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The Physical Properties of Glycosyldiacylglycerols. Calorimetric Studies of a Homologous Series of 1,2-Di-*O*-acyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerols†

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ABSTRACT: The polymorphic phase behavior of aqueous dispersions of a homologous series of 1,2-di-*O*-acyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerols was studied by differential scanning calorimetry. At fast heating rates, unannealed samples of these lipids exhibit a strongly energetic, lower temperature transition, which is followed by a weakly energetic, higher temperature transition. X-ray diffraction studies have enabled the assignments of these events to a lamellar gel/liquid crystalline (chain-melting) phase transition and a bilayer/nonbilayer phase transition, respectively. Whereas the values for both the temperature and enthalpy of the chain-melting phase transition increase with increasing acyl chain length, those of the bilayer/nonbilayer phase transition show almost no chain-length dependence. However, the nature of the bilayer/nonbilayer transition is affected by the length of the acyl chain. The shorter chain compounds form a nonbilayer 2-D monoclinic phase at high temperature whereas the longer chain compounds from a true inverted hexagonal (H_{II}) phase. Our studies also show that the gel phase that is initially formed on cooling of these lipids is metastable with respect to a more stable gel phase and that prolonged annealing results in a slow conversion to the more stable phase after initial nucleation by incubation at appropriate low temperatures. The formation of these stable gel phases is shown to be markedly dependent upon the length of the acyl chains and whether they contain an odd or an even number of carbon atoms. There is also evidence to suggest that, in the case of the shorter chain compounds at least, the process may proceed via another gel-phase intermediate. In annealed samples of the shorter chain compounds, the stable gel phase converts directly to the L_α phase upon heating, whereas annealed samples of the longer chain glycolipids convert to a metastable gel phase prior to chain melting. The chain-melting phase transition temperatures of the various 1,2-di-*O*-acyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerols studied here are comparable to those reported for the phosphatidylethanolamines containing identical fatty acids but are higher than those of most other phospholipids. Moreover, the total enthalpy change associated with the conversion of the L_c-like phase to the L_α phase is higher than that reported for either the phosphatidylethanolamines or phosphatidylcholines. These results suggest that the stable gel states formed by these glycolipids are characterized by relatively strong interactions between the sugar polar headgroups. However, the bilayer to nonbilayer phase transition temperatures of these glycolipids are lower than those of the corresponding phosphatidylethanolamines, indicating a greater tendency of these glycolipids to adopt a nonlamellar configuration.

Sugar-containing lipids are now known to be important structural and immunological constituents in almost all cells (Quinn & Williams, 1978; Rogers et al., 1980; Gigg, 1980; Wiegandt, 1985; Slomiany et al., 1987; Curatolo, 1987a,b). Among these lipids, the glycosyldiacylglycerols, in which a sugar residue is attached via an α- or β-linkage to 1,2-di-*O*-acyl-*sn*-glycerol, are major structural components of the membranes of many plants and microorganisms (Quinn & Williams, 1978, 1983; Rogers et al., 1980). Despite the importance of these compounds in nature, there have been relatively few studies aimed at a thorough characterization of their physical properties when dispersed in water. This is mainly because of the relative scarcity of native materials with a homogeneous fatty acid composition and the difficulties encountered in the synthesis of optically active compounds of high anomeric purity (Wickberg, 1958). As a result, previous investigations have concentrated on the native β-D-galactosyldiacylglycerols isolated from chloroplasts and blue-green algae (Quinn & Williams, 1983; Mannock et al., 1985b), their hydrogenated derivatives (Sen et al., 1983; Mannock et al., 1985a), and the native α-D-glucosyldiacylglycerols from organisms like *Acholeplasma laidlawii* B (Wieslander et al., 1978, 1981a,b; Khan et al., 1981; Silvius et al., 1980). Those studies have shown that the native lipids tend to form inverted hexagonal (H_{II}) phases when dispersed in water at or near physiological temperature and have led several workers (Quinn & Williams 1983; Wieslander et al., 1981a) to suggest that these compounds may have a more important structural role in biological membranes than had hitherto been realized.

A number of recent calorimetric, spectroscopic, and X-ray diffraction studies on the thermotropic properties of semisynthetic saturated β-D-galactosyldiacylglycerols (Sen et al., 1983; Mannock et al., 1985a; Lis & Quinn, 1986) and of synthetic 1,2-di-*O*-alkyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerols (Endo et al., 1982; Hinz et al., 1985; Blöcher et al., 1985; Jarrell et al., 1986, 1987a,b) have appeared. These studies have established that these two glycolipids exhibit some thermotropic properties, in particular, gel-state polymorphism and the ability to undergo lamellar/reverse hexagonal phase transitions, which closely resemble those of the saturated,

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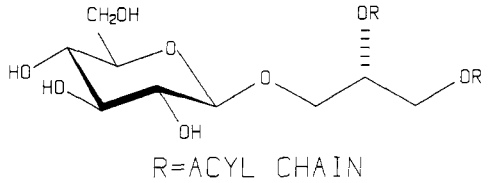

FIGURE 1: Chemical structure of 1,2-di-*O*-acyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerols.

straight-chain phosphatidylethanolamines [see Wilkinson and Nagle (1981), Mantsch et al. (1983), and Seddon et al. (1983a,b, 1984)]. Although considerable insight into some of the physical properties of "glycolipid bilayers" has been obtained from these and other studies, the bulk of the data acquired so far has been obtained from studies on relatively few compounds. From recent studies on phosphatidylcholine bilayers, it has become apparent that there are inherent risks in using any one member of a homologous series as a model for other homologues, since there can be marked behavioral discontinuities between the short- and long-chain homologues and between those with acyl chains containing an odd or even number of carbon atoms (Lewis & McElhaney, 1985a,b). Given this, we have recently synthesized a homologous series of β-D-glucosyldiacylglycerols with saturated *n*-acyl chains from 12 to 20 atoms (Mannock et al., 1987) and have begun a systematic characterization of their physical properties. The results of the differential scanning calorimetric (DSC)[1] studies are described in this paper.

Materials and Methods

Details of the synthesis and purification of this series of glycolipids have been described elsewhere (Mannock et al., 1987). The general chemical structure of the glucolipids employed in this study is shown in Figure 1. To facilitate reference to these compounds, the correct chemical name, 1,2-di-*O*-acyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol, will be abbreviated to diacyl(*N*)-β-D-Glc-*sn*-glycerol, where *N* equals the number of carbon atoms per acyl chain.

Preparation of Samples for Calorimetry. DSC was performed by using a Perkin-Elmer DSC-2C. As has been observed previously (Hinz et al., 1985), these samples, unlike the phosphatidylcholines, do not readily hydrate and form stable homogeneous dispersions by the conventional vortexing or even sonication of aqueous mixtures. It was therefore necessary to devise alternative dispersion procedures. In the case of the short-chain compounds ($N \leq 16$), samples were prepared by mixing 2–3 mg of freeze-dried lipid with 50 μL of distilled water in a stainless steel, large-volume capsule. For the long-chain compounds ($N \geq 16$), 1–2 mg of lipid was added to the capsule dissolved in $CHCl_3$/MeOH (2:1 v/v), the solvent removed under a stream of nitrogen, and the sample dried overnight in vacuo. After addition of distilled water, all samples were sealed and repeatedly heated and cooled to ensure thorough mixing and complete hydration. The reference consisted of a similar capsule containing 50 μL of distilled water.

Samples were heated and cooled between −3 and 97 °C under the conditions specified in the figure legends and the data collected and analyzed with a Perkin-Elmer 3600 data station using TADS software (Perkin-Elmer) and other computer programs developed in this laboratory. The transition temperatures reported are maxima in the case of heating thermograms and minima in the case of cooling thermograms. After the calorimetric measurements, the capsules were opened and the lipid contents estimated by gas chromatography of the fatty acid methyl esters after transesterification using acidic methanol and an appropriate internal standard (Lewis & McElhaney, 1985a).

Preparation of Samples for X-ray Diffraction. Freeze-dried lipids were placed in thin-walled glass capillaries (1.5-mm diameter), and distilled, deionized water (four times the weight of the sample) was added. The contents were mixed with a stainless steel plunger and the capillaries then sealed with a silicone rubber sealant (Dow Corning Corp.). To fully hydrate the samples, the sealed capillaries were heated to 80–85 °C, cooled to room temperature (20 °C), and then incubated at 40 °C for 24 h, following which they were centrifuged to pellet the hydrated lipids at the bottom of the capillaries. A Rigaku Rotaflex (Model RU-200B) rotating-anode X-ray generator equipped with a Franks-type camera and a thermoelectric temperature control system was used for the measurements. Diffraction patterns were recorded either on film (Kodak, X-ray film DEF-5) or by using a TEC Model 205 position-sensitive proportional counter (PSPC) interfaced to an IBM PC-AT personal computer through a multichannel analyzer add-on board. Typical times for recording diffraction patterns were 30 min for PSPC and 3 h for film.

Results

Thermotropic Phase Behavior. The DSC thermograms of unannealed dispersions of the 1,2-di-*O*-acyl-β-D-Glc-*sn*-glycerols studied show a complex pattern of polymorphic phase behavior (see Figure 2). The pattern is not the same for all samples, and our observations seem consistent with the occurrence of gel-phase polymorphism and metastability which is strongly influenced by the length of the hydrocarbon chains. Our approach to the characterization of this metastability involved studies on the influence of scan rates, annealing times, and storage times prior to heating on the observed phase behavior of these compounds and the use of X-ray diffraction measurements to determine the nature of the structural changes occurring at the observed phase transitions.

(*i*) *Effects of Scan Rate.* DSC thermograms of unannealed samples of the 1,2-diacyl-β-D-Glc-*sn*-glycerols acquired at scan rates of 10, 5, 1, and 0.31 °C min^{-1} are shown in Figure 2. At fast heating rates (≥5 °C min^{-1}), the compounds with fewer than 18 carbons per acyl chain all exhibit a strongly energetic, lower temperature transition and a weakly energetic, higher temperature transition, which have been assigned to a lamellar gel/liquid crystalline (L_β/L_α) phase transition and a lamellar liquid crystalline to nonbilayer (L_α/NBL) phase transition, respectively (see below). For the shorter chain compounds ($N \leq 15$), the thermotropic event assigned to the L_α/NBL transition is fairly broad and is only easily discernible at fast scan rates. Unlike the L_β/L_α phase transition, the L_α/NBL transitions of these compounds appear not to be very chain length dependent and get progressively sharper and closer to the L_β/L_α transition as the length of the acyl chain increases. In the case of the longer chain compounds ($N = 19, 20$) where only one transition is observed, the single thermotropic event has been assigned as a net transition from the L_β gel state to an inverted hexagonal phase, i.e., an L_β/H_{II} transition (see below).

At slower heating rates (≤1 °C min^{-1}), the three shorter chain compounds ($N = 12, 13, 14$) show a second, highly

[1] Abbreviations: DSC, differential scanning calorimetry; NMR, nuclear magnetic resonance; PC, phosphatidylcholine; PE, phosphatidylethanolamine; NBL, nonbilayer; 1,2-diacyl-3-*O*-β-D-Glc-*sn*-glycerol, 1,2-di-*O*-acyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol. The fatty acyl chains of the lipids used in this study are described by the shorthand notation *N*:0, where *N* denotes the number of carbon atoms per acyl chain with the zero signifying the absence of double bonds.

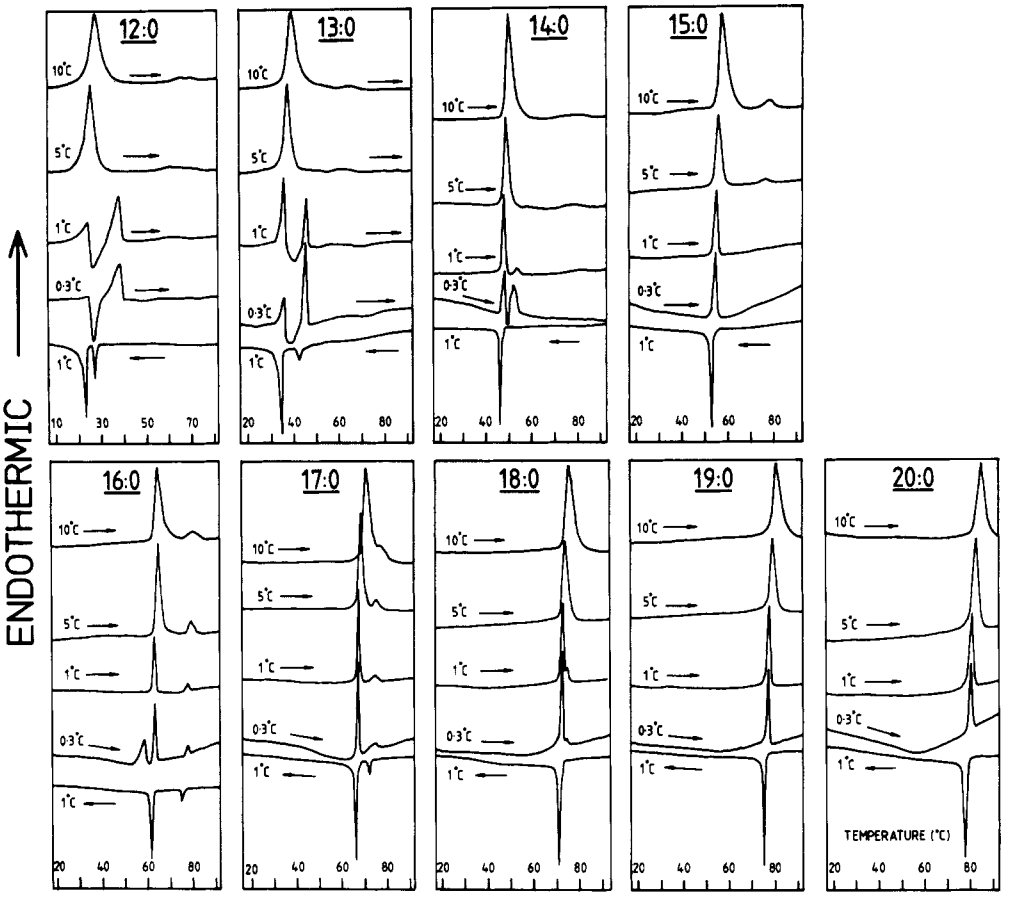

FIGURE 2: Heating and cooling thermograms of di-12:0- to di-20:0-β-D-glucopyranosyl-*sn*-glycerols heated and cooled as indicated on each thermogram. The arrows indicate the direction of temperature change. Corresponding cooling thermograms at 10, 5, and 0.3 °C min^{-1} are not shown and differ from that at 1 °C min^{-1} only in terms of peak resolution and scan rate effects.

energetic endothermic event which occurs at temperatures higher than that of the lamellar gel/liquid crystalline phase transition but lower than that of the L_α/NBL phase transition. The occurrence of this event is accompanied by a reduction in the area of the endotherm assigned to the lamellar gel/liquid crystalline phase transition and the appearance of a substantial exotherm between the two events. With a decrease in the scan rate the size of the higher temperature endotherm increases, apparently at the expense of the lower temperature endotherm. This, along with the appearance of the exotherm in the heating thermograms, indicates that the gel phase formed on initial cooling of these compounds is a metastable state which, at low temperatures, is being converted to a more stable gel state. A close inspection of the thermograms presented in Figure 2 shows that, for any given scan rate (≤1 °C min^{-1}), the high-temperature endotherm, and the exotherm that precedes it, becomes less dominant as the acyl chain length increases. This indicates that an increase in the acyl chain length results in slower rates of conversion from the metastable to the stable gel phase.

The longer chain compounds ($N \geq 15$) are considerably less sensitive to changes in the heating rate, and with the exception of the di-16:0 species, changes in the heating rates only affect the resolution between the gel/liquid crystalline phase transition and the L_α/NBL phase transition. However, at the slowest heating rate used (0.31 °C min^{-1}), the 16:0 species exhibits an additional endothermic event at temperatures below the onset of the normal lamellar gel/liquid crystalline phase transition. It is clear therefore, that, like the shorter chain compounds ($N = 12, 13, 14$), the gel phase formed upon initial cooling of the 16:0 species is metastable with respect to a more stable gel phase. Unlike the shorter chain homologues, however, the stable gel phase formed becomes unstable at temperatures below those of the lamellar gel/liquid crystalline phase transition.

(*ii*) *Effects of Low-Temperature Annealing.* All of the compounds (except the di-19:0 species) exhibit gel-state polymorphism when subjected to appropriate regimes of low-temperature annealing. Some of the conditions used to induce the formation of the more stable gel phases of these lipids are listed in Table I, and representative heating endotherms obtained after the stated annealing periods are shown in Figure 3. Once fully equilibrated by appropriate annealing at suitable low temperatures, the shorter chain compounds ($N \leq 15$) each exhibit a single, more energetic transition at tempeatures higher than those assigned to the melting of the gel phase formed upon initial cooling of these samples, whereas their longer chain counterparts (except the di-19:0 compound) exhibit additional endothermic transitions at temperatures lower than that assigned to the lamellar gel/liquid crystalline phase transition. The conditions necessary to induce the formation of the stable gel phases of these lipids (see Table I) become more exacting as the length of the acyl chain increases. This is more apparent with the longer chain odd-numbered compounds, for which the conversion to the stable gel phase generally requires longer incubation periods than their even-numbered neighbors stored under similar conditions. It is thus clear that the rate of formation of the stable gel phases of this homologous series of β-D-glucolipids becomes slower with increasing acyl chain length and that the process is more kinetically favorable in the case of the even-numbered compounds.

A close inspection of the thermograms of the di-13:0 and di-14:0 compounds shown in Figure 3 also suggests that the

Table I: Storage Conditions Used To Induce the Stable Gel Phases of the 1,2-Diacyl-3-*O*-β-D-Glc-*sn*-glycerols

sample	storage conditions[a]	estimated minimum time for conversion at 22 °C	longest storage period at 22 °C (days)
12:0	(a) 48 h at −3 °C; or (b) 2–3 h at 17 °C; or (c) 70 min at 29 °C	1–2 h	40
13:0	(a) 6 h at 39 °C	10–15 h	71
14:0	(a) 25 days at 22 °C	18–20 days	80
15:0[b]	(a) 58 days at 22 °C	40–50 days	84
16:0[c]	(a) 7 days at 22 °C; or (b) 5 h at 42 °C	5–7 days	75
17:0	(a) 2 days at −3 °C	~50 days	90[d]
18:0	(a) 34 days at 22 °C	25–30 days	80
19:0	no new gel phase observed		~120
20:0	(a) 65 days at 22 °C	50–60 days	78

[a] Storage conditions that induced complete conversion to the stable gel phase after an initial seeding period at −3 °C for 5 min. Conversion to the stable gel phase was judged to be complete if an incubation at 22 °C for the maximum observation period specified did not result in any changes in the thermodynamic parameters of the stable gel phase formed. [b] A stable gel phase was not initiated by incubation at 22 °C for 16 days. [c] A stable gel phase was not induced by incubation at −3 °C for 13 days. [d] A stable gel phase was not observed after incubation at −3 °C for several hours, followed by storage at 22 °C for 70–90 days, suggesting a longer nucleation time may be necessary for some of these samples.

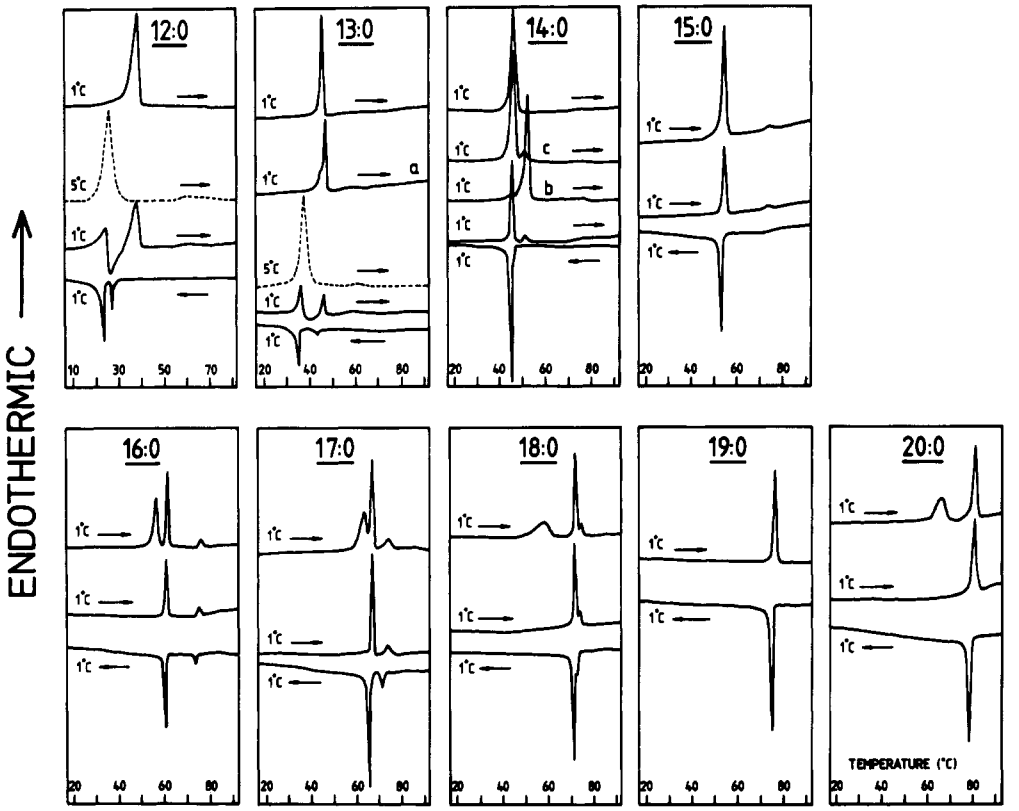

FIGURE 3: DSC thermograms of the di-12:0- to di-20:0-β-D-glucopyranosyl-*sn*-glycerols after annealing as described in Table I. The data shown were acquired at the scan rates indicated. In each block the bottom two thermograms are the heating and cooling curves obtained without annealing, whereas the uppermost curve is that obtained after extensive annealing. The heating thermogram of the di-19:0 species obtained after extensive annealing is indistinguishable from that obtained without annealing and is not shown. In the case of the di-12:0 and di-13:0 species, which show exotherms in the heating thermograms of the unannealed samples, a fast scan rate of an unannealed sample is also included (broken curves) to illustrate the melting of the metastable gel phase. The additional curves shown for the di-13:0 and di-14:0 species show the time course of the conversion to the stable gel phase and were obtained after (a) 12 h at 27 °C, (b) 36 h at 47 °C, and (c) 13 days at 22 °C, respectively.

process by which the stable gel phases of these compounds form is fairly complex. Upon annealing of the metastable gel phase that is formed upon initial cooling, there is first a conversion to a more stable gel phase which, upon heating, undergoes a transition at temperatures near 47.3 and 52.1 °C (di-13:0 and di-14:0, respectively). However, upon further annealing, the stable gel phase that is formed initially converts to an even more stable gel phase (as judged from the increases in enthalpy, see Table III) which, upon heating, melts at temperatures near 45.8 and 46.5 °C (di-13:0 and di-14:0, respectively). This indicates that the formation of the most stable gel phase of these lipids is in fact preceded by another metastable gel phase intermediate.

The metastable gel phase intermediates of the shorter chain compounds are not usually observed on account of the very rapid rates at which they convert to their respective stable gel phases. However, with those compounds we have detected such intermediates in kinetic experiments using short annealing times (minutes to hours) and fast scan rates (≅20 °C min^{-1}). In the case of the longer chain compounds ($N \geq 15$), we have no evidence for the formation of such intermediates, and at this stage we are unsure whether the "stable" gel phases so far observed are intermediate or final forms, since the very long annealing times required preclude any easy experimental verification.

(*iii*) *X-ray Diffraction Studies.* The X-ray scattering patterns obtained with these compounds were consistent with the expectations of gel-phase polymorphism and metastability.

Table II: X-ray Diffraction Spacings Obtained from Aqueous Dispersions of 1,2-Di-*O*-acyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerols at Various Temperatures

lipid	temperature (°C)	phase	*D* spacing (Å)
14:0	20	lamellar equilibrium gel	47.8
	55	lamellar liquid crystalline	49.8
	85	nonbilayer 2-D monoclinic	64.6
	20	lamellar metastable gel	53.5
16:0	20	lamellar equilibrium gel	51.6
	55	lamellar metastable gel	60.3
	70	lamellar liquid crystaline	52.9
	85	nonbilayer (hexagonal II)	55.8
17:0	20	lamellar equilibrium gel	53.3
	55	lamellar metastablge gel	62.9
	70	lamellar liquid crystalline	55.3
	85	nonbilayer (hexagonal II)	57.6

For the shorter chain compounds ($N \leq 15$; exemplified here by 1,2-di-14:0-β-D-Glc-*sn*-glycerol), the X-ray data provided evidence for at least two lamellar "gel" phases. The first is obtained upon initial cooling of the samples from temperatures above those of their respective gel/liquid crystalline phase transition temperatures. This phase exhibited a series of low-angle reflections consistent with the so called L_β-type lamellar gel phase. In the particular case of the di-14:0 species (see data in Table II), an L_β phase with a lamellar repeat of 53.5 Å is formed. This phase is metastable, and upon equilibration at low temperatures (see above), it transforms (via an intermediate) to an apparently stable phase with X-ray spacings characteristic of a more highly ordered lamellar structure (L_c) with a repeat of 47.9 Å. Upon heating, both the stable and metastable lamellar gel phases of the di-14:0 species convert directly to a form that exhibits low-angle reflections characteristic of a lamellar liquid crystalline (L_α) phase with a repeat of 49.8 Å. From these types of data, the transitions observed with the shorter chain compounds can be assigned as L_β/L_α (unannealed samples) and L_c/L_α (annealed samples) transitions, respectively. Such transitions coincide with sharp increases (L_c/L_α), and sharp decreases (L_β/L_α) in the *D* spacings at their respective transition temperatures (see data for the di-14:0 species in Table II). At temperatures above the transition to the L_α phase, the shorter chain compounds exhibit a broad, weakly energetic transition that is only calorimetrically detectable at fast heating rates (see Figure 2). This transition coincides with a sharp increase in the *D* spacing (see data for the di-14:0 species in Table II) and a major change in the X-ray scattering pattern in the low-angle region. The low-angle pattern exhibited by the new phase formed is characteristic of a nonbilayer 2-D monoclinic phase (M. Akiyama, personal communication, unpublished results).

Gel-phase polymorphism was also apparent with all of the longer chain compounds studied with the exception of the di-19:0 species. The X-ray data (exemplified here by the di-16:0 and di-17:0 compounds) also showed evidence of at least two gel phases. Like their shorter chain homologues, the gel phase that is initially formed upon cooling from the liquid crystalline phase exhibits low-angle reflections typical of a L_β-type gel phase, and after equilibration by incubation under appropriate conditions (see Table I), low-angle reflections characteristic of a lamellar phase with a decreased *D* spacing (see Table II) are observed. We have attributed this to the formation of an L_c-like phase. However, unlike their shorter chain homologues, the stable L_c gel phase first converts to the L_β gel phase upon heating, and this is reflected by a sharp increase in the *D* spacing (see Table I). With the exception of the two longest chain compounds studied ($N = 19, 20$), further heating of the L_β phase results first in a sharp decrease in the *D* spacing (see data in Table II), with the appearance of the low-angle pattern typical of the lamellar liquid crystalline (L_α) phase followed, at still higher temperatures, by a large increase in the *D* spacing with the appearance of the low-angle pattern characteristic of an inverted hexagonal (H_{II}) phase. Thus, the three transitions observed in the DSC heating thermograms of fully equilibrated samples of the di-16:0, di-17:0, and di-18:0 compounds can be assigned as L_c/L_β, L_β/L_α, and L_α/H_{II} transitions, respectively. With the di-19:0 and di-20:0 compounds, the L_β phase converts directly to the inverted hexagonal phase upon heating, and the transitions so far observed for extensively annealed samples of these compounds can be assigned as an L_β/H_{II} transition for di-19:0 and, for the di-20:0 species, L_c/L_β and L_β/H_{II} transitions, respectively.

Thermodynamic Data. The transition temperatures and enthalpy changes associated with endothermic transitions of the 1,2-diacyl-3-*O*-β-D-Glc-*sn*-glycerols are listed in Table III. For the purpose of this presentation, we have designated the gel phases of the shorter chain lipids as gel phases G_I, G_{II}, and G_{III} and those of the longer chain compounds as gel phases G_I and G_{II}'. These designations basically reflect the order in which the gel phases are formed [i.e., metastable gel phase, intermediate gel phase (where observed) and stable gel phase, respectively], while the phases designated as L_α and H_{II} are in accordance with the usual notation for the lamellar liquid crystalline and inverted hexagonal phases, respectively. The G_I/L_α transitions (i.e., the normal chain-melting transitions) are strongly chain length dependent with respect to both their transition temperatures and transition enthalpies and, when plotted as a function of acyl chain length (see Figures 4 and 5), describe a smooth function, as would be expected of simple chain-melting processes. In the case of the corresponding parameters of the L_α/NBL transitions, there is some chain-length dependence, which presumably reflects differences in

Table III: Phase Transition Properties of a Series of Synthetic 1,2-Di-*O*-acyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerols

	phase transition temperature (°C)[a]					enthalpy ΔH (kcal·mol^{-1})				
lipid	G_{II}' to G_I	G_I to L_α	G_{II} to L_α	G_{III} to L_α	L_α to NBL	G_{II}' to G_I	G_I to L_α	G_{II} to L_α	G_{III} to L_α	L_α to NBL
12:0		26.0[b]	33.1[c]	38.6	57.7[c]		4.9		14.3	0.3
13:0		35.7	47.3	45.8	59.0[c]		5.8	14.7	16.3	0.35
14:0		45.5	52.1	46.5	72.0[d]		6.7	15.7	18.3	0.77[d]
15:0		54.2	54.8		73.4		8.2	18.0		0.67
16:0	56.2	61.0			75.0[e]	14.0	9.0			1.47[e]
17:0	63.1	67.0			73.9[e]	9.1	10.2			1.45[e]
18:0	58.1	71.7			73.8	8.8	11.2			1.10
19:0		76.5[f]					13.3[f]			
20:0	62.5	79.7[f]				10.8	14.7[f]			

[a] All temperature measurements have been corrected for the instrument's dependence on scan rate and were obtained at a heating rate of 1 °C min^{-1}, except where noted. [b] Obtained at a heating rate of 5 °C min^{-1}. [c] Obtained by extrapolation to a heating rate of 1 °C min^{-1} from values obtained at faster heating rates. [d] 2-D monoclinic phase. [e] Hexagonal II phase (see Table I). [f] G_I to H_{II}.

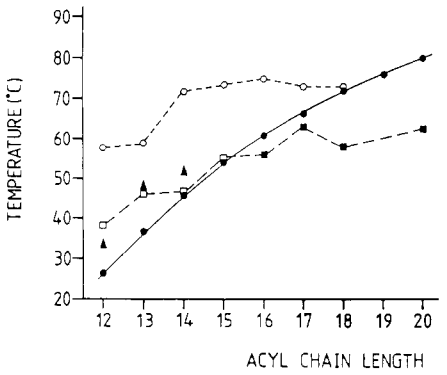

FIGURE 4: Acyl chain length dependence of the transition temperatures of a homologous series of 1,2-di-*O*-acyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerols. Data are presented for (●) $G_I \rightarrow L_\alpha$ transitions, (○) $L_\alpha \rightarrow$ NBL transitions, (■) $G_{II}' \rightarrow G_I$ transitions, (□) $G_{III} \rightarrow L_\alpha$ transitions, and (▲) G_{II} to L_α transitions. The lines shown are merely a guide for the eye. In the case of the di-19:0 and di-20:0 compounds, the chain-melting event is a transition from a metastable gel phase (G_I) to a H_{II} phase without an intermediate L_α phase (see text).

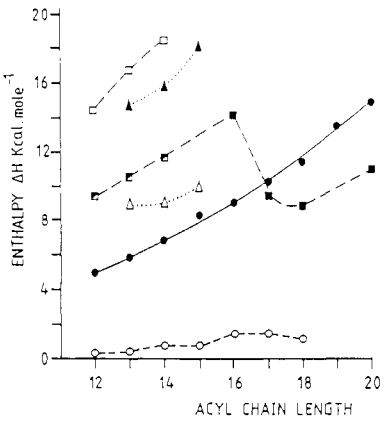

FIGURE 5: Acyl chain length dependence of the transition enthalpies of a homologous series of 1,2-di-*O*-acyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerols. Data are presented for (●) $G_I \rightarrow L_\alpha$ transitions, (○) $L_\alpha \rightarrow$ NBL transitions, (■) $G_{II}' \rightarrow G_I$ transitions, (□) $G_{III} \rightarrow L_\alpha$ transitions, (▲) $G_{II} \rightarrow L_\alpha$ transitions, (△) estimated $G_{II} \rightarrow G_I$ transitions (▲ minus ●), and (◩) estimated $G_{III} \rightarrow G_I$ transitions (□ minus ●). The lines are merely a guide for the eye. In the case of the di-19:0 and di-20:0 compounds, the chain-melting event is a transition from a metastable gel phase (G_I) to a H_{II} phase without an intermediate L_α phase (see text).

the nature of the nonbilayer phase. However, within each class there is almost no chain-length dependence of either the transition temperature or enthalpy. The properties of the thermotropic transitions involving the stable gel states of these lipids [i.e., those designated as $G_{III}/L_\alpha (N \leq 15)$ and G_{II}'/G_I $(N \geq 16)$] are also chain length dependent, and it is clear from Figure 4 that there are pronounced discontinuities in the transition temperatures of the odd- and even-numbered members of this homologous series. An evaluation of the effect of chain length on the enthalpy changes involving the stable gel states of these lipids is complicated by the fact that the transition enthalpies calculated for the G_{III}/L_α transitions of the shorter chain compounds undoubtedly include a contribution from the normal chain-melting process (i.e., the G_I/L_α transition). When the enthalpies are corrected for this (subtraction of the enthalpy of the G_I/L_α transition from that of the G_{III}/L_α transition should give good estimates of the enthalpy of the G_{III}/G_I transition) and plotted as a function of acyl chain length (see Figure 5), a pronounced discontinuity is observed between the trends set by the shorter chain compounds and those containing 17 or more carbon atoms per acyl chain. A lack of data prevents a similar characterization of the chain-length dependence of the properties of the intermediate gel state (G_{II}).

Discussion

The pattern that emerges from the calorimetric studies reported here is that the 1,2-diacyl-3-*O*-β-D-Glc-*sn*-glycerols studied can all form a metastable lamellar gel phase (G_I phase) which, depending on the chain length, may upon heating convert either to a lamellar liquid crystalline (L_α) phase ($N \leq 18$) or a nonbilayer inverted hexagonal (H_{II}) phase ($N \geq 19$). In the case where an L_α phase is formed, further heating results in a transition to a nonbilayer structure, the nature of which is also dependent on the chain length; the shorter chain compounds ($N < 15$) form 2-D monoclinic phases, while their longer chain counterparts ($N = 16, 17, 18$) form inverted hexagonal phases. In addition, all of the compounds studied (except the di-19:0 species) exhibit some form of gel-phase polymorphism which typically involves the slow conversion of the metastable gel phase to a more ordered L_c-type of structure (G_{II}, G_{II}', and G_{III}). The above structural assignments have been made on the basis of the X-ray diffraction measurements, which were consistent with the chain length dependent pattern of polymorphic phase behavior reported by DSC. A detailed x-ray diffraction study of this series of 1,2-diacyl-3-*O*-β-D-Glc-*sn*-glycerols will be published elsewhere.

In some respects, the behavior of these compounds is analogous to a number of other monoglycosyldiacylglycerols that have been studied (Rivas & Luzzati, 1969; Shipley et al., 1973; Sen et al., 1981, 1983; Jarrell et al., 1986). However, some aspects of the polymorphic phase behavior reported here (e.g., the formation of 2-D monoclinic phases by the shorter chain lipids and the direct transitions from an L_β-type gel phase to an H_{II} phase exhibited by the longer chain homologues) are not frequently reported phenomena. Nevertheless, an L_α/cubic transition has been identified in studies with a short-chain dialkyl-PE (Seddon et al., 1984), and L_α/2-D monoclinic phase transitions have been seen in both the short-chain 1,2-di-*O*-acyl-3-*O*-α-D-Glc-*sn*-glycerols (A. Sen et al., unpublished observations) and the short-chain 1,2-di-*O*-alkyl-3-*O*-β-D-Glc-*rac*-glycerols (M. Akiyama, personal communication), while L_β/H_{II}-type transitions have been detected in a study of a PE dispersed in a saturated salt solution (Marsh & Seddon, 1982), in ^2H NMR studies of 1,2-ditetradecyl-3-*O*-(α-D-mannopyranosyl)-*sn*-glycerol (Jarrell et al., 1987a) and by low-angle X-ray diffraction studies of the longer chain homologues of both the 1,2-di-*O*-acyl-3-*O*-(α-D-glucopyranosyl)-*sn*-glycerols (A. Sen et al., unpublished observations) and racemic di-*O*-alkyl-β-D-glucopyranosylglycerols (M. Akiyama, personal communication).

The gel-phase polymorphism exhibited by the longer chain compounds ($N \geq 16$) closely resembles the transformations involving the "subgel" phases of a number of phosphatidylcholines [see Chen et al. (1980), Stumpel et al. (1983), Lewis and McElhaney (1985a), and Lewis et al. (1987a,b)] and some N-methylated PEs (Gagne et al., 1985). In these cases the stable gel phases become unstable with respect to an L_β-like gel phase at temperatures below that of the onset of the cooperative melting of the acyl chains, with the result that discrete L_c/L_β and L_β/L_α thermotropic transitions are observed. With the longer chain 1,2-di-*O*-acyl-3-*O*-β-D-Glc-*sn*-glycerols studied here, the rate of conversion from the L_β-like G_I phase to the stable G_{II}' gel phase is very slow and can be seen to decrease as the length of the acyl chain increases. In addition, there are pronounced discontinuities in the kinetics of formation of the stable gel phases of the odd- and even-numbered homologues, as has been observed with

a number of phosphatidylcholines [see Lewis and McElhaney (1985a), Yang et al. (1986), and Lewis et al. (1987a,b)]. With these glucolipid species, the formation of the stable gel phases of the odd-numbered homologues is less kinetically favorable, and with the di-19:0 species, its formation was not detected even after incubation periods in excess of 1 year. The slow rate of formation of the stable gel phases of these longer chain compounds (N = 16, 17, 18, 20) also makes it difficult to determine whether the apparently stable gel phases we have so far observed are in fact the most stable that can be formed. Because of this, we have designated those phases as G_{II}' to distinguish then from the stable gel phases of the shorter chain compounds.

In the case of the shorter chain homologues, the pattern of gel-phase polymorphism takes the form of a slow conversion (though considerably faster than their longer chain counterparts) of the metastable L_β-like G_I gel phase to a stable L_c-like G_{III} gel phase via another metastable L_c-like gel phase intermediate (the G_{II} phase). Unlike their longer chain counterparts, however, the L_c-like phases that are formed are thermodynamically stable with respect to the L_β-like G_I phase at all temperatures at which they are observed, with the result that, upon heating, they undergo direct transitions to the L_α phase. Thus, L_β/L_α transitions are only observed in unannealed samples of these shorter chain lipids, and the endothermic transitions exhibited by equilibrated samples are net L_c/L_α transitions which occur at temperatures higher than those of the L_β/L_α transitions. Direct L_c/L_α transitions have been observed with a number of short-chain phosphatidylcholines (Lewis & McElhaney, 1985a; Finegold & Singer, 1986; Lewis et al., 1987a), phosphatidylcholines with unsaturated or ω-cyclohexyl fatty acyl chains (Lewis & McElhaney, 1985b; Lewis et al., 1988), and some phosphatidylethanolamines (Seddon et al., 1983a; Wilkinson & Nagle, 1984) and have been inferred in studies with two monogalactosyldiacylglycerols (Sen et al., 1983; Mannock et al., 1985a). The gel-phase polymorphism exhibited by these shorter chain glucolipids also indicates that the formation of their stable L_c-like gel phases is a complex, multistage process which probably involves an initial nucleation process followed by the growth of the more stable structures. Recent studies on phosphatidylcholine and phosphatidylethanolamine model systems (Seddon et al., 1983a; Finegold & Singer, 1986; Tristram-Nagle et al., 1987; Lewis et al., 1987a; Slater & Huang, 1987) suggest that this may be a general characteristic of the formation of L_c-like gel phases of lipid bilayers.

An examination of the data presented here reveals several points pertinent to the organization of bilayers formed from these lipids. First, the occurrence of odd–even discontinuities in the properties of the transformations involving the stable gel phases observed are probably indicative of the formation of a quasicrystalline, stable gel phase in which the acyl chains are tilted with respect to the bilayer normal [see Broadhurst (1962)], while the absence of such odd–even discontinuities in the properties of the G_I/L_α phase transitions suggests that the gel phase from which the L_α phase is nucleated is probably a relatively loosely packed structure in which the acyl chains are normal with respect to the bilayer plane. Second, a comparison of the properties of these β-D-glucosyldiacylglycerols with those of other saturated, straight-chain polar glycerolipids [see Silvius (1982), Seddon et al. (1983b), Sen et al. (1983), Hinz et al. (1985), Blöcher et al. (1985), Mannock et al. (1985a), Small (1986), and Lewis et al. (1987a) and references cited therein] indicates that their properties closely resemble those of the so-called "non-bilayer-forming lipids" such as the PEs and other monoglycosyldiacylglycerols. The enthalpies of their G_I/L_α transitions (i.e., the normal chain-melting transition) and the temperatures at which they occur are close to those of comparable phosphatidylethanolamines (which form nonbilayer structures) and considerably higher than those of comparable phosphatidylcholines, phosphatidylglycerols, and digalactosyldiacylglycerols (which do not normally exhibit any tendencies to form nonbilayer structures). The overall similarities in the properties of these β-D-glucosyldiacylglycerols and the phosphatidylethanolamines may be indicative of similar functional roles for both classes of lipids in the biological membranes in which they naturally occur.

Despite the above similarities, it is apparent that there are also some differences between these glucolipids and the phosphatidylethanolamines. In particular, the enthalpy values for the G_{III}/L_α phase transitions are considerably greater than those obtained for the stable L_c/L_α phase transitions of the corresponding PEs (Chang & Epand, 1983; Mantsch et al., 1983). Furthermore, the enthalpies for the G_{II}'/G_I phase transitions are also considerably larger than those of the L_c/L_β' phase transitions recently reported for a series of straight-chain PCs (Lewis et al., 1987a). These results suggest that the L_c phases formed in these glycolipids are stabilized by strong headgroup interactions, which probably involve intermolecular hydrogen bonds. It is also apparent that the glucolipids which undergo L_α/H_{II} transitions do so at temperatures that are considerably lower than those of PEs with similar acyl chains [see Harlos and Eibl (1981) and Seddon et al. (1983b)]. Moreover, the L_α/H_{II} transition temperatures of these glucolipids are not very chain length dependent, whereas the L_α/H_{II} transition temperatures of the PEs exhibit a strong inverse dependence on chain length [see Seddon et al. (1983b)]. Although these observations appear not to be consistent with the proposal that the H_{II} phase is stabilized by an increase in acyl chain length (Seddon et al., 1983b), our results have also shown that, with the longer chain compounds, H_{II} phases are formed in preference to other nonlamellar structures. Thus, despite the weak (if any) chain-length dependence of the L_α/H_{II} transition temperaures, a general stabilization of the H_{II} phase by the elongation of the acyl chains is still apparent. Most investigators currently believe that factors which can stabilize or destabilize the H_{II} phase exert their effects primarily by their ability to affect the dynamic cross-sectional area of the lipid polar headgroup relative to that of the hydrocarbon chains in the liquid-crystalline state (Seddon et al., 1983b; Cevc & Marsh, 1985). Such factors are known to include pH, ionic strength, and polar headgroup hydration where ionizable headgroups are involved (Seddon et al., 1983b). In addition, such factors must also include alterations in the polarity of the hydrophobic/hydrophilic interface of the lipid assembly, since it has been shown with some non-bilayer-forming lipids that replacement of acyl chains with alkyl chains results in only a slight increase in the gel/liquid crystalline phase transition temperature but a dramatic reduction in the L_α/H_{II} transition temperature (Boggs et al., 1981; Seddon et al., 1983b). A comparison of the data from our diester glucolipids with the data available for their diether analogues (Hinz et al., 1985; Blöcher et al., 1985; Jarrell et al., 1986; unpublished experiments from this laboratory) shows that this is also true of these glucolipids. In sugar-containing lipids where the direct effects of pH and ionic strength are probably negligible, the "dynamic cross-sectional area" of the polar headgroups will be determined primarily by the configuration, conformation, and hydration of the carbohydrate moieties. Our studies on a comparable homologous series of

the 1,2-di-*O*-acyl-3-*O*-(α-D-glucopyranosyl)-*sn*-glycerols (unpublished experiments from this laboratory) have shown that a change in the configuration of the glycosidic linkage alters both the pattern of gel-phase polymorphism and the properties of the L_α/NBL phase transition. This suggests that there may be significant differences in the conformation and/or dynamic cross-sectional areas of the lipid polar headgroups of the α- and β-anomers, as indicated by ^2H NMR studies of some of the diether analogues by Jarrell and co-workers (Jarrell et al., 1986, 1987a,b). Further studies of glycolipids in which the configuration of the polar headgroup is systematically varied should contribute greatly to an understanding of the principles underlying the thermotropic phase behavior of these lipids and may provide insight into their functional roles in biological membranes.

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