Articles

Physical Properties of Glycosyl Diacylglycerols. 1. Calorimetric Studies of a Homologous Series of 1,2-Di-*O*-acyl-3-*O*-(α-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*-(α-D-glucopyranosyl)-*sn*-glycerols was studied by differential scanning calorimetry. At fast heating rates unannealed samples of these lipids exhibit a strongly energetic transition, which has been identified as a lamellar gel/liquid crystalline (L_β/L_α) phase transition (short- and medium-chain compounds) or a lamellar gel to inverted hexagonal (L_β/H_{II}) phase transition (long-chain compounds) by X-ray diffraction studies (Sen et al., 1990). At still higher temperatures, some of the lipids that form lamellar liquid-crystalline phases exhibit an additional transition, which has been identified as a transition to an inverted nonbilayer phase by X-ray diffraction studies. The lamellar gel phase formed on initial cooling of these lipids is a metastable structure, which, when annealed under appropriate conditions, transforms to a more stable lamellar gel phase, which has been identified as a poorly hydrated crystal-like phase with tilted acyl chains by X-ray diffraction measurements (Sen et al., 1990). With the exception of the di-19:0 homologue, the crystalline phases of these lipids are stable to temperatures higher than those at which their L_β phases melt and, as a result, they convert directly to L_α or H_{II} phases on heating. Our results indicate that the length of the acyl chain affects both the kinetic and thermodynamic properties of the crystalline phases of these lipids as well as the type of nonbilayer phase that they form. Moreover, when compared with the β-anomers, these α-D-glucosyl diacylglycerols are more prone to form ordered crystalline gel phases at low temperatures and are somewhat less prone to form nonbilayer phases at elevated temperatures. Thus the physical properties of glucolipids (and possibly all glycolipids) are very sensitive to the nature of the anomeric linkage between the sugar headgroup and the glycerol backbone of the lipid molecule. We suggest that this is, in part, due to a change in orientation of the glucopyranosyl ring relative to the bilayer surface, which in turn affects the way(s) in which the sugar headgroups interact with each other and with water.

Although glycolipids constitute a sizable fraction of the structural matrix of many biological membranes (Quinn & Williams, 1978, 1983; Williams & Quinn, 1987; Ishizuka & Yamakawa, 1985; Sprague, 1987), the major thrust of glycolipid research to date has been directed toward the biological and medical aspects rather than toward the physical properties of this important class of lipids (Makita & Taniguchi, 1985; Wiegandt, 1985; Hemming, 1985; Curatolo, 1987a,b). This is primarily due to the difficulties in the chemical synthesis of glycolipids of high anomeric and chiral purity [see Gigg (1980)] and thus the unavailability of the comparatively large quantities of material that are required for thorough physical studies. However, recent improvements in the syntheses of both glycosphingolipids (Gigg, 1980; Kiso et al., 1986, 1987; Bruzik, 1988; Bruzik & Tsai, 1987; Schmidt & Zimmermann, 1986; Schmidt et al., 1987; Schmidt & Maier, 1988; Ito et al., 1986; Sato et al., 1986; Hino et al., 1986; Numata et al., 1987; Sugimoto et al., 1986; Mulzer & Brand, 1986; Herold, 1988) and glycosyl diacylglycerols [see Mannock et al. (1987, 1990) and Sugawara et al. (1986a,b) and references cited therein] have made practical the routine syntheses of many of these compounds, with the result that the long overdue systematic studies on the physical properties of such compounds are now feasible. We have recently undertaken a major study of synthetic glycosyl diglycerides, including a systematic characterization of the effects of variations in the anomeric and isomeric configuration of the sugar headgroups, the linkage of the hydrocarbon chains to the glycerol moiety, and the structure of hydrocarbon chains, on the physical properties of such lipids. The first paper in this series (Mannock et al., 1988) concentrated on a homologous series of saturated, straight-chain β-D-glucosyl diacylglycerols, natural counterparts of which are known to occur in the cell membranes of *Staphylococcus* sp (Macfarlane, 1962; Quinn & Sherman, 1971), *Pseudomonas* sp (Wickberg, 1958), and *Mycoplasma neurolyticum* (Smith, 1972). The present investigation concentrates on a similar series of α-D-glucosyl diacylglycerols (anomeric isomers of the β-D-glucolipids) with acyl chains ranging from 10 to 20 carbon atoms, which have been recently synthesized in this laboratory (Mannock et al., 1990). The α-D-glucosyl diacylglycerols (α-GlcDGs)[1] are also known to occur naturally and have been found in the cell membranes of many groups of procaryotic microorganisms, for example, *Pneumococcus* sp (Kaufman et al., 1965; Brundish et al.,

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[1] Abbreviations: DSC, differential scanning calorimetry; NMR, nuclear magnetic resonance; PC, phosphatidylcholine; PE, phosphatidylethanolamine; NBL, nonbilayer; FTIR, Fourier-transform infrared; α-GlcDG, 1,2-di-*O*-acyl-3-*O*-(α-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.

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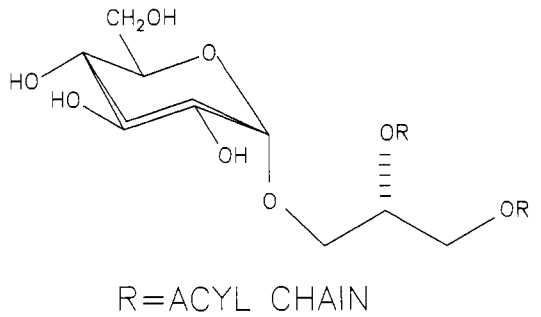

FIGURE 1: Chemical structure of 1,2-di-*O*-acyl-3-*O*-(α-D-glucopyranosyl)-*sn*-glycerols.

1967), *Diplococcus* sp (Ishizuka & Yamakawa, 1968), *Pseudomonas* sp (Wilkinson, 1969; Wilkinson & Galbraith, 1979), *Streptococcus* sp (Fischer, 1976, 1977; Pieringer, 1968), and *Acholeplasma* sp (Shaw et al., 1968; Slomiany et al., 1978). Evidently this class of glycolipids is widely occurring and is found in many microorganisms that are of considerable medical and microbiological interest.

There is relatively little known about the physical properties of the α-D-glucosyl diacylglycerols at present. To date, the bulk of the data published on this class of lipids have been on native lipids isolated from *Acholeplasma laidlawii* (Wieslander et al., 1978, 1981a,b; Silvius et al., 1980; Khan et al., 1981). Although useful, these preliminary studies have not been very detailed and for the most part have not been directed at an overall characterization of the thermotropic phase behavior of these lipids. Rather, many of these studies have been directed toward their ability to form nonbilayer structures, since the levels of these lipids in their natural membranes appear to be regulated in accordance with their nonbilayer-forming potential (Wieslander et al., 1978, 1981a,b; Khan et al., 1981). This paper describes a differential scanning calorimetric (DSC) investigation of the thermotropic phase behavior of aqueous dispersions of a homologous series of such lipids. X-ray diffraction studies were also performed to characterize the thermally induced structural changes observed and to identify the various phases formed. Those studies are reported in the following paper (Sen et al., 1990).

Materials and Methods

The details of the synthesis, purification, and chemical characterization of this series of glucolipids have been described elsewhere (Mannock et al., 1990). The general chemical structure of the glucolipids used 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*-(α-D-glucopyranosyl)-*sn*-glycerol, will be abbreviated to di-*N*:0 α-GlcDG, where *N* equals the number of carbon atoms per acyl chain. Differential scanning calorimetric measurements were performed with a Perkin-Elmer DSC-2C calorimeter equipped with a Perkin-Elmer 3700 thermal analysis data station. The DSC samples were prepared by essentially the same methodology as that used for the β-anomers (Mannock et al., 1988), except for the longer chain compounds ($N > 17$), for which the methodology was as follows. Samples of 3–4 mg of the dry lyophylized lipid were placed in a large, stainless-steel sample capsule, which was placed on a heated stage and allowed to warm up to temperatures higher than the softening point of the lipid (50–90 °C, depending on the length of the acyl chain) to facilitate the absorption of water by the sample. At this point 50 μL of distilled water was added, and the capsule was sealed and then repeatedly heated and cooled at 10 °C/min to ensure complete hydration. The sample capsule containing the hydrated lipid was then rapidly centrifuged in a microcentrifuge to ensure good contact between the lipid and the bottom of the capsule, whereupon it was checked by reheating in the calorimeter. DSC heating and cooling thermograms were acquired between −3 and 97 °C and the data were processed by using TADS software (Perkin-Elmer) and other computer programs developed in this laboratory. After the DSC measurements, the capsules were opened and their contents quantified as previously described (Mannock et al., 1988).

Results

Thermotropic Phase Behavior. A preliminary study of the thermotropic phase behavior of these α-D-glucosyl diacylglycerols revealed a complex pattern of phase behavior. We found that these glucolipids exhibit gel-phase polymorphism and that at least one of their gel phases is metastable with respect to a more stable structure. Therefore the polymorphic phase behavior of these α-D-glucosyl diacylglycerols was characterized by a careful examination of the effects of scan rate and storage conditions prior to heating on their observed phase behavior. Such measurements were designed to characterize the thermodynamic aspects of the interconversions between the various phases (where feasible) and to examine some aspects of the kinetics of the interconversions. These dynamic measurements were also supported by static X-ray diffraction measurements to determine the nature of the structural changes occurring at the various phase transitions [see Sen et al. (1990)].

(*1*) *Effects of Scan Rate.* DSC heating thermograms of unannealed samples of the α-GlcDGs acquired at scan rates of 20, 10, 5, 1, and 0.31 °C/min are shown in Figure 2. It is clear that for most of these compounds the DSC pattern obtained is strongly dependent on the heating rate employed. In contrast, the patterns exhibited by the DSC thermograms obtained upon cooling are essentially independent of the scan rate (except for the resolution of the thermotropic events) and only those acquired at 1 °C/min are shown. With these compounds the observed pattern of phase behavior is also strongly dependent upon the lengths of their acyl chains and on whether they contain an odd or an even number of carbon atoms. For the purposes of this presentation, these lipids will be broadly classified according to their acyl chain lengths into short-chain (N = 10–13), medium-chain (N = 14–18), and long-chain (N = 19, 20) compounds.

The characteristic pattern of scan-rate-dependent phase behavior exhibited by the short-chain compounds is best illustrated by the series of heating thermograms of the di-13:0 α-GlcDG (see Figure 2). At fast heating rates (≥10 °C/min for the di-13:0 compound but considerably faster for the shorter homologues), unannealed samples of these compounds all exhibit a single fairly energetic transition, which we have assigned to the conversion from a lamellar (L_β-like) gel phase to a lamellar liquid crystalline (L_α) phase using X-ray diffraction techniques (Sen et al., 1990). However, at slower heating rates, a second thermotropic event is observed at a temperature higher than that of the L_β/L_α phase transition. With the appearance of this second peak, there is a diminution in the area of the first peak and this is usually accompanied by a pronounced exotherm between the two heating endotherms (see Figure 2). Moreover, the contribution of the first endothermic event to the total enthalpy change progressively decreases as the heating rate decreases. This type of behavior has also been observed with the β-linked glucosyl diacylglycerols (Mannock et al., 1988) and indicates that the L_β-like gel phase formed upon initial cooling of these compounds is metastable with respect to a more stable gel phase. At temperatures below some critical nucleation temperature, the

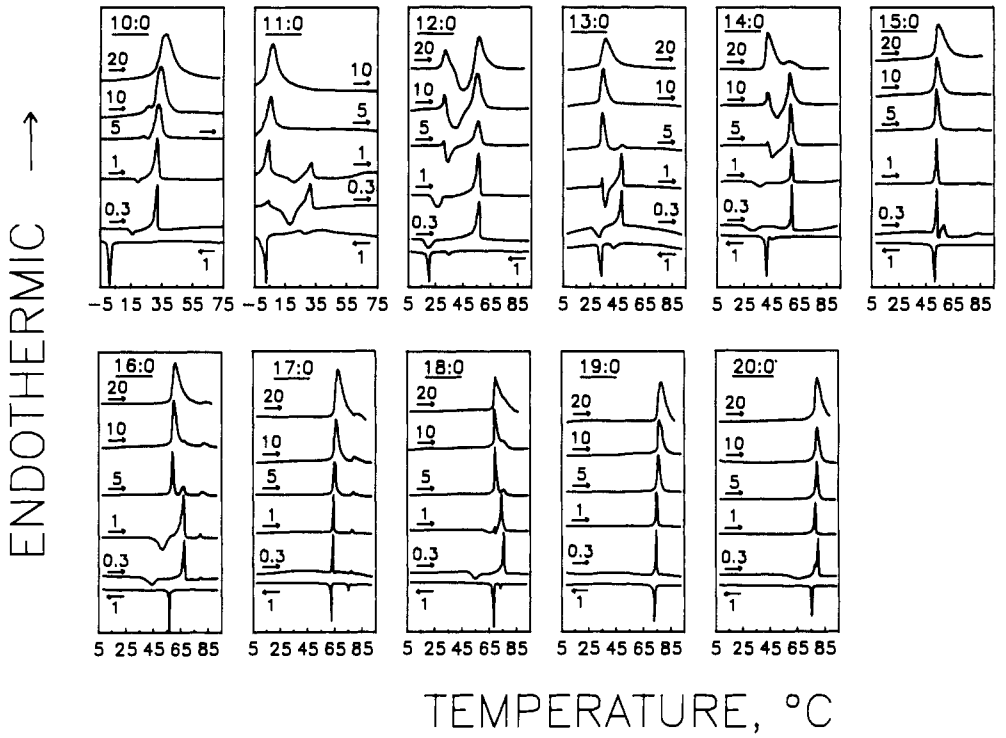

FIGURE 2: Heating and cooling thermograms of di-10:0- to di-20:0-α-D-glucopyranosyl-*sn*-glycerols heated and cooled as indicated on each thermogram. The arrows indicate the direction of the temperature change. Corresponding cooling thermograms at 10, 5, and 0.31 °C/min are not shown and differ from that at 1 °C/min only in terms of peak resolution and scan-rate effects.

metastable gel phase gradually transforms to the more stable gel phase, and the conversion of this stable gel phase to the L_α phase gives rise to the higher temperature peak in the DSC thermograms. Thus, the appearance of the exotherms in the thermograms acquired at slower heating rates represents a conversion of the metastable L_β-like gel phase to the more stable gel phase on the timescale of those measurements. The stable gel phase that is formed has been identified as a poorly hydrated crystalline phase with tilted acyl chains by X-ray diffraction studies [see Sen et al. (1990)]. Although the general pattern of scan-rate-dependent behavior observed with these short-chain glucolipids is as described above, the details vary markedly from one compound to the next. For example, only a single peak corresponding to the melting of the metastable L_β-like gel phase of the di-12:0 compound is observed at scan rates in excess of 40 °C/min (thermograms not shown), while in the case of the di-10:0 compound there is almost complete conversion to the L_c-like stable gel phase even on the time scale of the 20 °C/min DSC measurement. In addition, at all scan rates, the extent of conversion to the stable gel phase is consistently less with the odd-numbered homologues. The above observations are a reflection of chain-length-dependent differences in the rates of conversion from the metastable to the stable gel phases. The rates of formation of the L_c phases of the odd-numbered compounds are much slower than those of their neighboring even-numbered homologues, and in the series as a whole they both decrease as the length of the hydrocarbon chains increase. These and other aspects of the kinetics of formation of the stable gel phases of these lipids will be discussed later.

The pattern of phase behavior exhibited by unannealed samples of the medium-chain compounds (N = 14–18) is essentially similar to that exhibited by the short-chain homologues except that an additional, weakly energetic transition is also observed at elevated temperatures. This thermotropic event occurs at temperatures above that of the chain-melting phase transition temperatures (i.e., the L_c/L_α or L_β/L_α transition temperature) and has been identified as a transition from the lamellar liquid crystalline phase (L_α phase) to an inverted nonbilayer (NBL) phase by our X-ray diffraction measurements (Sen et al., 1990). The X-ray studies have also shown that the inverted nonbilayer phases formed by the di-15:0 and di-16:0 compounds are cubic phases, belonging to the *Pn*3*m* or *Pn*3 space groups, in marked contrast to those of the di-17:0 and di-18:0 species, which are true inverted hexagonal (H_{II}) phases. The other differences between the behavior of these medium-chain α-GlcDGs and that of their short-chain homologues are directly attributable to the slower rates at which their metastable gel phases convert to their respective stable gel phases. From this it follows that the overall pattern of endothermic transitions seen at fast heating rates with these medium-chain compounds results from the melting of the metastable L_β-like gel phase to form their lamellar liquid crystalline states (i.e., L_β/L_α transitions) and the subsequent conversion of their L_α phases to their respective inverted nonbilayer phases (i.e., L_α/NBL transitions). At the slower heating rates, the additional heating endotherms and exotherms that result from the conversion of the metastable L_β-like gel phase to the stable L_c gel phase become visible and the differences in the conversion rates between these gel phases in the odd- and even-chain compounds, as judged by the relative areas of the endothermic peaks, become more obvious. In the case of these medium-chain compounds (with the exception of the di-18:0 species), the additional endotherm arising from the melting of the L_c phase to the L_α phase (i.e., the L_c/L_α transition) occurs at temperatures between those of the L_β/L_α and the L_α/NBL transitions. With the di-18:0 species, however, the L_c/L_α and the L_α/NBL transitions are not resolvable as discrete thermotropic events and a single transition representing a net L_c/H_{II} conversion is observed at temperatures above that of the L_β/L_α transition. This indicates that for the di-18:0 species, both the L_β and L_α phases are metastable with respect to the L_c phase at all temperatures at which they are observed.

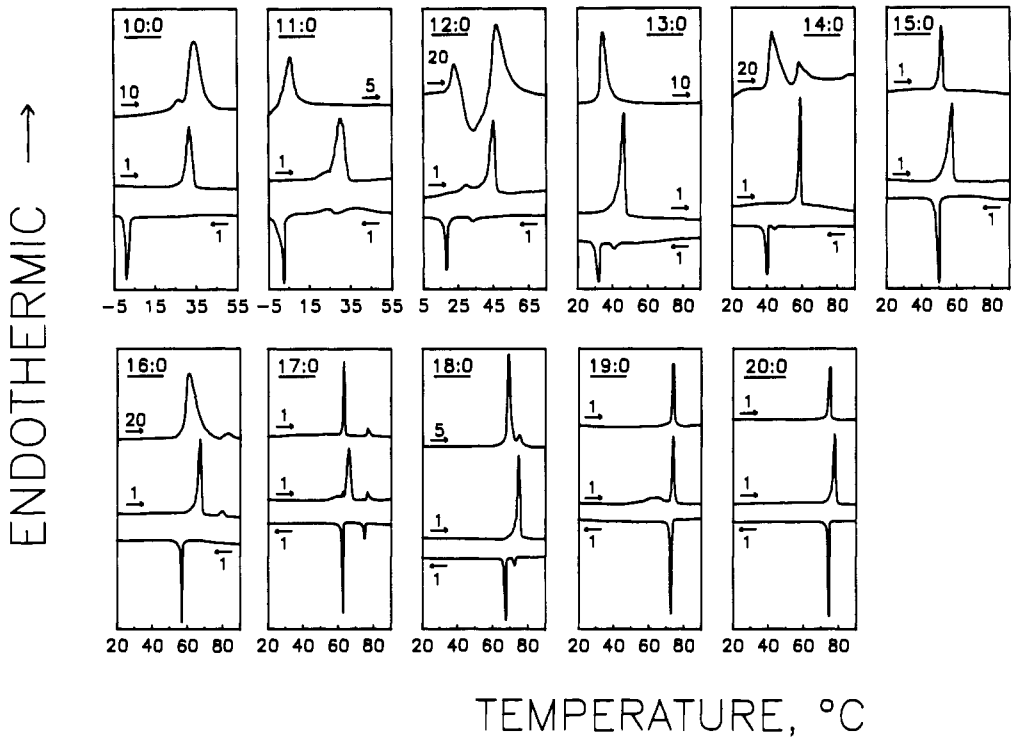

FIGURE 3: Heating thermograms of fully annealed samples of the 1,2-di-*O*-acyl-α-D-glucopyranosyl-*sn*-glycerols. The heating thermograms of the fully annealed samples are the middle curves in each panel. Heating and cooling thermograms of unannealed samples are included to facilitate comparison and the scan rates are indicated on each thermogram.

The thermotropic phase behavior of the long-chain compounds (N = 19, 20) shows virtually no heating rate dependence. In the case of the di-19:0 compound, unannealed samples show a single thermotropic transition at all scan rates investigated. This transition has been identified as an L_β/H_{II} transition by our X-ray diffraction studies (Sen et al., 1990). There is no evidence of any conversion to the stable gel phase on the time scale of these experiments. This is most likely the result of the extremely slow rate at which the L_β phase of the di-19:0 compounds converts to its stable L_c phase (see below). In the case of the di-20:0 compound, unannealed samples exhibit a single transition attributed to an L_β/H_{II} conversion at all but the slowest heating rates feasible with our instrument (0.31 °C/min). However, at the slowest heating rates studied, a partial conversion to the stable gel phase occurs on the time scale of the DSC experiment and there is a diminution in the area of the peak observed at fast scan rates. This is accompanied by the appearance of an exotherm in the heating thermogram and the appearance of a more energetic peak at higher temperatures. The second more energetic peak has been assigned to a direct conversion of the L_c phase to the H_{II} phase.

(*2*) *The Effects of Annealing.* All of the diacyl-α-D-Glc-*sn*-glycerols studied show evidence of gel-state polymorphism after being suitably annealed at low temperatures. This is manifest by marked changes in the calorimetrically observable thermotropic properties of the lipids (see Figure 3) and by marked changes in the X-ray diffraction patterns observed (Sen et al., 1990). The formation of the stable gel phases of these lipids is initiated by a relatively short period of incubation at low temperatures to nucleate the process (usually −3 °C under our conditions), and its completion could then be accelerated by a longer period of annealing at a higher temperature (room temperature, 22 °C under our conditions). However, with these lipids the conditions needed for the conversion of the metastable gel phase to the stable gel phase are considerably less exacting than is the case with comparable β-linked anomers (Mannock et al., 1988). We found that with these α-GlcDGs the critical temperature at which nucleation of the stable gel phase takes place is higher than is the case with the β-anomers, and once nucleated, the rate of formation of their stable gel phases is considerably faster than is the case with their β-linked counterparts. However, like the β-linked anomers, the rate of formation of the stable gel phases of these compounds decreases markedly as the length of the acyl chains increases, and the formation of the stable gel phases of the odd-numbered homologues is considerably slower than that of their neighboring even-numbered homologues (see Table I). This odd–even alternation with respect to the kinetics of formation of the stable gel phase is qualitatively similar to that observed with the β-linked anomers (Mannock et al., 1988), though the magnitude of the effect is considerably greater with those compounds.

Table I: Estimated Times for Complete Formation of the Stable Gel Phases of the 1,2-Di-*O*-acyl-3-*O*-(α-D-glucopyranosyl)-*sn*-glycerols

acyl chain length	mean completion time[a]
10	<10 s
11	≅30 min
12	<10 min
13	15 h
14	<40 min
15	10 days
16	<5 h
17	75 days
18	5 days
19	190[b] days
20	10 days

[a] Completion times were all estimated at 22 °C for samples that were preincubated at −3 °C for 5–10 min and reflect the minimum time required for at least 95% conversion to the stable gel phase. [b] This is probably the lower limit of the completion time. There is some uncertainty as to whether the stable gel phase observed is the most stable phase that can be formed.

The representative initial heating thermograms shown in Figure 3 were obtained after annealing appropriately for "complete" conversion to the stable gel phase. It is clear that

Table II: Thermodynamic Characterization of the Transitions Exhibited by the 1,2-Diacyl-3-*O*-(α-D-glucopyranosyl)-*sn*-glycerols

	phase transition temperature (°C)				phase transition enthalpy (kcal/mol)			
lipid *N*:0	L_β/L_α	L_c/L_β	L_c/L_α	L_α/NBL	L_β/L_α	L_c/L_β	L_c/L_α	L_α/NBL
10:0			31.1				21	
11:0	1.9		29.4		3.7		14.2	
12:0	19.5[a]		45.9		5.8[b]		18.5	
13:0	32.9		46.7		6.1		18.5	
14:0	40.5[a]		58.2	105.0[c]	7.4[b]		21.2	0.3[c]
15:0	50.7		56.7	82.0[c]	8.9		21.2	0.4[c]
16:0	57.2		66.7	79.1[c]	9.5		23.6	1.1[c]
17:0	63.4		66.8	76.6[d]	10.4		21.7	1.4[d]
18:0	68.4		73.9[f]	74.5[a,d]	12.2		29.0[f]	1.0[a,d]
19:0	73.7[e]	59.9			14.4[e]	6.6		
20:0	76.8[e]		78.9[f]		15.4[e]		31.8[f]	

[a] Data were acquired at fast heating rates. Values quoted were obtained by extrapolation to a heating rate of 1 °C/min. [b] Values obtained from cooling exotherms. [c] Inverted nonbilayer phases are cubic phases. [d] L_α/H_{II} transitions. [e] L_β/H_{II} transitions. [f] L_c/H_{II} transitions.

once the stable gel phase is formed, the observed behavior of all of these lipids (except the di-19:0 species) is essentially the same. With the exception of the di-19:0 species, heating of the stable gel phase of these lipids results in a transition to either the lamellar liquid crystalline state ($N \leq 17$) or the inverted hexagonal phase ($N = 18, 20$), at temperatures higher than that assigned to the melting of the L_β-like gel phase. In samples where such behavior is evident, the transition attributed to the melting of the metastable L_β phase is absent from the DSC thermograms of fully annealed samples and is replaced by a more energetic transition resulting from the melting of the L_c phase. In the case of the di-19:0 species, the stable gel phase formed upon annealing becomes unstable with respect to the L_β-like gel phase at temperatures below that of the L_β/L_α phase transition, with the result that discrete L_c/L_β and L_β/L_α phase transitions are observed (see Figure 3). Two of the short-chain lipids ($N = 10, 11$) also exhibit an additional broad, weakly energetic thermal event upon prolonged incubation (7–10 days). This is observed at temperatures below that of the main L_c/L_α phase transition (see Figure 3) and is apparently absent in all of the longer chain compounds except probably the di-17:0 species, for which a similar but broader component becomes visible when the conversion of the initial metastable L_β-like gel phase to the more stable L_c-like phase is almost complete (60 days annealing). The X-ray diffraction studies indicate that this "new" phase does not appear to be very different from the crystalline phase initially formed and it may be the result of small changes in the organization of that L_c phase.

(*3*) *Thermodynamic Data.* The transition temperatures and enthalpy changes associated with the thermotropic transitions of the α-GlcDGs are listed in Table II. The L_β/L_α transition temperatures and associated enthalpy changes are strongly chain-length dependent and, when plotted as a function of acyl chain length (Figure 4), both parameters show relatively smooth monotonic increases with acyl chain length without any discontinuities between the odd- and even-numbered homologues. This is generally what is expected of simple chain-melting phenomena in which the melted phase (in this case the L_α phase) is nucleated from a loose hexagonally packed structure (see Broadhurst, 1962). However, a close inspection of Figure 4b shows that the enthalpy values plotted for the di-19:0 and di-20:0 compounds are slightly higher than what may be extrapolated from the trends set by the shorter chain homologues. This is probably a result of the fact that the enthalpy change measured for the two long-chain compounds contains a small additional contribution (expected range 1.5–2 kcal/mol) for the conversion to the H_{II} phase. The chain-length dependence of the lamellar liquid crystalline to nonbilayer transition (i.e., the L_α/NBL transition) is not as

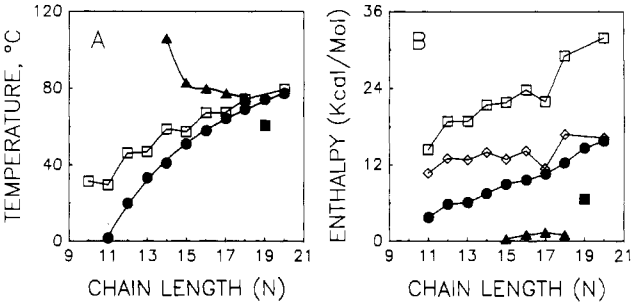

FIGURE 4: Chain-length dependence of the thermodynamic parameters of the thermotropic transitions of the 1,2-di-*O*-acyl-α-D-glucopyranosyl-*sn*-glycerols. (A) Chain-length dependence of the transition temperatures of the L_β gel/liquid crystalline phase transition (●), the L_c/liquid crystalline phase transition (□), the L_c/L_β gel phase transition (■), and the bilayer/nonbilayer phase transition (▲). (B) Chain-length dependence of the enthalpy of the L_β gel/liquid crystalline phase transition (●), the L_c/liquid crystalline phase transition (□), the L_c/L_β gel phase transition (estimated from difference measurements) (◇), the L_c/L_β gel phase transition (direct measurement) (■), and the bilayer/nonbilayer phase transition (▲).

straightforward, since the nature of the nonlamellar phase formed is itself chain-length dependent. Nevertheless, it is clear that the temperatures at which the L_α phases of these lipids become unstable with respect to an inverted nonlamellar phase decrease as the lengths of the acyl chains increase. Also illustrated in Figure 4 are plots of chain-length dependence of the transition temperatures and associated enthalpy changes of the interconversions involving the L_c phases of these glycolipids. In this case, there are distinct odd/even discontinuities in the chain-length dependence of the observed transition temperatures and associated enthalpy changes. Such odd–even alternation in the properties of a homologous series of paraffinic compounds is believed to be the result of the formation of a strongly tilted crystal-like phase (Broadhurst, 1962), and this has been confirmed by our X-ray diffraction studies (Sen et al., 1990). In addition, it is also clear that the transition parameters of the di-19:0 species are discontinuous from the trend set by the other lipids. The fact that the thermotropic transition involving the crystalline phase of the di-19:0 species, unlike that of the other lipids, does not contain a chain-melting component (i.e., it is a L_c/L_β transition) is probably the major reason for this "anomalous" behavior.

A close inspection of Figure 4b also suggests that the L_c/L_α transition enthalpy of the di-17:0 compound is lower than what would be predicted from the trends set by the shorter chain homologues. This discontinuous behavior of the di-17:0 species is made all the more apparent when the chain-length dependence of the estimated values of the L_c/L_β transition enthalpies[2] is examined (see Figure 4b). The data show that the

L_c/L_β transition enthalpies of both the di-17:0 and di-19:0 compounds are discontinuous from the general trends set by the other homologues. In the case of the di-17:0 compound there is no obvious reason for this, since its general thermotropic phase behavior is similar to that of all other homologues (except the di-19:0 compound). However, the fact that the kinetics of formation of its L_c phase is so much slower than that observed with all of the other homologues (except the di-19:0 compound) does leave open the possibility that the L_c phase of the di-17:0 species was not completely formed over the time course of these studies. In the case of the di-19:0 compound, however, the major behavioral discontinuity is apparent even with the L_c/L_β transition enthalpies for which there is no contribution from the chain-melting process, as is the case with the L_c/L_α transitions. Thus, like the di-17:0 species, the L_c phase of the di-19:0 compound may have been incompletely formed over the time course of this study.

Discussion

The data presented here and in the accompanying paper (Sen et al., 1990) clearly indicate that gel-phase polymorphism and the formation of inverted nonlamellar structures are intrinsic properties of these α-GlcDGs. In these respects they show a general similarity to the phosphatidylethanolamines (Chang & Epand, 1983; Mantsch et al., 1983; Seddon et al., 1983a,b, 1984; Wilkinson & Nagle, 1981, 1984; Brown et al., 1986; Silvius et al., 1986; Caffrey, 1985) and a number of other glycosyl glycerolipids (Rivas & Luzzatti, 1969; Shipley et al., 1973; Sen et al., 1981, 1983; Mannock et al., 1985, 1988; Jarrell et al., 1986, 1987a,b; Blöcher et al., 1985; Hinz et al., 1985; Lis & Quinn, 1986). However, despite the general similarities between these α-GlcDGs and a number of other nonbilayer-forming glycerolipids, there are also some important differences, which will be illustrated here by a comparison of these lipids with their β-linked counterparts and with the diacyl PEs. Listed in Table III is a compilation of thermodynamic data on the α- and β-D-glucosyl diacylglycerols and the diacyl PEs (this work; Mannock et al., 1988; Seddon et al., 1983b; Lewis et al., 1989). The data listed therein reveal several interesting points. First, it is clear from Table IIIA that, for any given acyl chain length, the lamellar to nonlamellar phase transition temperatures of the glucolipids are considerably lower than those of the PEs and that the α-linked glucolipids are less prone to form inverted nonlamellar phases than are the corresponding β-linked anomers. In principle, the fact that the α-linked glucolipids are less prone to form nonlamellar structures than are their corresponding β-linked anomers seems logical when viewed from the perspective of recent monolayer film and ^2H NMR studies. The monolayer film studies provide direct evidence that both the close-packed and expanded areas of the α-linked anomers are greater than those of their β-linked counterparts (Asgharian et al., 1989) and are thus compatible with the conformational evidence obtained form the ^2H NMR studies, which indicate that, in the L_α phase, the sugar headgroup of the β-anomer is extended away from the bilayer surface, in marked contrast to that of the α-anomer, which is aligned nearly parallel to the bilayer surface (Jarrell et al., 1986, 1987a,b). From these results it seems reasonable to suggest that the "dynamic cross-sectional areas" of polar headgroups of the α-linked glucolipids may be larger than those of their β-anomers, and, given this, the greater tendency of the β-anomers to form an inverted nonbilayer phase would be compatible with the geometric concepts postulated by Israelachvilli et al. (1977, 1980). However, such arguments will not explain why the glucolipids are considerably more prone to form nonbilayer structures than are PEs of comparable chain length, especially since the monolayer film measurements indicate that both the close-packed and expanded molecular areas of PE are smaller than those of either the α- or β-linked glucolipids (Asgharian et al., 1989). Thus relative molecular areas deduced from monolayer film experiments are not always good indicators of the bilayer/nonbilayer phase preferences of lipids, especially in cases where the chemical structures of the polar headgroups are markedly different. Clearly there are other factors that influence the phase preferences of lipids in general, and these need to be investigated further.

Second, a comparison of the thermodynamic parameters for the L_β/L_α phase transitions of the PEs and both anomers of the glucosyl diacylglycerols (Table IIIB) shows that, whereas the temperatures of the L_β/L_α phase transitions of the glucolipids are lower than those of comparable PEs, the enthalpy changes are consistently larger. On closer inspection, it is also apparent that whereas the temperatures of the L_β/L_α phase transitions of the α-linked compounds are lower than those of the corresponding β-anomers, the enthalpy changes are considerably higher. These observations can be explained if their are significant differences in the entropy changes that occur at their respective L_β/L_α phase transitions. Indeed, a simple calculation shows that for these three classes of lipids,

Table III: Thermodynamic Properties of the Glucosyl Diacylglycerols and the Phosphatidylethanolamines

acyl chain length	diacyl-α-D-Glc		diacyl-β-D-Glc		diacyl-PEs	
	T_m[a] (°C)	ΔH_c[b]	T_m[a] (°C)	ΔH_c[b]	T_m[a] (°C)	ΔH_c[b]
(A) The Bilayer/Inverted Nonbilayer Phase Transition						
12	c		57.8[d]		c	
13	c		59.0[d]		c	
14	105.0[d]		72.0[d]		c	
15	82.0[d]		73.4[d]		c	
16	79.1[d]		75.0[e]		118.0[e]	
17	76.6[e]		73.0[e]		107.6[e]	
18	74.5[e]		73.8[e]		99.2[e]	
19	73.7[f]		76.5[f]		98.0[e]	
20	76.8[f]		79.9[f]		97.2[e]	
(B) The Metastable Gel Chain Melting Phase Transition						
12	19.5	5.8	26.0	4.9	30.5	3.7
13	32.9	6.1	35.7	5.8		
14	40.5	7.4	45.5	6.7	50.5	5.8
15	50.7	8.9	54.2	8.2		
16	57.2	9.5	61.0	9.0	64.4	7.9
17	63.4	10.4	67.0	10.2		
18	68.4	12.2	71.7	11.2	73.5	10.5
19	73.7[g]	14.4[g]	76.5[g]	13.3[g]		
20	76.8[g]	15.4[g]	79.7[g]	14.7[g]	82.0	12.5
(C) The Stable Gel Phase Transition						
12	45.9	18.5	38.6	14.3	44.5	13.3
13	46.7	18.5	45.8	16.3		
14	58.2	21.2	46.5	18.3	57.0	14.8
15	56.7	21.2	54.8	18.0		
16	66.1	23.6	56.2[h]	14.0[h]	64.9	18.5
17	66.8	21.7	63.1[h]	9.1[h]		
18	73.9[i]	29.0[i]	58.1[h]	8.8[h]		
19	59.9[h]	6.6	j	j		
20	78.9[i]	31.8[e]	62.5[h]	10.8[h]		

[a] T_m, phase transition temperature. [b] ΔH_c, transition enthalpy. [c] Values unknown and presumed to be $\gg$100 °C. [d] Inverted cubic phase formed. [e] Inverted hexagonal phase formed. [f] A lamellar gel to inverted hexagonal phase transition. [g] L_β/H_{II} transitions (all other values listed in this section are for L_β/L_α transitions). [h] L_c/L_β transitions. [i] L_c/H_{II} transitions. [j] No L_c phase has been observed (all values listed are for L_c/L_α transitions, unless otherwise noted).

[2] L_c/L_β transition enthalpies estimated by the difference between the L_c/L_a and L_β/L_α transition enthalpies.

entropy changes for the α-linked glucolipids are generally some 4 and 8 cal/deg greater than those of comparable β-linked glucolipids and PEs, respectively. One obvious difference between the PEs and the anomeric glucolipids is the chain-like flexibility of the PE headgroup, which markedly contrasts with the intrinsic rigidity of the glucopyranose ring. Thus, the PE headgroup is probably capable of many degrees of motional freedom in both the L_β and L_α phases, whereas the glucolipid headgroups are comparatively motionally restricted, particularly when the lipids are in the gel state. Hence, the relative change in headgroup mobility which would occur at the L_β/L_α phase transition may be significantly greater with the glucolipids than with the PEs. These ideas are generally supported by previous ^2H NMR studies (Browning, 1981; Jarrell et al., 1987a), which suggest that changes in the mobility of the PE headgroup at the L_β/L_α phase transition may be smaller than those of the glucolipid headgroups. In the case of the glucolipids, where the difference between the headgroups is exclusively in the nature of the anomeric linkage, it has been shown that a change in configuration at the anomeric center alters the orientation of the glucopyranose headgroup with respect to the bilayer surface (Jarrell et al., 1986, 1987a,b). The sugar headgroup of the β anomer is believed to be extended away from the bilayer surface, whereas that of the α-anomer is aligned almost parallel to the bilayer surface. These differences in conformation should result in more restrictions on the mobility of the glucose ring of the α-anomers in the L_β phase, with the result that the changes in overall order which occur at the L_β/L_α phase transition may be greater than that exhibited by the β-anomers. The above arguments may help to explain why the entropy changes calculated for the α-linked glucolipids at the L_β/L_α transition are so much higher than those of both the PEs and β-linked anomers, since the α-linked glucolipid bilayers probably undergo greater changes in the overall ordering of the constituent lipid molecules at the L_β/L_α phase transition.

Third, a comparison of the data (particularly the enthalpy changes) on the thermotropic transitions involving the crystal-like phases of these α-GlcDGs with that available for PEs and the β-linked anomers (see Table IIIC) suggests that, of these three classes of lipids, the α-linked glucolipids tend to form crystalline gel phases that are thermodynamically more stable than those of the β-linked anomers, which in turn form more stable gel phases than do the PEs. Moreover, an examination of the kinetic observations presented in Table I and comparable data available for the β-linked anomers (Mannock et al., 1988) and the PEs (Seddon et al., 1983a,b; Mantsch et al., 1983; Wilkinson & Nagle, 1981, 1984; unpublished observations from this laboratory) would also indicate that, under comparable conditions, the α-linked glucolipids form their stable gel phases at faster rates than do the β-linked anomers, which also form their L_c-like gel phases at considerably faster rates than do the PEs. This correspondence between the thermodynamic and kinetic predisposition to form the stable crystal-like phases by these α-linked glucolipids may not be coincidental, since it appears that both factors are intricately linked to the overall balance of forces between the headgroup–headgroup interactions and the headgroup–solvent interactions at the lipid bilayer surface [L_c-like structures tend to be stabilized by headgroup–headgroup interactions and destabilized by interactions between the headgroup and the solvent; see Cevc and Marsh (1985) and Cevc (1987)]. Given that this balance must be affected by changes in the structure of the lipid polar headgroup, the fact that the PEs are kinetically and thermodynamically less predisposed to form ordered L_c-like gel phases than the glucolipids can probably be attributed to the greater polarity of its phosphorylethanolamine headgroup. The greater polarity conferred by the charged groups present would be expected to tilt the balance of forces toward stronger interactions between the zwitterionic phosphorylethanolamine headgroup and water, with the result that the partial dehydration of the headgroup and interfacial regions of the lipid bilayer [an essential process in the formation of stable L_c-like structures; see Cameron and Mantsch (1982) and Cevc (1987) and references cited therein] would be made less favorable.

With the anomeric glucolipids there are no obvious reasons why the α-anomers should be so much more prone to form highly ordered crystalline phases than their β-linked counterparts. Evidently the physical properties of glucolipids are very sensitive to the configuration at the anomeric center and the current glycolipid literature carries few clues as to the molecular basis of this phenomenon. However, some insight may be obtained by an examination of the hydration and solution properties of free sugars, their methyl glycosides and glycoside detergents. It is generally accepted that, in aqueous solution, simple carbohydrate molecules interact with a tetrahedrally arranged framework of water molecules (the so-called hydration sphere), which is probably more highly ordered than the bulk water [see Walrafen (1966), Franks et al. (1972), and Harvey and Symons (1978) and references cited therein]. It has also been suggested that the optimal configuration for a hexopyranose, which would cause minimal distortion of the "hydration sphere" and at the same time be capable of forming the strongest hydrogen bonding interactions with the solvent, is one in which the hydroxyl groups are equatorally oriented around the ring, i.e., β-D-glucopyranose (Kobayama & Patterson, 1958; Kobayama et al., 1958; Tait et al., 1972). From this it follows that, for a given hexopyranose, the extent of intermolecular hydrogen bonding interactions with its neighboring molecules, as well as the balance between inter- and intramolecular hydrogen bonding, is determined by molecular geometry, i.e., the number of equatorial versus axial hydroxyl groups. Titration measurements of both reducing sugars [see Beenackers et al. (1985) and references cited therein] and methyl glycosides [see Rendleman (1973) and references cited therein] have shown that the strength of the hydrogen bonding interactions between the sugar hydroxyls and the hydration sphere is also determined by the configuration of the anomeric oxygen, which, by means of stereoelectronic effects, can alter the "acidity" of the sugar hydroxyls. This is the so-called anomeric effect [see Praly and Lemieux (1987) and references cited therein]. Thus, the interaction between the carbohydrate molecule and its "hydration sphere" is strongly dependent upon the orientation of the sugar hydroxyls and the configuration at the anomeric center [for a more thorough discussion, see Franks (1977, 1989), Suggett (1975), and Barone et al. (1981, 1984a,b)]. Given the above, one would expect that, with glycolipid bilayers, factors such as the orientation of the sugar hydroxyls, the configuration of the anomeric center, and consequently the orientiation of the glycolipid headgroup should be important determinants of polar headgroup hydration number as well as the strength of the headgroup–solvent interactions. These factors would be expected to affect the structure and stability of any given gel phase by their effects on the balance between headgroup–headgroup and headgroup–solvent interactions at the bilayer surface.

Another factor that merits consideration is the hydrophobic component of the molecular interactions involving sugar

molecules. From an examination of molecular models, it becomes apparent that in the $^1C_4$ conformation, the underside of the glucopyranose ring is fairly hydrophobic because all of the ring hydroxyls are equatorially oriented. Thus, the glucose molecule itself has amphipathic properties. Given this one would expect that the nature of any interactions involving glucopyranose molecules and indeed other sugar isomers would reflect a balance between hydrophobic and hydrophylic components. This is an intrinsic property of molecular interactions involving carbohydrates and has been invoked to explain aspects of the specificity of carbohydrate-mediated recognition events, both at the cell surface and in certain carbohydrate-binding proteins (Lemieux, 1989; Quiocho, 1989, and references cited therein). This aspect of the properties of carbohydrate molecules is particularly relevant to our considerations, since the amphipathic properties of the carbohydrate molecules are themselves affected by the anomeric configuration [see Praly and Lemieux (1987) and references cited therein]. Moreover, in glycolipid bilayers, the anomeric configuration also determines the orientation of the headgroup relative to the bilayer surface (Jarrell et al., 1987a) and as a consequence controls the orientation of the hydrophobic and hydrophylic surfaces of the headgroup relative to both the hydrocarbon chain region and the aqueous environment. We suggest that through its effect on these factors, the anomeric configuration could indirectly affect the balance between headgroup–headgroup and headgroup–solvent interactions at the bilayer surface and in this way control both the structure and the stability of the gel phases formed.

The effects of the anomeric configuration on molecular interactions involving the sugar headgroups are especially important where lipid molecules are concerned, since in such systems the presence of long hydrocarbon chains directly (or indirectly) linked to the anomeric center can also have an additional effect. A good example of this is provided by the α- and β-linked octyl glucosides, whose lyotropic polymorphism (these molecules are carbohydrate liquid crystals) has been well documented (Goodby, 1984; Jeffrey, 1986), as has their use as detergents for the isolation of membrane proteins (Rosevear et al., 1980). With these compounds their polymorphism, and to some extent the differences in their solution properties, can be explained in terms of the carbohydrate solution studies discussed above, whereas the effect of the long hydrocarbon chain at the anomeric center is reflected in the differences in their spontaneous association to form micelles when dissolved in water. Brown et al. (1970), on the basis of surface tension measurments of others (Shinoda et al., 1961) and their own measurements of solubility and apparent molal volumes, concluded that the β-linked octyl glucoside was the more water soluble and formed micelles less readily than the α-anomer (critical micellar concentration $\cong$ 10 mM for the α- versus $\cong$ 23 mM for the β-compound). They suggested that these differences in solubility were governed by the balance between the interchain interactions in the hydrophobic domain of the micelle and the hydration properties of the carbohydrate headgroup, which together determined the penetration of water into the interfacial region of the micelle. Moreover, using space-filling molecular models, Brown et al. (1970) showed that the extent of interfacial hydration of the micelle is correlated with the penetration of the 2-hydroxyl group into the polar–apolar interfacial region and that this penetration is greater with the β-anomer (i.e., there is a greater penetration of water into the polar–apolar interface of micelles composed of octyl β-D-glucopyranoside). The results of a similar study of glycoside detergents (Drummond et al., 1985) and some studies on the conformations of the α- and β-D-glucopyranose headgroups of liquid crystalline dialkyl glucolipid bilayers (Jarrell et al., 1987a) are also consistent with the above findings. The results of Jarrell et al. (1987a) are particularly interesting in this regard, since they suggest that such processes may play an important part in determining the physical properties of glycolipids as well as glycoside detergents.

From all of the above arguments, it is apparent that the differing physical properties of the individual anomers of both D-glucopyranose and the alkyl D-glucopyranosides can be explained in terms of a balance between solute–solute and solute–solvent interactions. This is an intrinsic property that is, in part, determined by the stereochemistry of the carbohydrate molecule itself and regulates factors such as hydrogen bonding, hydration numbers, and the stability of the "hydration sphere" around the sugar molecule. A logical extrapolation of the above arguments to glucolipid bilayers is that the balance between headgroup–headgroup and headgroup–solvent interactions is a major determinant of the kinetics and thermodynamics of the interconversions between the stable and metastable gel phases of these lipids. We suggest that with these α-GlcDGs, the balance between headgroup–headgroup and headgroup–solvent interactions is more conducive to the formation of stable "crystalline" gel phases, and that their polar headgroups may be interacting less strongly with water than is the case with the corresponding β-anomers. Such factors would almost certainly manifest themselves in the greater kinetic and thermodynamic predisposition of the α-linked anomers to form ordered L_c-like gel phases and in the types of L_c-like phases that are formed. The structures of the these and the other polymorphic phases of these α-D-glucosyl diacylglycerols are described in the accompanying X-ray study (Sen et al., 1990).

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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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