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Physical Properties of Glycosyl Diacylglycerols. 2. X-ray Diffraction Studies of a Homologous Series of 1,2-Di-*O*-acyl-3-*O*-(α-D-glucopyranosyl)-*sn*-glycerols†

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ABSTRACT: X-ray diffraction methods were used to characterize the thermotropic polymorphism exhibited by aqueous dispersions of a homologous series of 1,2-*O*-acyl-3-*O*-(α-D-glucopyranosyl)-*sn*-glycerols. Upon cooling from temperatures at which the acyl chains of these lipids are melted, all of these compounds form structures that exhibit both low-angle and wide-angle diffraction patterns consistent with the formation of lamellar L_β gel phases. After a suitable protocol of low-temperature annealing, complex diffraction patterns consistent with the formation of highly ordered, lamellar, crystal-like phases are obtained. These patterns are similar for all of the compounds studied, suggesting that the unit cell structure is invariant. The assumption that the unit cell structure is invariant permits the assignment of phases to the diffraction orders, thereby making possible the construction of electron density profiles. These electron density profiles indicate that the crystal-like phases of these lipids are poorly hydrated structures with the hydrocarbon chains inclined at 35° to the bilayer normal. The diffraction patterns of the crystal-like phases of these lipids changed abruptly at the calorimetrically determined phase transition temperatures to those characteristic of either lamellar liquid crystalline phases ($N \leq 17$) or inverted nonbilayer phases. With these X-ray diffraction data we demonstrate that, at elevated temperatures, the shorter chain homologues ($N \leq 16$) form cubic phases of the *Pn3m* space group, whereas the longer chain compounds form inverted hexagonal phases.

Despite the obvious importance of this class of lipids in nature [see Mannock et al. (1990a) and references cited therein], there have been relatively few studies aimed at a thorough characterization of their physical properties, mainly because the synthesis of useful quantities of these lipids of assured anomeric purity and acyl chain homogeneity was fairly difficult. However, with recent advances in carbohydrate chemistry [see Mannock et al. (1990a,b) for references], it has now become feasible to synthesize significant quantities of pure monoglycosyl diacylglycerols on a routine basis, with the result that systematic physical studies on these lipids are now possible. We have recently used such methods to synthesize a homologous series of α- and β-D-glucosyl diacylglycerols (Mannock et al., 1987, 1990b) and have begun a thorough study of the physical properties of these lipids. In the first phase of our studies we undertook an investigation of the physical properties of the β-linked anomers using DSC,[1] X-ray diffraction, FTIR spectroscopy, and monolayer film techniques (Mannock et al., 1988; Asgharian et al., 1989; Lewis et al., 1990). In this phase, we have begun a similar set of studies on the α-D-glucosyl diacylglycerols, the first part of which deals with the dynamic thermal phenomena studied by DSC (Mannock et al., 1990a). In this, the second part, X-ray diffraction methods are used to study the structure of

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[1] Abbreviations: DSC, differential scanning calorimetry; PC, phosphatidylcholine; PE, phosphatidylethanolamine; α-GlcDG, 1,2-di-*O*-acyl-3-*O*-(α-D-glucopyranosyl)-*sn*-glycerol; β-GlcDG, 1,2-*O*-acyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol; FTIR, Fourier-transform infrared.

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Table I: Low-Angle and Wide-Angle X-ray Diffraction Measurements on a Series of Synthetic 1,2-Di-*O*-acyl-3-*O*-(α-D-glucopyranosyl)-*sn*-glycerols

chain length	diffraction spacings (nm)			
	$d(L_c)$	$d(L_\beta)$	$d(L_\alpha)$	d(NBL)
10	3.38	[a]	4.08 (10°C); 3.80, 1.92[b]	
	0.40, 0.41, 0.55	[a]	0.46	
11	3.58	[a]	4.41 (10°C); 3.94, 2.00[b]	
	0.40, 0.41, 0.55	[a]	0.46	
12	3.78	[a]	4.57 (25°C); 3.94, 2.01[b]	
	0.40, 0.41, 0.43, 0.55	[a]	0.46	
13	4.01	5.17	4.80 (47°C)	6.70, 5.47, 3.84, 3.31[c]
	0.40, 0.42, 0.54	0.42	0.46	
14	4.15	5.35	4.97 (44°C)	7.16, 6.32, 4.16, 3.84[c]
	0.41, 0.43, 0.55	0.42	0.46	
15	4.37	5.78	5.19 (53°C)	6.70, 5.83, 3.99, 3.44[c]
	0.40, 0.41, 0.55	0.42	0.46	
16	4.55	5.90	[a]	7.92, 6.31, 4.46, 3.79[c]
	0.40, 0.41, 0.55	0.42	[a]	
17	4.84	6.10	5.51 (64°C)	5.87, 3.37, 2.64[d]
	0.40, 0.41, 0.43, 0.55	0.42	0.46	
18	5.01	6.29	5.58 (69°C)	5.99, 3.41, 2.99[d]
	0.40, 0.41, 0.55	0.42	0.46	
19	5.27	6.52		0.07, 3.50, 3.05[d]
	0.40, 0.41, 0.43, 0.55	0.42		0.46
20	5.43	6.70		6.23, 3.59, 3.09[d]
	0.40, 0.41, 0.55	0.42		0.45

[a] We were unable to make this measurement for technical reasons (see text for details). [b] The following reflections (*hkl*) were measured at 76 °C: (100) and (200), which correspond to a lamellar liquid crystalline. [c] The following reflections (*hkl*) were measured at 80 °C: (110), (111), (211), and (220), which correspond to a cubic (Q_{II}) phase belonging to the $Pn3m/Pn3$ space group. [d] The following reflections (*hkl*) were measured at 80 °C: (100), (110), and (200), corresponding to an inverted hexagonal (H_{II}) phase.

the various thermotropic phases detected in the DSC experiments.

Materials and Methods

Preparation of Samples for X-ray Diffraction. The lyophilized lipids were transferred to 1.5-mm glass capillaries and water (four times the weight of the lipids) was added. The contents of the capillaries were then thoroughly mixed with a stainless steel wire, sealed with a silicone sealant (Dow Chemicals Corp., Midland, MI), and cycled between 85 °C and room temperature to ensure full hydration of the samples. Prior to the X-ray diffraction measurements, the samples were centrifuged to pellet the hydrated lipids and preincubated under the conditions needed for the formation of the various phases detected by DSC [see Mannock et al. (1990a)].

X-ray Diffraction Measurements. The diffraction experiments were performed with a rotating-anode X-ray generator (Rigaku Rotaflex, Model RU-200B) operating at 40 kV and 25 mA and a Frank's-type camera fitted with a thermoelectric temperature-controlled sample holder. Diffraction patterns were recorded with film (Kodak, X-ray film DEF-5) and with a position-sensitive proportional counter (TEC Model 25, Knoxville, TN). The counter was interfaced to an IBM PC-AT personal computer fitted with a pulse height analyzer add-on board. Typical diffraction measurements involved 2–3-h exposures for film recordings and 15–30-min exposures for the counter. A Joyce-Loebl densitometer was used for the measurement of the intensities of the bands recorded on film.

Results

In the preceding paper (Mannock et al., 1990a), DSC measurements on the 1,2-di-*O*-acyl-3-*O*-(α-D-glucopyranosyl)-*sn*-glycerols detected a complex polymorphic phase behavior in which the thermotropic phase changes observed are, in part, strongly influenced by the thermal history of the particular sample. In these X-ray studies we have observed thermally induced interconversions between diffraction patterns diagnostic of highly ordered lamellar subgel-like phases (L_c), lamellar gel phases (L_β), lamellar liquid crystalline phases (L_α), reversed cubic phases (Q_{II}), and reversed hexagonal phases (H_{II}). For the most part the changes in the diffraction patterns are correlated with the phase changes observed calorimetrically, and this has enabled an unambiguous identification of the types of phase changes exhibitied by the individual lipids concerned.

Identification of the Phase Changes

(*a*) *Short-Chain Homologues* ($N \leq 10$–12). The DSC results (Mannock et al., 1990a) indicate that rapid heating of unannealed samples of these lipids results in a single cooperative and highly energetic phase transition at low to moderate temperatures without any evidence for structural changes at higher temperatures (up to 120 °C). In the corresponding X-ray diffraction experiment performed at temperatures up to 80 °C, two X-ray diffraction patterns characteristic of lamellar phases were observed (see Figure 1 and Table I). At low temperatures a single reflection at 0.42 nm was observed in the wide-angle region, indicative of gel-state hydrocarbon chains packed on a hexagonal lattice (Luzzati, 1968). On continued heating of the sample, the reflection at 0.42 nm was replaced by a diffuse band at 0.46 nm, characteristic of melted hydrocarbon chains hexagonally packed but undergoing rapid motion. Thus, these samples are lamellar throughout the temperature range 0–80 °C and the single transition observed is the typical gel/liquid crystalline (L_β/L_α) phase transition. Upon annealing [as described previously in Mannock et al. (1990a)], the L_β phases transform into their respective equilibrium gel phases, which have been shown to "melt" at temperatures above those of their respective L_β/L_α transitions. This new, equilibrium gel phase exhibits a complex diffraction pattern, with low-angle reflections occurring in the ratio 1:2:3:4 indicative of a lamellar structure, as well as a complex series of reflections in the wide-angle region (see Figure 1B and Table I). Such a pattern is indicative of the formation of a highly ordered crystal-like lamellar (L_c) phase, similar to those reported for the subgel phase of PCs (Ruocco & Shipley, 1982a,b; Church et al., 1986) and the crystalline

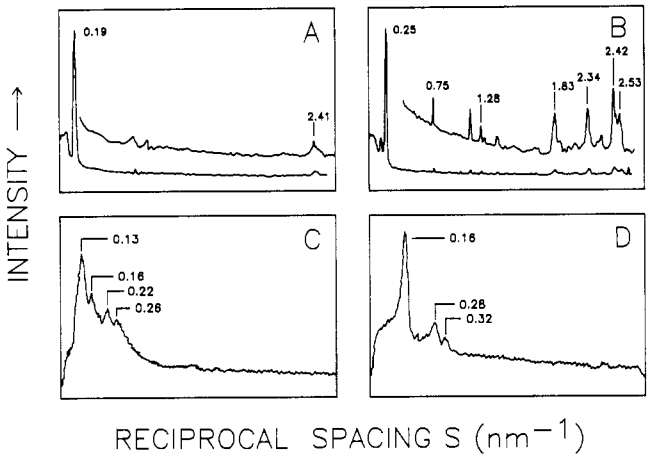

FIGURE 1: Representative X-ray diffraction patterns exhibited by the polymorphic phases of the α-D-glucosyl diacylglycerols. The patterns shown are for (A) small-angle and wide-angle diffraction of the L_β phase of di-13:0-α-GlcDG, (B) small-angle and wide-angle diffraction of the L_c phase of di-13:0-α-GlcDG, (C) small-angle diffraction of the cubic (Q_{II}) phase of di-16:0-α-GlcDG, and (D) small-angle diffraction of the hexagonal (H_{II}) phase of di-20:0-α-GlcDG. Diffraction patterns A and B are densitometer scans of films; C and D are computer generated data from the PSP counter.

phases formed by PEs (Mulukutla & Shipley, 1984) and some other glycolipids (Sen et al., 1983; Mannock et al., 1985). From these data it is clear that the transitions exhibited by annealed and unannealed samples of these short-chain α-linked glucosyl diacylglycerols correspond to L_c/L_α and L_β/L_α transitions, respectively.

(*b*) *Medium-Chain Homologues* (*N* = *13–16*). DSC studies on unannealed samples of these lipids all show a sharp transition at moderate temperatures and, with the exception of the di-13:0 species, a weaker transition at higher temperatures [see Mannock et al. (1990a)]. The low-angle diffraction patterns obtained from these samples at temperatures below the weakly energetic, higher temperature thermal event are entirely consistent with a lamellar structure. The corresponding measurements in the wide-angle region (Figure 1 and Table I) reveal a sharp 0.42-nm band at low temperatures, which is replaced by a diffuse 0.46-nm reflection upon heating, indicating that the highly energetic thermal event observed calorimetrically is a typical lamellar gel/liquid crystalline (L_β/L_α) phase transition. Upon further heating of these samples (to temperatures near 80 °C), the diffraction pattern changes markedly to that typified in Figure 1C (see Table I for the measured spacings). The spacings for the Bragg reflections are in the ratio $\sqrt{2}:\sqrt{3}:\sqrt{6}:\sqrt{8}$, which is consistent with both the *Pn3m* and the *Pn3* space groups (Shyamsunder et al., 1988). This *Pn3m*/*Pn3* cubic diffraction pattern was consistently obtained for lipids with 13–16 carbon chains, but as previously observed by Shyamsunder et al. (1988), the unit cell spacings often varied for different samples of the same lipid and even for the same sample after different thermal pretreatments. The formation of the *Pn3m*/*Pn3* cubic diffraction pattern coincides with the less energetic, higher temperature transition exhibited by the di-15:0 and di-16:0 compounds and clearly identifies these events as lamellar liquid crystalline/cubic (L_α/Q_{II}) phase transitions. In the case of the di-13:0 and di-14:0 species, this change in diffraction pattern is not correlated with any calorimetrically detected thermal event seen on heating over the temperature ranges studied. However, upon cooling of these samples a weakly energetic event, which may correspond to the Q_{II}/L_α transition, was observed [see Figures 2 and 3 in the preceeding paper by Mannock et al. (1990a)].[2] We suspect that the L_α/Q_{II} transitions of these particular lipids are weakly energetic and/or poorly cooperative thermal events and are thus very difficult to detect by DSC. Like the short-chain compounds described above, these samples also transform to an equilibrium gel phase after a suitable annealing protocol [see Mannock et al. (1990a)]. The X-ray measurements indicate that the formation of this equilibrium gel phase also coincides with the appearance of a diffraction pattern consistent with the formation of a highly ordered lamellar, crystal-like structure (Figure 1A,B). Thus, unannealed samples of the medium-chain homologues of these α-GlcDGs exhibit L_β/L_α and L_α/Q_{II} transitions, whereas annealed samples exhibit L_c/L_α and L_α/Q_{II} phase transitions.

(*c*) *Long-Chain Homologues* (*N* = *17–20*). Unannealed samples of the long-chain homologues all exhibit a highly energetic thermotropic transition at relatively high temperatures. The di-17:0 and the di-18:0 compounds also exhibit an additional weakly energetic transition at even higher temperatures [see Mannock et al. (1990a)]. The X-ray studies indiate that all of these compounds exhibit diffraction patterns consistent with a lamellar L_β-type gel phase (Figure 1A and Table I) at temperatures below their respective highly energetic thermotropic transitions seen in the DSC studies (Mannock et al., 1990a). In the case of the di-17:0 and the di-18:0 compounds, lamellar diffraction patterns are also observed upon heating these samples to just above the completion temperature of that transition. The change in the diffraction pattern observed is consistent with the conversion from a lamellar gel (L_β) to a lamellar liquid crystalline (L_α) phase and is similar to that reported for the short- and medium-chain compounds. Upon further heating of these samples, the diffraction pattern changes abruptly to that exemplified in Figure 1D (see Table I for a list of the measured spacings). Here the spacings for the Bragg reflections are in the ratio $1:\sqrt{3}:\sqrt{2}$ and are characteristic of an inverted hexagonal phase (Luzzati, 1968). The formation of this phase coincides with the weakly energetic higher temperature transition observed in the DSC curves of the di-17:0 and di-18:0 compounds (Mannock et al., 1990a) and thus identifies that event as a L_α/H_{II} transition. However, on heating samples of the di-19:0 and di-20:0 species, the changes in the X-ray diffraction patterns show that the L_α phase is absent, indicating that the single thermotropic transition observed by DSC is a direct conversion from a L_β-type gel phase to a reversed hexagonal phase (i.e., a L_β/H_{II} transition). The X-ray data also show diffraction patterns consistent with the formation of highly ordered L_c structures after these long-chain homologues are suitably annealed. Furthermore, as was suggested by the DSC studies, the nature of the thermal transitions exhibited by the annealed samples is also chain-length dependent. We found that after being suitably annealed, the di-17:0 species exhibits L_c/L_α and L_α/H_{II} transitions and the di-19:0 compound exhibits L_c/L_β and L_β/H_{II} transitions, whereas both the di-18:0 and the di-20:0 homologues exhibit a singe thermal event that we have assigned to a L_c/H_{II} transition.

X-RAY CHARACTERIZATION OF THE GEL PHASES

(*a*) *The Metastable* (L_β) *Gel Phase*. The DSC studies have clearly shown that the L_β-type gel phase of these α-GlcDGs

[2] A similar event is also visible in the thermograms of the di-12:0 species when those samples are cooled from temperatures near 100 °C. Thus, it is possible that the di-12:0 compound forms a Q_{II} phase at temperatures above 85 °C. However, we found that with these samples the capillary tubes were unable to survive very high temperatures for the long periods of time necessary to make the X-ray measurements and so the maximum temperature at which we measured these samples was 80 °C.

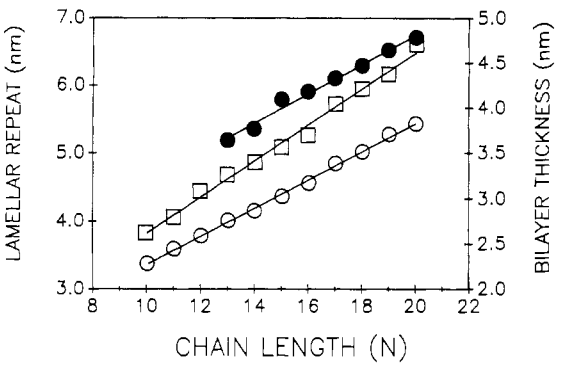

FIGURE 2: Lamellar repeats of the L_β (●) and the L_c (○) phases of the α-D-glucosyl diacylglycerols and the corresponding "bilayer thickness" of the L_c phase (□) obtained from the electron density profiles shown in Figure 5, plotted as a function of chain length.

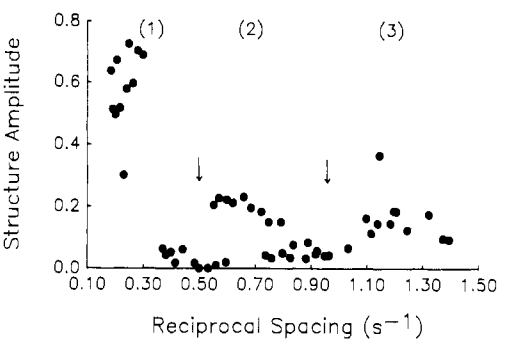

FIGURE 3: Structure amplitudes of the L_c phases of the α-D-glucosyl diacylglycerols (N = 10–20) plotted as a function of the reciprocal spacing. The structure amplitudes plotted are the absolute values of the diffraction amplitudes of the hth-order reflections normalized by the factor h. See text for details.

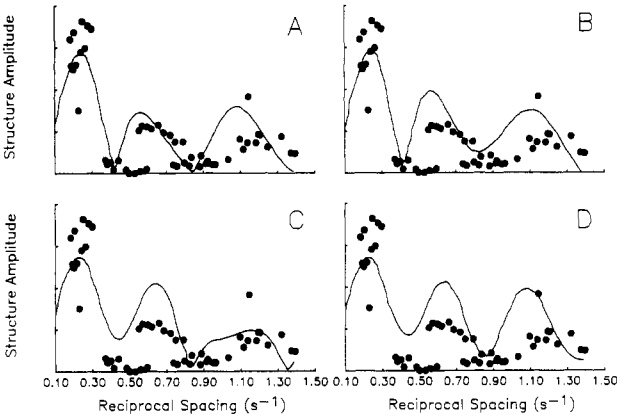

FIGURE 4: Plots of structure amplitudes of the α-D-glucosyl diacylglycerols against reciprocal spacing. The filled circles are the experimental data obtained for the entire homologous series of lipids, and the solid line is the structure amplitude calculated for di-19:0-α-GlcDG by using the sampling theory. The phase angle combinations shown are (A) π, 0, π, (B) π, 0, 0, (C) 0, 0, π, and (D) 0, 0, 0.

is metastable with respect to a highly ordered, crystal-like L_c phase. The L_β-type gel phase is readily formed upon cooling from high temperature, and with the exception of those formed by the short-chain homologues (N = 10–12), the L_β phase is sufficiently stable for study provided that it is not cooled to low temperatures. The d spacings of the L_β phases of the lipids studied ($N \geq 13$) increase linearly when plotted as a function of acyl chain length (Figure 2). Since an increase in one C–C bond length gives a projected length of 5 nm along the long axis of the fatty acyl chain, it is possible to calculate the tilt of the fatty acid to the bilayer normal if one assumes that there is no change in the thickness of the headgroup/water region. From the slope of the regression line (0.218) the calculated tilt of the fatty acyl chains is 31° to the bilayer normal. Thus, the metastable gel phase of these α-GlcDGs should actually be denoted as L_β' to indicate tilting of the hydrocarbon chains.

(*b*) *The Stable Crystal-like (L_c) Phase.* The stable crystal-like gel phase of these lipids can be readily formed by the temperature annealing protocol reported in the DSC study [see Mannock et al., (1990a)]. The X-ray measurements on the L_c phases of these lipids were all conducted at 20 °C, with the exception of the two shorter chain compounds studied (N = 10, 11). In the two latter cases, their L_c phases became unstable at room temperature and the experimental measurements were done at 0 °C. As was observed with the L_β phases, the first-order reflections of the L_c phases increase linearly as a function of acyl chain length (Figure 2) with no evidence for odd/even discontinuities. The measurements in the wide-angle region throughout the entire series show almost no chain-length dependence, indicating that the structure of the L_c phase is invariant with chain length. Using the assumptions described above for the L_β' phase, we estimate from the slope of the regression line (0.207) that in the L_c phase of these lipids the acyl chains are tilted by some 35° with respect to the bilayer normal.

In these studies we have also attempted to obtain additional structural information on the L_c phase of these lipid bilayers by the construction of electron density profiles. The electron density profiles of phospholipid bilayers have generally been calculated from measured intensities of the reflections in diffraction patterns and by using phase combinations obtained through swelling measurements (Moody, 1963; Worthington et al., 1973; McIntosh et al., 1984). Such an approach is not very useful with glycolipids and the crystalline phases of PEs, since they do not swell appreciably owing to the small amounts of water bound (Sen et al., 1983; Sen & Hui, 1988). However, given the availability of a homologous series of these lipids and the fact that the X-ray data suggest that this series of compounds is isostructural for the L_c phase, since the low-angle reflections increase linearly with chain length while the wide-angle reflections remain almost constant, we thought it feasible to obtain the phase combinations needed for the construction of the electron density profile by considering these measurements as "swelling experiments" of the hydrocarbon domain instead of the aqueous phase. Given that the absolute electron density is not measured in these types of measurements, the variation of the hydrocarbon domain with a constant interheadgroup distance can be viewed as being equivalent to a "swelling" series, with a shift in the origin of π.

Illustrated in Figure 3 are the structure amplitudes (absolute values of the diffraction amplitudes of the hth-order reflections normalized by the factor h [see McIntosh (1978) and Hui and Huang (1986)]) plotted as function of their reciprocal spacing for the lipids with acyl chains ranging from 10 to 20 carbon atoms. This plot simplifies the assignment of the phase, since in the range $0 < s < 0.13$ (where $s = 2 \sin [\theta/l]$ nm^{-1}) there are only two possible points (indicated by the arrows in Figure 4) where the continuous Fourier transform could go to zero and a phase change could occur. Thus, for a centrosymmetric structure, there are three regions in the structure amplitude plot (labeled 1, 2, and 3 in Figure 3) that can have a different phase angle and 2^3 phase angle combinations are possible. These eight possible phase angle combinations give rise to four unique possible solutions and their mirror images. For each of these lipids, the continuous transform for each possible phase angle combination was calculated by using the structure factors

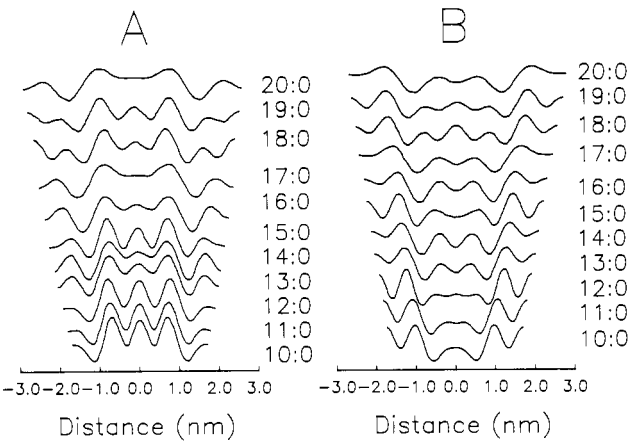

FIGURE 5: One-dimensional electron density profiles of the L_c phase of the α-D-glucosyl diacylglycerols. The profiles are plotted with the origin at the center of the water/headgroup region (Panel A) and with the origin at the center of the hydrocarbon region (Panel B).

derived from each phase combination and typical results (for the di-19:0 species) are shown in Figure 4. The best match to the experimental data was obtained with phase angle combinations π, 0, π and its mirror image 0, π, 0 (Figure 4A). Of these two possibilities, the combination 0, π, 0 can be eliminated since the electron density of the hydrocarbon chains at the center of the bilayer must be lower than that of the polar headgroups at the bilayer surface. Thus, the phase angle combination π, 0, π was used to construct electron density profiles of the L_c phases of these lipids (resolution 0.75–1.1 nm). Plots of the electron density profiles are shown in Figure 5. In centrosymmetric systems such as lipid bilayers, it is possible to shift the origin by half of the unit cell length and such a shift in the origin results in a change of sign for all reflections of odd h but no change in the absolute value of the structure amplitude (Stout & Jensen, 1968). The electron density profiles are thus shown in parts A and B of Figure 5 with the origin at the center of the water layer and at the center of the hydrocarbon layer, respectively. The results show that the thickness of the hydrocarbon region (the region between the two major maxima in Figure 5B) increases continually with increasing hydrocarbon chain length, whereas the thickness of the water/polar headgroup region (the region between the two major maxima in Figure 5A) remains relatively constant. However, we observed that there is a small increase in the thickness of the water/polar headgroup region as the length of the hydrocarbon chain increases. It is possible that these small changes may reflect a chain-length-dependent moderation of the polar interactions between the sugar headgroups. We also found relatively high electron densities in the water/polar headgroup region and this is consistent with the low hydration levels found in the stable gel phases of other glycolipids (Sen et al., 1983; Sen & Hui, 1988). With these lipids, however, the absence of a heavy atom such as phosphorus results in lower electron densities in the headgroup/water region than is usually seen with phospholipids. The bilayer thickness as measured from the electron density profiles describe a linear function of chain length (Figure 2) with a slope of 0.2. The slope of the line suggests a chain tilt of 35 °C, which is in excellent agreement with the chain tilt estimated from the slope of the lamellar repeat spacing versus chain length plot also shown in Figure 2.

Discussion

This X-ray diffraction study has provided the structural basis of the polymorphism evident from the DSC studies of these α-GlcDGs (see Mannock et al. (1990a)]. It is clear that the 1,2-diacyl-3-*O*-α-D-Glc-*sn*-glycerols studied here can all form a metastable lamellar gel ($L_{\beta'}$) phase, which may convert to either a lamellar liquid crystalline (L_α) phase ($N \leq 18$) or a nonlamellar inverted hexagonal (H_{II}) phase ($N \geq 19$) upon heating. In samples where an L_α phase is formed, further heating in some cases results in a transition to a nonlamellar phase whose structure is chain-length dependent, in agreement with earlier observations of a similar series of β-GlcDGs (Mannock et al., 1988). With the shorter chain compounds ($N = 15, 16$), a cubic phase (*Pn3m* or *Pn3*) is formed, whereas the longer chain compounds form an H_{II} phase. The type of cubic phase observed here is similar to that formed by repeated thermal cycling of dioleoyl PE through its L_α/H_{II} transition (Shyamsunder et al., 1988) and by the β-GlcDGs.[3] This phase has also recently been identified in studies of some short-chain β-D-glucosyl dialkylglycerols, where it appears to be a stable intermediate en route to the formation of an inverted hexagonal phase (M. Akiyama et al., unpublished data). Thus, it is possible that the inverted cubic phases formed by the short-chain glucolipids may themselves convert to inverted hexagonal phases at higher temperatures. The chain-length dependence of the type of nonlamellar phase formed by these glucolipids is similar to that exhibited by their β-anomers (Mannock et al., 1988). At this stage it is unclear whether the same is also true of the PEs, since a similar characterization of the effect of acyl chain length on the nature of the inverted nonlamellar phases formed by the PEs has not yet been reported. However, the fact that the shorter chain glucolipids form cubic phases, whereas the longer chain homologues form H_{II} phases, coupled with the evidence that the cubic phase is probably a stable intermediate en route to the formation of an H_{II} phase (M. Akiyama et al., unpublished data), is compatible with the postulates of Israelachvilli et al. (1977, 1980), which predict that the formation of H_{II} phases would be favored by an increase in hydrocarbon chain length.

All of the compounds studied also exhibit some form of gel-phase polymorphism, which typically involves the conversion of the metastable gel phase to a poorly hydrated crystalline phase upon annealing at low temperatures. With the exception of the di-19:0 compound, the crystalline phases are stable to temperatures above those at which their L_β phases melt and are transformed to either an L_α phase ($N \leq 17$) or an H_{II} phase ($N = 18, 20$) on heating, producing defined L_c/L_α or L_c/H_{II} phase transitions, respectively. In the case of the di-19:0 compound, the L_c phase formed becomes unstable at temperatures below that of the onset of the acyl chain melting event and as a result it is the only member of the series for which discrete L_c/L_β and L_β/H_{II} transitions are observed. Here the data also suggest that these lipids form L_c phases with strongly tilted hydrocarbon chains. The tilting of the hydrocarbon chains probably forms the physical basis for the odd/even discontinuities reported in the accompanying calorimetric study [see the preceding paper by Mannock et al. (1990a)]. In comparing the X-ray data on these α-D-glucosyl diacylglycerols with that available for both the β-GlcDGs (Mannock et al., 1988) and the β-GalDGs (Sen et al., 1983; Quinn & Lis, 1987; Lis & Quinn, 1986), we find that the lamellar repeat spacings of the L_c phases of the α-anomers are some 0.6–0.7 nm smaller than that of β-anomers of similar chain length. In principle, this could be the net result of differences in hydrocarbon chain tilt, headgroup orientation,

[3] In the studies on the β-anomers (Mannock et al., 1988), the cubic *Pn3m* phase was called a two-dimensional monoclinic phase.

headgroup and interlamellar hydration, and possibly the degree or extent of interdigitation of the hydrcarbon chains. Of these possibilities, differences in hydrocarbon chain tilt may be the least significant, since our estimations based on the chain-length dependence of the *d* spacings of the β-GlcDGs suggest that the hydrocarbon chain tilt in the L_c phase of the β-anomers should be comparable to that of the L_c phase of the α-linked glucolipids. In addition, if we assume that with the diacyl glycolipids the orientation of the sugar headgroups is similar to that envisaged for the dialkyl compounds by Jarrell et al. (1987), then using molecular models, one can easily show that the "headgroup orientation effect" would be too small to account for the magnitude of the differences in the *d* spacings. Thus, we suggest that the observed difference between the measured *d* spacings of the two anomeric glucolipids may originate from greater headgroup and interlamellar hydration of the β-anomers and/or some interdigitation of the hydrocarbon chains of the α-linked glucolipids. The suggestion that the β-linked glucolipids could be more hydrated than their α-anomers seems consistent with the arguments presented in the accompanying DSC paper (Mannock et al., 1990a, preceding paper). Moreover, the bilayer thicknesses determined from the electron density profiles are small enough to suggest that there may even be some interdigitation of the acyl chains in the L_c phases of the α-anomers, in agreement with our own infrared spectroscopic studies of these compounds (unpublished observations from this laboratory). Whereas the hydrocarbon chain tilt may be significantly different in the L_c phases of the α- and β-GlcDGs as well as the β-GalDGs, a comparison of the wide-angle X-ray data for the L_c phase of the α-GlcDGs and L_{c2} phase of the β-GalDGs reveals a number of similarities (this work; Lis & Quinn, 1986; Quinn & Lis, 1987). Although the reflections between 0.40 and 0.43 nm can confidently be assigned to hydrocarbon chain packing components, it is unlikely that the remaining reflections from 0.5 to 0.85 nm seen in the L_c phases of the lipids studied here (see Figure 1B and Table I) can be definitively identified. Lis and Quinn (1986) have suggested that these reflections originate from the sugar headgroup by virtue of their absence from diffraction patterns obtained from L_c phases in some phospholipid species and by comparison with the crystallographic studies of both galactosylceramide (Pasher & Sundell, 1977) and *n*-alkyl glycosides (Moews & Knox, 1976; Dorset & Rosenbusch, 1981). This is possible. However, the packing of these molecules is extremely complex, and in the absence of a definitive crystallographic study of the glycoglycerolipids, this assignment must remain tentative.

This work, the accompanying DSC study (Mannock et al., 1990a, preceding paper), and previously published studies on the β-D-glucosyl diacylglycerols (Mannock et al., 1988) have clearly demonstrated that the physical properties of glycolipid bilayers are very sensitive to the configuration at the anomeric center. The fact that a small change in configuration can dramatically alter the properties of the lipid bilayers formed is very significant and underscores the fact that where glycolipids are concerned there is a considerable potential to vary the properties of a bilayer by the implementation of relatively small changes in the structure of the polar headgroup. Unlike the phospholipids, however, structural changes in glycolipids such as these probably exert their effects exclusively by small changes in the hydration of the lipid bilayer and by subtle changes in the interaction between the polar headgroups and water. The effects of such changes on the properties of lipid bilayers in general and natural membranes in particular are yet to be rigorously addressed, despite the fact that such properties are obviously exploited by a large number of living organisms.

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