Physical Behavior of the Hydrophobic Core of Membranes: Properties of 1-Stearoyl-2-linoleoyl-*sn*-glycerol†

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ABSTRACT: Phospholipids containing a saturated fatty acid in the primary position and an unsaturated fatty acid in the secondary position are a major structural part of biological membranes. The mixed-chain hydrophobic core of the membranes is the diacylglycerol part. To better understand the core properties of membranes we have studied the physical behavior of 1-stearoyl-2-linoleoyl-*sn*-glycerol (SLDG) by X-ray diffraction and differential scanning calorimetry (DSC) in the dry and hydrated states. Dry SLDG has four polymorphic phases: α (transition temperature, 11.6 °C; ΔH = 7.5 kcal/mol); sub-α_1 (3.0 °C; 0.6 kcal/mol); sub-α_2(−1.0 °C; 0.5 kcal/mol); and β' (16.1 °C; 15.4 kcal/mol). The α, sub-α_1, and sub-α_2 phases are metastable with a probable extended bilayer structure ($d_{001} \approx 59.5$ Å). The chain packing of the α phase is hexagonal, while sub-α_1 and sub-α_2 have pseudohexagonal chain packing. The β' phase has a tilted bilayer structure (46.9 Å) with strong wide-angle diffractions, suggesting elements of orthorhombic perpendicular packing. Compared to saturated 1,2-diacylglycerols, SLDG packs much less efficiently, but, when compared to 1-stearoyl-2-oleoyl-*sn*-glycerol, it appears to pack somewhat more efficiently. Thus polyunsaturated linoleate chains appear to pack marginally more effectively with the saturated stearate chains than do monounsaturated chains. SLDG hydrates with 0.5 mol of H_2O, which prevents the β' phase from forming. Only one hydrated α phase (α_w) and two hydrated sub-α (sub-α_{w1}, sub-α_{w2}) phases are formed. These phases are similar in structure to the nonhydrated α phases, but the bilayer period is increased by about 2 Å ($d_{001} \approx 61.5$ Å). This causes minor changes in polymorphism, including lower melting temperatures and enthalpy. A comparison of diacylglycerols to phosphatidylcholines with the same chains shows that the addition of a strong polar group (e.g., phosphocholine) to the free hydroxyl of the glycerol depresses chain melting and prevents the more efficient packing of the SLDG core of the bilayer.

The continuous phase of biological membranes is a bilayer composed largely of phospholipids. Most membrane phospholipids are of the mixed-chain type, usually with a saturated long-chain fatty acid (e.g., palmitate or stearate) in the primary position and an unsaturated fatty acid (oleate, linoleate, etc.) in the secondary position. These phospholipids are usually glycerolipids which consist of a 1,2-diacyl-*sn*-glycerol with a highly polar, often charged group like phosphoethanolamine, phosphoserine, phosphoinositol, phosphoglycerol, or phosphocholine (zwitterionic) at the 3-position. The physical properties of many phospholipids have been studied widely. However, the polar constituent of phospholipid modulates the behavior of the membrane lipid greatly. Strong hydration of the polar region tends to greatly depress the chain melting transition. For instance, the chain melting transition (T_m) of 1,2-dipalmitoyl-*sn*-glycerol is 70.1 °C (Kodali et al. 1990a) while that of dipalmitoyl phosphatidylcholine is 41 °C (Janiak et al., 1976). If a net charge is present as in phosphotidyl serine, the transition may be even lower, due to repulsive forces between adjacent molecules. Most phospholipids having mixed saturated and unsaturated chains have a very low T_m, often below 0 °C. However, even a few percent incorporation of diacylglyceride into phospholipid will raise the transition temperature abruptly (Heimberg et al., 1992).

In an attempt to understand the properties of the largely hydrocarbon core of membranes, we have initiated studies on the 1,2-diacyl-*sn*-glycerol core of membrane lipids. The properties of the diacylglyceride core are those of a membrane without the modulating effects of the polar head group. The physical properties of mixed-chain diacylglycerols therefore form a structural base line to which the properties of more complex phospholipids can be compared and contrasted.

Diacylglycerols are also transiently present in membranes and in the phospholipid monolayers surrounding fat globules and plasma lipoproteins. The 1,2-diacyl-*sn*-glycerols play critical roles in biological processes, acting as secondary messengers (Bell & Burns, 1991). To elucidate their physical and chemical properties, the authors have recently studied the polymorphic behavior of simple saturated (Kodali et al., 1990a; Hamilton, et al., 1991) and mixed-chain (Di & Small, 1993) diacylglycerols. The presence of a double bond in the diacylglycerol changes the polymorphic behavior dramatically. For instance, 1-stearoyl-2-oleoyl-*sn*-glycerol (SODG) has at least eight polymorphic structures whereas 1,2-distearoyl-*sn*-glycerol (SSDG) has only two. The transition temperatures, enthalpies, and entropies are much lower for all the SODG polymorphs. These characteristics suggest

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difficulties in accommodating the double bond in the crystal structure and thus give rise to marked polymorphism of energetically similar states.

The present study deals with the polymorphism of 1-stearoyl-2-linoleoyl-*sn*-glycerol (1,2-*sn*-SLDG). This molecule contains the essential fatty acid, linoleate, at the 2-position, which has two cis double bonds at the Δ-9- and Δ-12-positions. This molecule, like SODG, is a major hydrophobic core constituent of many phospholipids. However, its properties may be different from SODG. Molecular modeling suggested that aliphatic chains containing two or more cis double bonds will pack more effectively with a saturated chain than chains with only one cis double bond (Applegate & Glomset, 1986, 1991a,b). A careful study of 1,2-*sn*-SLDG packing will test that hypothesis and perhaps give some insight into how the different chains of membrane lipids can assemble in bilayers.

MATERIALS AND METHODS

Purity. 1,2-*sn*-SLDG (stated to be 99% pure) was purchased from Serdary International, Port Huron, MI. Upon arrival the materials were checked with TLC (Kodali et al., 1990b), and 10%−30% of 1,2-*sn*-SLDG in different samples migrated as the 1,3-SLDG isomer. 1,2-*sn*-SLDG and 1,3-SLDG isomers were separated by flash column chromatography on silica gel containing 2.5% boric acid (mobile phase: 5%−8% ethyl acetate/hexane) (Kodali & Duclos, 1992; Buchnea, 1974), and 1,2-*sn*-SLDG with purity >99% was isolated. To check the position of the stearate and linoleate after purification, the 1,2-*sn*-SLDG was reacted briefly with pancreatic lipase. The hydrolysis products, fatty acids and monoglycerols, were isolated, methylated, and examined by GLC. The results showed that more than 96% of stearate was on the primary (α glycerol) position and more than 96% of linoleate was on the *sn*-2 (β) position. According to the supplier (Serdary International) the compound is optically pure.

Physical Methods. Dry sn-SLDG. Crystals of the stable form (β′) were grown from a hexane solution of 1,2-*sn*-SLDG at −20 °C under argon. The hexane was evaporated slowly. The crystals obtained were filtered and dried under vacuum for 20 min. DSC was carried out on a Perkin-Elmer DSC-7 (Norwalk, CT) as described previously (Kodali et al., 1990a). Each sample (3−6 mg), weighed to the nearest 0.01 mg, was sealed in a stainless steel pan. Heating and cooling rates were 5 °C/min unless otherwise specified. The enthalpies were determined as previously described (Di & Small, 1993). The thermal protocols to obtain the different phases are as follows: α phase, a solidified sample was melted at 35 °C, and the isotropic liquid was cooled to 6 °C; sub-α_1 phase, a solidified sample was melted at 35 °C, and the isotropic liquid was cooled to 0 °C; sub-α_2 phase, a solidified sample was melted at 35 °C, and the isotropic liquid was cooled to −10 °C; β′ phase, slow evaporation of 1,2-*sn*-SLDG/hexane solution in a cold room at −20 °C under argon gave the β′ phase. A complicated thermal protocol can also produce β′ from other polymorphs (see Results below).

X-ray powder diffraction patterns of each sample were recorded using nickel-filtered Cu Kα radiation from an Elliot GX-6 (Elliot Automation, Borehamwood, U.K.) rotating-mode generator equipped with camera employing Franks's double-mirror optics (Franks, 1958) and toroidal-mirror optics. All the samples for the X-ray studies were subjected to the same thermal treatments as those for the DSC studies. Nomenclature of polymorphs was given according to Larsson (1966) and Small (1986). The β′ phase is characterized by strong reflections at 4.2 and 3.8 Å, as well as other weaker reflections. The α phase has hexagonal packing with a single strong reflection at ~4.2 Å. The sub-α phases are obtained by cooling the α phase. These polymorphic transitions have low ΔH. The X-ray diffraction of the sub-α phases has two wide-angle reflections at about 4.1 and 3.8 Å (Kodali et al., 1990a). These sub-α phases have pseudohexagonal packing similar to phospholipids and other lipids (Small, 1986).

Hydrated sn-SLDG. Methods of DSC and NMR previously developed (Di & Small, 1993) were employed for the hydrated samples of *sn*-SLDG. SLDG in hexane was evaporated and maintained in a vacuum for about 30 min. Excess water was added. The mixture was shaken and equilibrated for 15 min. The thermal protocols developed for the phases of *sn*-SLDG with water are as follows: α_w phase, samples were melted at 35 °C and then were cooled to 4 °C; sub-α_{w1} phase, samples were melted at 35 °C and then cooled to −3 °C; sub-α_{w2} phase, samples were melted at 35 °C and then were cooled to −10 °C. No hydrated β′ phase was produced by equilibration of β′ phase with water.

Table 1: Thermodynamic Data from DSC Analysis of Dry and Hydrated 1,2-*sn*-SLDG

phases	T_m[a] (°C)	ΔH[b] (kcal mol^{-1})	ΔS[c] (cal K^{-1} mol^{-1})	T_c[d] (°C)
dry				
α	11.6	7.5	26.3	7.7
sub-α_1	3.0[e]	~0.6[e]	2.2[e]	0.0[e]
sub-α_2	−1.0[e]	~0.5[e]	1.8[e]	−4.2[e]
β′	16.1	15.4	53.2	
hydrated				
α_w	9.8	6.7	23.7	6.4
sub-α_{w1}	0.2[e]	~0.6[e]	2.2[e]	−2.7[e]
sub-α_{w2}	−3.0[e]	~1.5[e]	5.6[e]	−6.0[e]

[a] Temperature of melting, transition peak value. *On repeated runs on different samples, the transition temperature (T_m) varies less than 0.3 °C and the enthalpy (ΔH_m) by less than 5%. On the same sample, using the same thermal protocol, the runs are superimposable.* [b] Enthalpy of melting. [c] Entropy of melting, calculated from T_m and the enthalpy of melting. [d] Temperature of crystallization, transition peak temperature on cooling. [e] Polymorphic transition process, not melting.

RESULTS

Dry 1,2-sn-SLDG. DSC Studies. DSC measurements were examined for the samples crystallized from melt and from hexane solution. The thermodynamic data are shown in Table 1. Figure 1a shows heating of β′ from −10 to 35 °C at 5 °C/min. β′ melts at 16.1 °C, ΔH = 15.4 kcal/mol. Figure 1b shows cooling of the melt from 35 °C. The melt begins to crystallize the α phase at 7.7 °C, and further cooling of α will transform α to sub-α_1 at 0.0 °C, and sub-α_1 will transform to sub-α_2 at −4.2 °C. Figure 1c shows heating from −10 to 35 °C. Sub-α_2 transforms to sub-α_1 at −1.0 °C (ΔH = 0.6 kcal/mol), and sub-α_1 transforms to α phase at 3.0 °C (ΔH, 0.5 kcal/mol). The α to sub-α_1 to sub-α_2 transitions are reversible polymorphic transitions with low enthalpies (<1 kcal/mol) which occur without melting. The difference between the peak of the transformations on heating and cooling is probably due to the rapid scanning rates. When scanned at slower rates the differences decrease. On further heating, α will melt at a peak temperature of 11.6 °C (ΔH

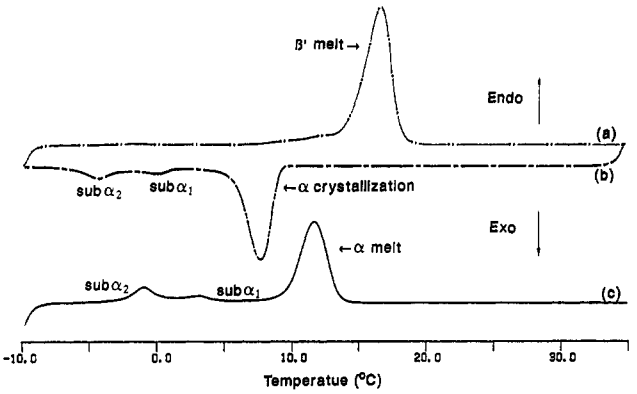

FIGURE 1: Polymorphic transitions in 1,2-*sn*-SLDG. (a) Heating of β' phase from −10 to 35 °C at 5 °C/min. (b) Cooling the melt from 35 to −10 °C at 5 °C/min gives α, which then transforms to sub-α_1 and then to sub-α_2. (c) Reheating from −10 to 35 °C at 5 °C/min transforms sub-α_2 to sub-α_1 to α.

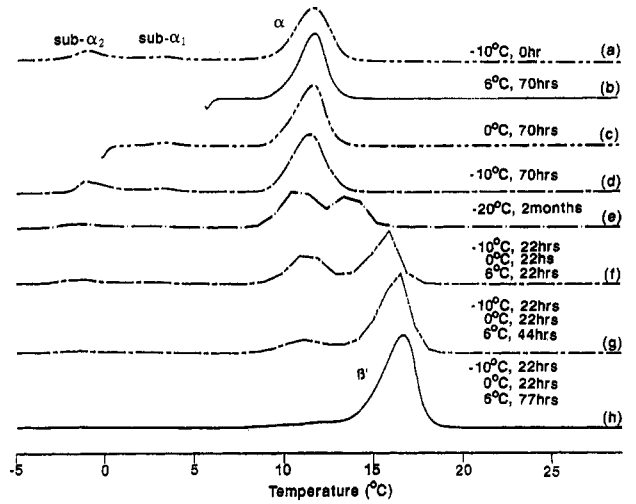

FIGURE 2: Conditions required to recrystallize the stable β' phase from the melt. (a) Cooling of a melt from 35 to −10 °C, immediate reheating (5 °C/min); sub-α_2 transforms to sub-α_1, sub-α_1 transforms to α, and then α melts. (b) Cooling of a melt from 35 to 6 °C, incubation at 6 °C for 70 h, heat; α melts. (c) Cooling of a melt from 35 to 0 °C, incubation at 0 °C for 70 h, heat; sub-α_1 transforms to α, α melts. (d) Cooling of a melt from 35 to −10 °C, incubation at −10 °C for 70 h, heat; sub-α_2 transforms to sub-α_1, sub-α_1 transforms to α, α melts. (e) Incubation at −20 °C for 2 months and then heating from −20 to 35 °C shows melting of both α and β'. (f−h) Cooling of a melt from 35 to −10 °C, incubation at −10 °C for 22 h, heating to 0 °C for 22 h, incubation at 0 °C for 22 h, heating to 6 °C, incubation at 6 °C for 22 (f), 44 (g), and 77 h (h). α gradually transforms to β'.

= 7.5 kcal/mol). The α phase can transform to β' phase through a combination of incubations at different temperatures. As shown in Figure 2, incubations of α phase at 6, 0, or −10 °C for 70 h alone will not give β' (Figure 2b−d). Storage of SLDG in a −20 °C freezer for 2 months gives ~45% β' (Figure 2e). Incubation at −10 °C for 22 h, followed by incubation at 0 °C for 22 h and another incubation for 22, 44, or 77 h at 6 °C is shown in Figure 2f−h, respectively. β' begins to form as early as 6 h at 6 °C (data not shown), and by 77 h almost all of the α phase has transformed to β' (Figure 2h).

X-ray Diffraction. The wide-angle X-ray diffraction patterns of the four polymorphs are shown in Figure 3. The long and short spacing and the relative intensities are summarized in Table 2. In the α form (6 °C) only one strong peak (4.1 Å) in the short spacing region and a long spacing

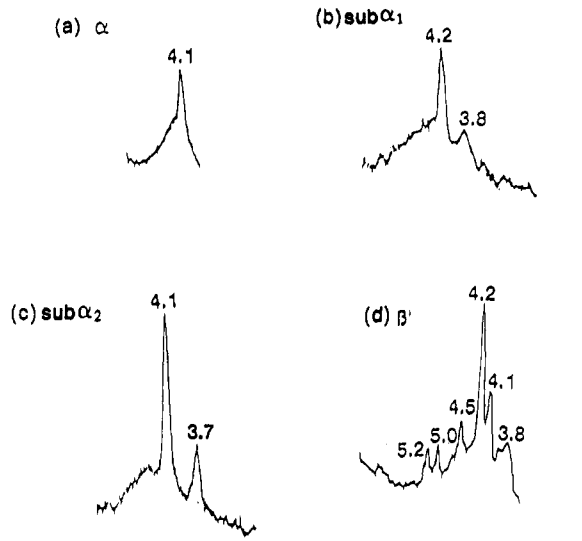

FIGURE 3: Wide-angle X-ray diffraction patterns showing different modes of chain packing: (a) α phase; (b) sub-α_1 phase; (c) sub-α_2 phase, and (d)β' phase. The numbers above the reflections are in angstroms.

(001) of 59.5 Å are present. In sub-α_1 form (0 °C), two strong reflections (4.2 and 3.8 Å) are revealed. The long spacing of sub-α_1 is 59.6 Å. In sub-α_2 form (−10 °C), two strong reflections (4.1 and 3.7 Å), one medium reflection (2.5 Å), and one weak reflection (3.0 Å). The long spacing of sub-α_2 is 59.4 Å. Finally, in the β' form, one very strong reflection (4.2 Å), one strong reflection (4.1 Å), and four medium reflections (3.8, 4.5, 5.0, 5.2 Å) are seen. The 001 diffractions show at least 7 orders and give an average long spacing of the β' phase of 46.9 Å.

Hydrated 1,2-sn-SLDG. DSC and NMR studies show that every SLDG molecule binds about half a water molecule. Comparison of ^{1}H NMR spectra between the neat liquid of 1,2-*sn*-SLDG and 1,2-*sn*-SLDG/water shows that there is an extra peak at 4.74 ppm with an area equivalent to 1H, which is different from that of the unbound water (4.82 ppm). The ^{1}H NMR of the −O*H* group shifts upfield by 0.1 ppm. The ^{13}C NMR spectrum shows the two carbonyls (C=O) shift downfield by 0.2 ppm after the hydrate formation. Three polymorphic phases were found, e.g., α_w, sub-α_{w1}, and sub-α_{w2}. The thermodynamic data are shown in Table 1. The α_w phase has T_m of 9.8 °C and ΔH_m = 6.7 kcal/mol. The transition temperature from α_w to sub-α_{w1} is 0.2 °C, ΔH = 0.6 kcal/mol, and that from sub-α_{w1} to sub-α_{w2} is −3.0 °C, ΔH = 1.5 kcal/mol. The melting/transition temperatures of the three phases (α_w, sub-α_{w1}, subα_{w2}) are 2−3 °C lower than the dry phases (Table 1). The X-ray diffractions from the α and sub-α phases of the hydrated 1,2-*sn*-SLDG were similar to dry 1,2-*sn*-SLDG, although the average d_{001} reflections of the α and sub-α phases were significantly increased by about 2 Å (Table 2). These changes indicate that the incorporation of about half a water molecule modestly lowered the transition temperatures and increased the long spacings by about 2 Å without affecting the overall structure of the phase.

DISCUSSION

Phase Transformations and Thermal Stability. The phase behavior of 1,2-*sn*-SLDG is shown in Figure 4. Cooling of a melt from 35 °C crystallizes the α phase. Further cooling of α can transform it to sub-α_1 and then to sub-α_2 reversibly.

Table 2: X-ray Diffractions for 1,2-*sn*-SLDG

dry				hydrated		
α	sub-α_1	sub-α_2	β'	α_w	sub-α_{w1}	sub-α_{w2}
59.5 (av)[c]	59.6 (av)	59.4 (av)	46.9 (av)[d]	61.79 (av)	61.36 (av)	62.48 (av)
59.5 (vs)[a], 1[b]	59.5 (vs), 1	59.4 (vs), 1	47.7 (vs), 1	61.48 (s), 1	60.19 (s), 1	63.53 (vs), 1
29.8 (vs), 2	29.8 (vs), 2	29.8 (vs), 2	23.9 (vs), 2	30.93 (s), 2	30.93 (s), 2	30.93 (s), 2
19.9 (w), 3			15.5 (s), 3			
14.8 (s), 4	14.9 (m), 4	14.8 (ms), 4	11.5 (m), 4	15.51 (m), 4	15.51 (w), 4	15.51 (w), 4
			9.4 (w), 5			
9.9 (w), 6		9.9 (w), 6	7.8 (w), 6			
			6.7 (w), 7			
			5.2 (m), 9			
					4.82 (m)	4.74 (w)
4.1 (vs)	4.2 (vs)	4.1 (vs)	5.0 (m)	4.32 (s)	4.35 (vs)	4.28 (s)
	3.8 (s)	3.7 (s)	4.5 (m)		3.93 (m)	3.83 (m)
		3.0 (w)	4.2 (vs)			
		2.49 (m)	4.1 (s)			
			3.8 (m)			

[a] Relative intensity is given in parentheses: vs = very strong, s, strong, m, medium, w, weak. [b] Reflection index. [c] "av" is the average 001 spacing calculated from all the orders. [d] Identical spacings and intensity distributions were present after prolonged incubation of β' in water. After being melted in water, β' could not be recrystallized. We conclude that no β'/H_2O phase is formed.

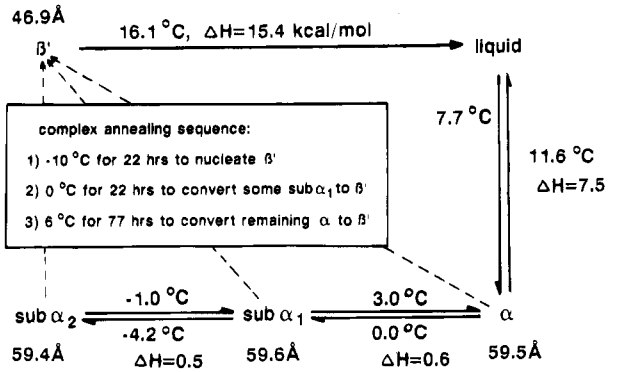

FIGURE 4: Phase transformations of dry 1,2-*sn*-SLDG.

Phases α, sub-α_1, and sub-α_2 are all likely to be able to transform to β' with nucleation at low temperature and a very long incubation. Combinations of incubation at the three different temperatures (−10 °C, 22 h; 0 °C, 22 h; 6 °C, 77 h) seem to be one of the short paths to convert these metastable phases to stable β' phase. It is likely that nucleation is essential and occurs from sub-α_2. Presumably the crystal growth rates are very slow at −10 °C since virtually no β' is found after 70 h of incubation at −10 °C. At 0 and 6 °C crystals must grow faster albeit still slowly (Figure 2). The β' phase can be readily obtained from crystallization in hexane solution at −20 °C. Since β' and α melt monotropically, β' is the most stable phase and α is the less stable phase. The α-phase can transform to the sub-α phases reversibly. Among these three phases, sub-α_2 is the stable phase below −1 °C, sub-α_1 is the stable phase between −1 and 3 °C, and α is the stable phase above 3 °C.

The α polymorph has only one strong reflection in the wide-angle region, indicating the chains have hexagonal packing. The two sub-α phases have pseudohexagonal packings as indicated by the reflections in the wide-angle region (Small, 1986). The transformations between α and sub-α phases are low-energy transformations (ΔH < 1 kcal/mol). The α to sub-α transformations are believed to be disorder to order processes involving chain packing (Kobayashi et al., 1986). The nature of the transformations is a conversion from gauche to trans conformation as the temperature is lowered. From the transformation enthalpies, we estimated that from α to sub-α_1 15%−20% of the gauche conformation per CH_2 has changed to the trans conformation; 13%−17%, from sub-α_1 to sub-α_2 (Small, 1986).

The (001) reflection of the three α and sub-α phases has a repeat of about 59.5 Å. Many bilayered structures of α phases of di- and triacylglycerides with oleic and stearic acids have a bilayer thickness of 51 ± 3 Å (Di & Small, 1993). Therefore 59.5 Å seems a bit long for an α phase bilayer. However, the bilayer spacing for saturated 1,2-distearoyl-*sn*-glycerol in the α phase is about 55 Å (Kodali et al., 1990a). In this phase the chains were presumed to be nearly perpendicular to the bilayer plane. This is based on the 001 diffractions of a homologous series of saturated chain diacylglycerols (Kodali et al. 1990a) in the α phase, which give an increment in the D spacings of 1.27 Å per carbon. The increment in the D spacings of 1.27 Å per carbon (2.54 Å per two CH_2 groups) is the average distance from carbon to carbon along the axis of the chain. Furthermore, it was assumed that the conformation of the glycerol part of the molecule is probably similar to that in the β' state, that is, the glycerol lies parallel to the bilayer plane. At least two known factors could lengthen the bilayer periodicity. The first involves the increment per CH_2 group in the α-state. As noted above, an *all-trans* hydrocarbon chain packed in the orthorhombic perpendicular state has an average increment per CH_2 group along the axis of the chain of about 1.27 Å [see Small (1986)]. However, Doucet et al. (1981a,b) and Denicolo et al. (1983) have shown quite clearly that there is an increase in the 001 spacing as odd-chained hydrocarbons go from the orthorhombic perpendicular packing to α phase packing. This increment is not large (the order of 1 Å), but it increases the mean increment per CH_2 in the α phase of hydrocarbons to something slightly over 1.3 Å per CH_2. If the chain were extended as it apparently is in saturated hydrocarbon, then this could at least partly account for increased bilayer periodicity in the α phase. The other possibility involves the conformation of the glycerol. If the glycerol formed a linear extension of the *sn*-1-stearoyl chain, as shown in Figure 5, then the bilayer would be about 59.5 Å thick. Such a structure would leave potential voids in the $-CH_3$ center of the bilayer which should give rise to a considerable increase in disorder in this part of the bilayer. In addition to the noted diffractions (Figure 3) the wide-angle region shows some broad scattering, consistent with

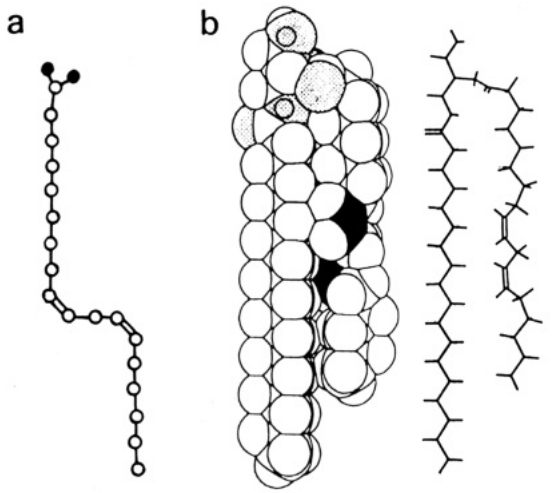

FIGURE 5: Conformation of (a) crystalline linoleic acid (Ernst et al., 1979) compared to (b) suggested conformation of 1,2-*sn*-SLDG (Applegate & Glomset, 1991a).

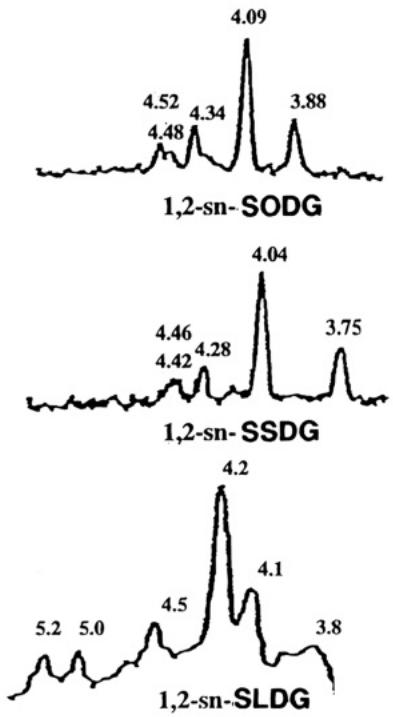

FIGURE 6: Wide-angle X-ray diffraction patterns for β' forms of 1,2-*sn*-SSDG and 1,2-*sn*-SODG compared to 1,2-*sn*-SLDG. *The diffraction from 1,2-sn-SSDG was recorded from pure samples as described in Kodali et al. (1990a), and the diffraction from 1,2-sn-SODG was recorded from pure samples as described in Di and Small (1993). All diffractions were collected on the same apparatus.*

some disorder in the chain packing. Although a bilayer is a reasonable suggestion for α and sub-α packing, some sort of trilayer is also possible. Trilayer structures have been observed in mixed-chain triglycerols (Kodali et al., 1989), but these have not been reported with diacylglycerols. However, it is difficult to see how the lattice could expand with hydration in a trilayer structure. The intensity distribution of all the phases is also slightly anomalous. The usual bilayer has intensity of $I(001) > I(003) > I(002)$ (Sato, 1989), and this intensity distribution is seen in most of the bilayered phases of saturated diacylglycerols (Kodali et al., 1990a) and SODG (Di & Small, 1993). However, the diffraction intensities of the first three reflections of all the phases show $I(001) > I(002) > I(003)$. The (00*l*) reflections

Table 3: Comparison of Dry 1,2-*sn*-SLDG, SODG, and SSDG

phases	SLDG	SODG	SSDG
β' phase			
T_m (°C)	16.1	25.7	77.2
ΔH_m (kcal mol^{-1})	15.4	11.9	30.6
ΔS_m (cal K^{-1} mol^{-1})	53.2	39.8	87.3
α phase			
T_m (°C)	11.6	16.4	61.6
ΔH_m (kcal mol^{-1})	7.5	6.8	16.8
ΔS_m (cal K^{-1} mol^{-1})	26.3	23.5	50.2

of β' phase have intensity distribution: $I(001) > I(002) > I(003) > I(004)$. Therefore, the β' phase probably does not have a typical bilayer structure either.

The molecular conformation around the double bonds of crystalline linoleic acid has the ...tsc̄ssc̄st... torsion sequence (−119.4, −2.3, 122.7, 123.5, −3.3, and −120.9°; I) (Ernst et al., 1979). The structure is shown in Figure 5a. The linoleoyl chain forms two sharp kinks, and it might be difficult for this jogged conformation to pack with the straight stearoyl chain in 1,2-*sn*-SLDG. Using this reasoning, and attempting to align the chains as closely as possible, Applegate and Glomset (1991) generated a more linear conformer for the linoleoyl chain in 1,2-*sn*-SLDG using molecular modeling. The linoleoyl chain was assigned the ...tgscssc̄sgt... torsion sequence (83.1, 139.1, −2.3, 122.4, 120.1, −0.7, 143.5, and 84.6°; II). Though sequences I and II both have angle-iron conformation (torsion angles around $-CH_2-$ between the two double bonds have either ss or s̄s̄ torsion sequence), II has two more gauche bonds than I and the skew bonds all have plus signs instead of antiskew as in conformation I. Conformation II can pack roughly parallel to the straight stearoyl chain (Figure 5b). The modeling study suggested that SLDG packs very similar to SSDG (1,2-distearoyl-*sn*-glycerol.) Wide-angle X-ray diffraction pattern of the β' forms of 1,2-distearoyl and 1-stearoyl-2-oleyl-*sn*-glycerol (SSDG, SODG) are compared to 1,2-*sn*-SLDG in Figure 6. SSDG and SODG are very similar (Di & Small, 1993), but the wide-angle region of 1,2-*sn*-SLDG is different and is more complex than those of SSDG and SODG. These results suggest that the chain packing of the β' form of 1,2-*sn*-SLDG is rather different from SSDG and SODG.

Comparisons of the melting points and melting enthalpies of 1,2-*sn*-SLDG, 1-stearoyl-2-oleoyl-*sn*-glycerol (SODG) (Di & Small, 1993), and 1,2-distearoyl-*sn*-glycerol (SSDG) (Kodali et al., 1990) are shown in Table 3. T_m, ΔH_m, and ΔS_m of SSDG are much higher than those of SLDG and SODG. This suggests the simple saturated diglycerols have much more favorable packing energy than mixed-chain diglycerols. Comparison between SLDG and SODG shows interesting results. In both β' and α phases, 1,2-*sn*-SLDG has lower T_m; however, melting enthalpies (ΔH_m) for SLDG are higher than SODG. The calculated entropy (ΔS_m) is also greater for 1,2-*sn*-SLDG, which probably means that the packing of 1,2-*sn*-SLDG is energetically more favorable than that of SODG. The result supports the theoretical prediction from molecular modeling study, i.e., unsaturated chains with two double bonds will pack more effectively with saturated chains than those containing only one cis double bond (Applegate & Glomset, 1986, 1991a,b). The phase behavior for 1,2-*sn*-SLDG (four phases) is simpler than for SODG (eight phases). SODG showed at least five phases of rather similar energy of packing (four β phases and one β' phase); however, 1,2-*sn*-SLDG has only one such phase (β'). This

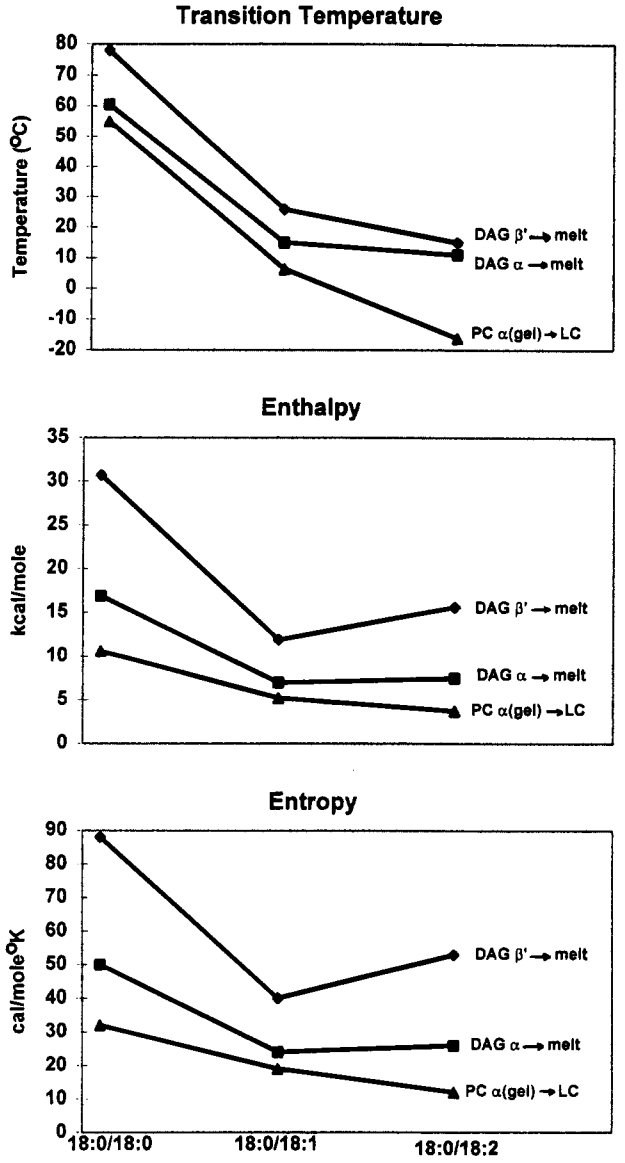

FIGURE 7: Comparison of the thermodynamic values for the 1,2-diacylglycerides, 1,2-distearoyl-*sn*-glycerol (18:0/18:0), 1-stearoyl-2-oleoyl-*sn*-glycerol (18:0/18:1), and 1-stearoyl-2-linoleoyl-*sn*-glycerol (18:0/18:2) to fully hydrated phosphatidylcholines (PC's) having the same diacylglycerol moiety. Data for DAG are from this study, Kodali et al. (1990a), and Di and Small (1993), and data for PC's are from Coolbear et al. (1983).

may indicate that the β' phase of 1,2-*sn*-SLDG is the only phase in which fairly stable (low-energy) chain packing is possible.

The effect of a strong polar head group on the thermodynamic parameters is shown in Figure 7. First, the most stable phase in DAG is the β' phase. Although a low-temperature phase with β'-like packing has been described for some saturated diacyl phosphatidylcholines (PC), it appears to be monotropic and transforms to a pseudohexagonal packing at temperatures well below the chain melting transition [see Small (1986) and Seddon and Cevc (1993)]. Such transitions have not been described for PC's with mixed saturated and unsaturated chains. The chain melting transition for hydrated PC's is the gel to liquid crystal transition. In this transition the gel phase, which has hexagonal chain packing quite similar to other lipids (Small, 1986), including DAG's, melts to a lamellar liquid crystalline phase, which has melted chains. Thus the best comparison between DAG and PC is that between the α phase to liquid melt of DAG and the gel phase to lamellar liquid crystal of PC. Both start with similar chain packing (hexagonal) and melt to liquid chains. It is evident that the presence of a phosphocholine group on DAG lowers this hexagonal to liquid chain transition. The ΔH and ΔS are also decreased. However, although the PC's seem to show a continuous decrease going from 18:0/18:0 to 18:0/18:1 to 18:0/18:2, this trend is not evident in the DAG's. The ΔH and ΔS of 18:0/18:2 is actually higher than 18:0/18:1, as noted in Table 3. Thus the presence of a large, hydrated polar group on DAG seems to prevent the more effective chain packing in the α phase of DAG. One might speculate that when phospholipase C removes phosphocholine from 18:0/18:2 PC the chains might become more ordered in the newly formed DAG.

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