & Davis, 1979). The remainder of the analysis was described previously (Keough & Davis, 1979).

Differential Scanning Calorimetry. Chloroform solutions of the lipids were dried under N_2 and evacuated for 16 h over P_2O_5. Water dispersions were made and thermal analyses performed on a Perkin-Elmer DSC-2 as described previously (Keough & Davis, 1979). Heating and cooling rates of 5 °C/min and sensitivities of 1–5 mcal/s full scale were employed. Because the lipid phase changes occurred close to, or under, the ice–water transition, heating runs were usually started between −12 and −8 °C. Water in samples of lipid dispersions can be routinely supercooled to −8 °C in the calorimeter. In some instances, supercooling to as much as −12 °C can be achieved before nucleation, and we used this ability to monitor the gel to lc transition of OPPC in water. Supercooled samples held at −12 °C will undergo nucleation in a matter of 1–3 min. Thus samples taken to −12 °C were generally only held there for about 15–30 s, except in one case when it was held for 2.5 min. Samples at −8 °C could be held at least up to 20 min without nucleation. Because the OPPC transition was close to −12 °C, it was difficult to establish a long base line before the transition. For these reasons, OPPC dispersions were also made in ethylene glycol–water (1:1 v/v) so that the samples could be cooled to a low temperature and held for some time. In all cases (except those with ethylene glycol), samples were taken through the ice–water transition at some point to ensure the presence of excess water. Transition temperatures were taken at the points of intersection of the tangents to the leading edges of the transitions and the extrapolated base lines. Enthalpies were determined as described previously (Keough & Davis, 1979).

Results

Typical DSC heating thermograms obtained for the dispersions of the four unsaturated mixed-acid lecithins are shown in Figure 1. All four have different transition temperatures, with OPPC having the lowest and OSPC having the highest. All the transitions show a high-temperature shoulder of varying intensity. Although we have observed some asymmetry in the transition profiles of single-acid and saturated mixed-acid lecithins, it has never been as great as that seen with these samples. The samples of SOPC having 18% acyl migration (not shown) had a fairly large asymmetry like that for OSPC, as opposed to the more symmetrical transition for the SOPC with only 6% migration. This suggests that the presence of the reversed isomer contributes to the broadening of the transition. However, the presence of the reversed isomer cannot be the only contributing factor. The sample of OPPC, which was essentially free from isomeric contamination, had a more pronounced shoulder than did POPC, which contained 8% of the reverse isomer. As can be seen in Table I, the samples with less positional isomers had smaller half-height widths (2–3 °C) as opposed to those with high migration (4–5 °C).

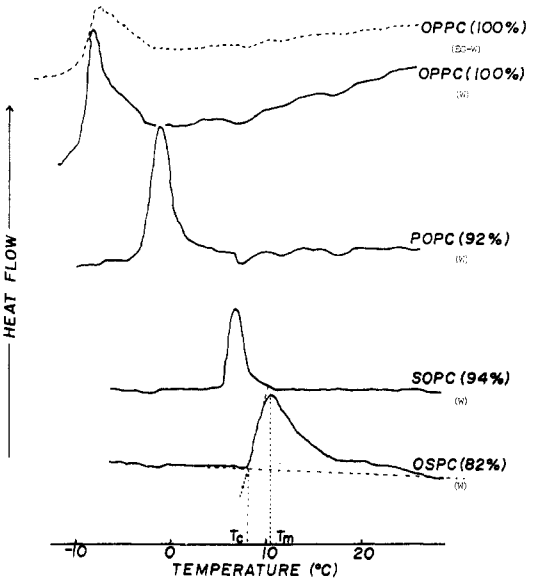

FIGURE 1: Tracings of DSC heating thermograms of dispersions of unsaturated mixed-acid phosphatidylcholines. Percentages indicate isomeric purity. The remaining lipid was the reversed positional isomer. Samples were dispersed either in water (W) or in ethylene glycol–water (1:1) (EG–W).

No pretransitions were detected in these thermograms, with the possible exception of the point made about OPPC below, and in this way, these lipids were more like unsaturated single-acid lecithins than saturated mixed-acid or saturated single-acid lecithins (Keough & Davis, 1979; Chen & Sturtevant,

1980). However, it is reasonable to expect that any pretransitions would occur well down in ice or under the ice–water transition and then be very difficult to detect. They would also not be expected in ethylene glycol (van Echteld et al., 1980).

If the water in samples of OPPC was frozen, a transition for OPPC was seen superimposed on the start of the ice melt. The transition appeared to start at ~−9 °C and have a component of very small enthalpy, followed by a broader high-enthalpy component where T_c was ~−6 °C and where T_m was ~−4 °C. This suggests an effect of the ice on the lipid transition. This behavior could, however, be due to a thermal lag caused by the ice. It also occurs in a region where the instrument is responding rapidly to changes in water. In any case, the transition temperature is still below that of POPC.

Analytical data for the thermal transitions of these lipids are given in Table I. All four unsaturated mixed-acid lecithins have unique T_c's and T_m's. Ethylene glycol did not substantially change the estimate of T_c or T_m for OPPC (Figure 1) on heating, but on cooling, the onset temperatures were raised by 4 °C because of the more pronounced high-temperature shoulder. The difference in T_c for the OPPC–POPC pair is at least 6.7 °C while that for the OSPC–SOPC pair is at least 2.3 °C (we consider the value of T_c = 6.3 °C for SOPC to be more reliable since it is for the least-migrated product with the narrowest transition). The differences were also similar for these parameters for the exotherms seen on cooling. The estimates of the differences in T_c's and T_m's are probably slight underestimates of those for the pure compounds because of the presence of reversed isomers.

The enthalpies of transition seemed to be affected by the broadness of the transitions. The enthalpy of the 94% pure SOPC was lower than that of the 82% pure materials. The enthalpy of OPPC in water was very slightly, but not significantly, lower than that in ethylene glycol. Enthalpies were different for each isomer of each mixed-acid pair, assuming the enthalpy of 94% SOPC to be the more reliable estimate. Estimates of ΔH for broader transitions are subject to more uncertainty because of difficulty of establishing exact points of base-line departure in broad transitions.

Discussion

The results indicate that water dispersions of the two positional isomers of unsaturated mixed-acid lecithins containing the same fatty acids have different transition temperatures and enthalpies of transition. This finding is consistent with that observed for positional isomers of saturated mixed-acid lecithins (Keough & Davis, 1979; Chen & Sturtevant, 1981). For both pairs of isomers, the T_c's are intermediate between those of the "parent" lecithins [see, e.g., Phillips et al. (1969); Mabrey & Sturtevant, 1976]. The transition temperature of SOPC of 6.3 °C is slightly higher than the value of 3 °C reported by Phillips et al. (1972), and our value for the T_c for OSPC is in the range of the broad transition found for OSPC by deKruyff et al. (1972). Similarly, our T_c for POPC of −2.6 °C is slightly higher than the value of −5 °C found by deKruyff et al. (1973). Of our values, we consider T_c = 6.3 °C and ΔH = 5.4 kcal/mol to be the more reliable for SOPC since they were obtained from the least-migrated sample with the narrowest transition width. This value for ΔH is similar to the 5 kcal/mol found by Phillips et al. (1972) for SOPC. Our value of ΔH = 6.7 kcal/mol for the enthalpy of OSPC is the same as that found by deKruyff et al. (1972). This estimate may be a bit high because of the transition broadness. We find a value of 5.4 kcal/mol for the ΔH of POPC. This is substantially below the values of 8.0 and 8.1 kcal/mol found by deKruyff et al. (1973) and ourselves (Davis et al., 1980) for preparations of POPC made by different procedures. The transition profiles of the POPC samples studied previously were substantially wider than the ones seen for this most recent preparation. Thus we consider the value of 5.4 to be more reliable.

Two factors could contribute to the difference in T_c's seen in the pairs of isomers of unsaturated mixed-acid lecithins. One of these is associated with the changing depth of the double bond in the bilayer. Barton & Gunstone (1975) have studied the thermotropic behavior of dispersions of a series of unsaturated single-acid lecithins containing octadecenoates with the double bond in different positions in the chain and some unsaturated mixed-acid lecithins containing stearate at the *sn*-1 position and various octadecenoates at the *sn*-2 position. They observed that the T_c and ΔH were influenced by the position of the double bond with a minimum in the T_c occurring when the double bond was at the 9 or 10 position of the fatty acid chain. Studies on crystals of phosphatidylcholine (Pearson & Pascher, 1979) and studies on dispersions of saturated single-acid and unsaturated mixed-acid phosphatidylcholines using deuterium nuclear magnetic resonance, neutron diffraction, and laser Raman spectroscopy (Seelig & Seelig, 1975; Haberkorn et al., 1977; Oldfield et al., 1978; Büldt et al., 1978; Seelig & Waespe-Šarčevič, 1978; Gaber et al., 1978; Zaccai et al., 1979) have indicated that the portions of the two acyl chains which are near the glycerol do not have the same orientation. This results in a difference in the depth of penetration into the bilayer by the two fatty acid chains, such that in dispersions the two chains are out of step by about 1.5 carbon–carbon bonds or 1.8–1.9 Å (projected distance on the bilayer normal). A consequence of this nonequivalence in chain penetration is that, for the two pairs of isomers studied here, moving the oleate chain from the *sn*-2 position to the *sn*-1 position would result in a lipid where the double bond is approximately 1.8 Å deeper in the bilayer. In that light, it would be consistent with the findings of Barton & Gunstone (1975) that OSPC would have a slightly higher T_c than SOPC.

The displacement of the double bond is not the only factor influencing the relative transition temperatures of isomeric pairs. If this were the case, OPPC should have a *higher* T_c than POPC, but that is the opposite of what is observed. Using the difference for OSPC–SOPC where the chain lengths are almost the same and assigning this difference to double bond movement, one would predict that the OPPC should have a T_c which was 2.3 °C higher than that of POPC. In fact, it is at least 6.7 °C *lower*. Thus, there is an effect of changing the chain lengths in the positions of the glycerol which supersedes the potential effect of moving the doubld bond further from the head group. The T_c's of the two compounds having stearate and palmitate at the 1 position (SOPC and POPC) are different by 9.9 °C—an amount consistent with a change of two methylene units in a single chain of a saturated single-acid lecithin (Phillips et al., 1969; Mabrey & Sturtevant, 1976; Nagle & Wilkinson, 1978). The T_c's of the PCs having stearate and palmitate at the 2 position (OSPC and OPPC) are different by 17.9 °C, almost twice that expected for just changing two methylene units in one chain. This reinforces the proposal that differences in chain penetration in the isomers strongly influence the T_c, even in the presence of double bonds.

For pairs of positional isomers of saturated mixed-acid lecithins, it has been observed that the member of the pair which has the longer chain at the 1 position is the one with the lower T_c (Keough & Davis, 1979; Chen & Sturtevant,

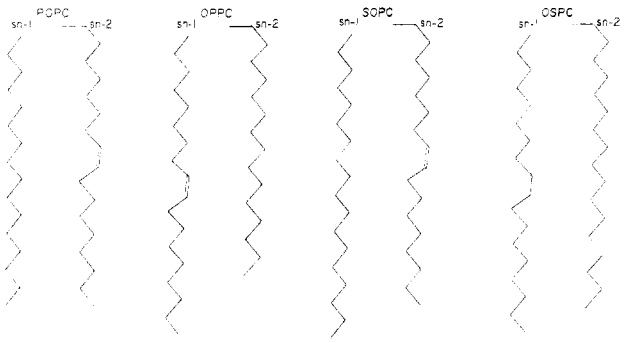

FIGURE 2: Possible chain orientations in the gel states of unsaturated mixed-acid lecithins.

1981). As noted above, because the chains are out of register, the difference between the chain penetration is accentuated in the 1-long–2-short isomer and reduced in the 1-short–2-long isomer. The pairs of isomers studied here follow the same pattern. In the OPPC–POPC pair, the oleate is substantially longer than the palmitate, and OPPC has the lower T_c. The difference in chain lenghts between oleate and stearate is small, but real. The difference in T_c's is also small, and consistently, it is the isomer with the 1-long–2-short fatty acid distribution (SOPC) which has the lower T_c of the pair. The way in which the double bond of the unsaturated chains pack in the gel state can influence the difference in effective chain penetration in addition to the difference induced at the head group. Seelig & Waespe-Šarčevič (1978) have found that the double bond of POPC in the lc phase is oriented with its axis almost parallel to the bilayer normal, and it would seem that this is a likely orientation in the gel. Such an orientation can be achieved by introducing a 30° twist in the carbon–carbon bond adjacent to the double bond plus a gauche rotamer in the bond between the carbons which are α and β to the double bond (Huang, 1977). Using CPK models, it can be ssen that this combination can be introduced on either side or on both sides of the double bond and still yield a chain whose double bond is almost parallel to the bilayer normal. If a twist plus gauche rotamer is introduced on only one side of the double bond, the axis of part of the oleate chain becomes displaced with respect to the remainder of that chain. Introduction of a twist plus rotamer on both sides of the double bond results in a chain with all parts on the same axis with the exception of the double bond. The double bond is displaced laterally, but its axis is nearly parallel to that of both chains as is shown diagrammatically in Figure 2. This type of arrangement results in a small perturbation at the site of the double bond. If this type of chain packing occurred in the gels of unsaturated mixed-acid lecithins, it would enlarge slightly the difference in effective chain penetration in SOPC and would reduce that difference slightly in OSPC. This would enhance the 1-long–2-short effect noted above. The effective difference in the chain penetrations would be about 1.3 carbon–carbon lengths for OSPC while it would be closer to 1.8 bond lengths for SOPC (Figure 2). For POPC such a chain orientation would yield almost equivalent penetration for both chains, but a difference of 3–3.5 bond lengths would occur for the chains of OPPC (Figure 2).

We had suggested that since T_c was presumably dependent on the total van der Waals interactions, and thus bilayer thickness, chain interdigitation was unlikely in isomers of saturated mixed-acid lecithins and that some foreshortening of the longer chain in the isomer with the 1-long–2-short configuration might occur to produce the lipid with the lower T_c. The unsaturated mixed-acid lecithins also follow a pattern which would be consistent with that possibility. Chen & Sturtevant (1981) feel we may have taken too simple a view of the packing in the gel. Whatever the arrangements of the chains in the gel, the T_c's of the unsaturated mixed-acid lecithins do follow the same general pattern as do saturated mixed-acid lecithins in that the isomer with the 1-long–2-short configuration has the lower T_c.

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