Differential Scanning Calorimetry and X-Ray Diffraction Studies of the Thermotropic Phase Behavior of the Diastereomeric Di-Tetradecyl-β-D-Galactosyl Glycerols and Their Mixture

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ABSTRACT We have investigated the thermotropic phase behavior of aqueous dispersions of the 1,2- and 2,3-di-*O*-tetradecyl-1(3)-*O*-(β-D-galactopyranosyl)-*sn*-glycerols and their diastereomeric mixture using differential scanning calorimetry and low-angle and wide-angle x-ray diffraction. Upon heating, unannealed aqueous dispersions of these compounds all exhibit a lower temperature, moderately energetic phase transition at ~52°C and a higher temperature, weakly energetic phase transition at ~63°C, both of which are reversible on cooling. X-ray diffraction measurements identify these events as the L_β (or $L_{\beta'}$)/L_α and L_α/H_{II} phase transitions, respectively. The structures of the L_β, L_α, and H_{II} phases of these lipids, as determined by x-ray diffraction measurements, are identical within the error bars for all of these lipids. On annealing below the L_β/L_α phase transition temperature, the L_β phase converts to an L_c phase at a rate which is strongly dependent on the chirality of the glycerol backbone (1,2-*sn*>1,2-*rac*>2,3-*sn*). The temperature of the phase transition from the L_c phase seen on reheating is also dependent on the glycerol chirality. In addition, the nature of the L_c phase changes on subsequent heating in the 1,2-*sn* and 1,2-*rac* lipids, but we have not been able to detect this L_{c1}/L_{c2} phase transition by calorimetry. However, wide-angle x-ray diffraction measurements indicate that these L_c phases differ mostly in their hydrocarbon chain packing modes. The L_{c2} phase does not appear to be present in the 2,3-*sn* compound, suggesting that its formation is not favored in this diastereomeric isomer. These observations are discussed in relation to the effect of glycerol chirality on the molecular packing of these glycolipids, particularly on hydrogen bonding and hydration in the interfacial region of the bilayer.

INTRODUCTION

Galactosyl diacylglycerols are found widely in nature as major lipid components of the chloroplast membranes of higher plants (Quinn and Williams, 1983) and of the cell membranes of prokaryotic blue-green algae and a number of other microorganisms (Ratledge and Wilkinson, 1988). There have been numerous physical studies of the native lipids (Sen et al., 1981; Mannock et al., 1985 and references cited therein) and of their hydrogenated derivatives (Sen et al., 1983; Mannock et al., 1985; Lis and Quinn, 1986; Quinn and Lis, 1987). More recently, we have studied the thermotropic properties of an homologous series of saturated, synthetic β-D-galactosyl diacylglycerols varying in their hydrocarbon chain length (Mannock and McElhaney, 1991). In order to supplement our work on the diacyl glycosyl glycerols (Mannock et al., 1987, 1988, 1990a,b; Mannock and McElhaney, 1991; Sen et al., 1990; Lewis et al., 1990), we have initiated a program of synthesis and characterization of the physical properties of some glycosyl dialkylglycerols (Mannock et al., 1992; Turner et al., 1992 and references cited therein). The absence of carbonyl groups in the interfacial regions of these compounds might be expected to confer on them physical properties more typical of the respective hexopyranoses. Here, we report DSC and x-ray diffraction measurements of the thermotropic phase properties of the diastereomeric 1,2(2,3)-di-*O*-tetradecyl-3(1)-*O*-(β-D-galactopyranosyl)-sn-glycerols (di-14:0-β-GalDAGs) and their equimolar mixture.

MATERIALS AND METHODS

The β-GalDAGs used in this study were synthesized according to literature methods (Ogawa and Beppu, 1982; van Boeckel et al., 1985; Glew et al., 1991; Mannock et al., 1987, 1992) using dialkylglycerols prepared from the 1,2-*sn* or racemic isopropylidene glycerols (Aldrich Chemical Co., Milwaukee, WI) or 2,3-isopropylidene-*sn*-glycerol (Pfanstiehl Laboratories, Waukegan, IL). The α- and β-anomers of the β-GalDAGs were separated as their peracetates by chromatography on a silica gel column (Davisil, 200–425 mesh) which was eluted with a gradient of hexane and ethyl acetate. The deacetylated lipids were purified on a silica gel column as above eluted with a gradient of chloroform and acetonitrile, and crystallized first from acetone, then from methanol. All analytical measurements were consistent with the defined structures. The uncorrected softening points (in parentheses) and melting points and the optical rotations (α_D^{20}) are as follows: 1,2-*sn*; mp (81–2°C) 128°C, α_D^{20} = −1.5 (c4, THF. Lit. α_D^{20} = −3.9, c0.665, THF (Ogawa and Beppu, 1982)); 1,2-*rac*; mp (77–8°C) 125°C, α_D^{20} = −1.85 (c4.9, THF); 2,3-*sn*; mp (73–5°C) 128°C, α_D^{20} = −2.2 (c4.9, THF). The purity of these compounds is at least 98% as estimated by elemental analysis and NMR spectroscopy techniques.[1] All solvents were reagent grade and were distilled before use.

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Abbreviations used: DSC, differential scanning calorimetry; PE, phosphatidylethanolamine; β-GalDAG, β-D-galactopyranosyl dialkylglycerol; β-GlcDAG, β-D-glucopyranosyl dialkylglycerol; β-GalDG, β-D-galactopyranosyl diacylglycerol; THF, tetrahydrofuran; ORD; optical rotatory dispersion.

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[1] The ORD measurements proved to be a considerable problem. At shorter chain lengths (n = 10 – 14 carbon atoms), it is more difficult to separate

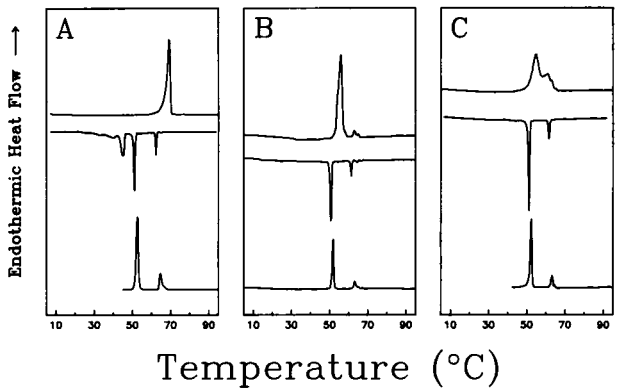

FIGURE 1 DSC thermograms of (*A*) 1,2-di-*O*-tetradecyl-3-*O*-(β-D-galactopyranosyl)-*sn*-glycerol, (*B*) 2,3-di-*O*-tetradecyl-1-*O*-(β-D-galactopyranosyl)-*sn*-glycerol, (*C*) 1,2-di-*O*-tetradecyl-3-*O*-(β-D-galactopyranosyl)-*rac*-glycerol. In each panel the upper trace originates from heating of the annealed sample, the middle trace is the subsequent cooling experiment, and the lower curve is that obtained on reheating the unannealed sample. All annealed samples were stored at 22°C for the following time periods: 1,2-*sn*, 2 h; 2,3-*sn*, 5 days; 1,2-*rac*, 24 h. All thermograms were obtained at heating and cooling rates of 1°C min^{-1} with the exception of the unannealed sample of the 1,2-*sn* lipid, which was obtained at a heating rate of 2°C min^{-1}. Heating thermograms of the unannealed samples of the 1,2-*sn* and 1,2-*rac* compounds had to be initiated from 46 and 42°C, respectively, in order to avoid nucleation of the L_c phase.

DSC measurements were performed with a Perkin Elmer DSC-2C calorimeter equipped with a thermal analysis data station. Lipid samples for DSC were prepared and quantified as reported earlier (Mannock et al., 1992). DSC curves were recorded between −3 and 97°C.

Samples for x-ray diffraction were prepared by transferring 3–5 mg of dry lipid into a thin-walled quartz capillary (1.5 mm). Deionized water (one to two times the lipid weight) was added, and the two components were mechanically mixed in the x-ray capillary with a 10-μl Drummond microdispenser. The capillary was then sealed using 5-min epoxy. The equipment and conditions employed for both wide-angle and low-angle x-ray diffraction measurements are as previously reported (Gruner et al., 1982; M. W. Tate et al. (manuscript in preparation); Shyamsunder et al., 1988).

RESULTS AND DISCUSSION

Mesophase transitions

The pattern of thermotropic phase behavior in the unannealed samples of all of the di-14:0-β-GalDAGs, which were heated from between 42 and 46°C to 97°C, typically consists of a moderately energetic, lower temperature, chain-melting phase transition with a higher temperature, weakly energetic, lamellar/nonlamellar phase transition (Fig. 1 and Table 1). Both of these phase transitions are reversible on cooling. Corresponding x-ray diffraction measurements confirm the lamellar nature of the lower temperature phase transition and show a characteristic decrease in the first order spacing on going from the gel (L_β or L_β') phase to the lamellar liquid crystalline (L_α) phase in all samples (See Figs. 2 and 3, *A* and *B*, and Table 1). This is supported by a change in the wide-angle diffraction pattern from a single sharp peak centered at 0.42 nm, which is characteristic of ordered hydrocarbon chains packed on a hexagonal lattice, to a broad band centered at 0.45 nm, which is characteristic of melted hydrocarbon chains. There is little change in the wide-angle region above 65°C. However, in the low angle region at this temperature, the diffracted orders are in the ratio $1:\sqrt{3}:\sqrt{4},\sqrt{7},\sqrt{9},\sqrt{12},\sqrt{13}$ which is characteristic of an inverted hexagonal (H_{II}) phase (See Fig. 3). Thus, the higher temperature, reversible event at 60–65°C is an L_α/H_{II} phase transition in both lipid diastereomers and their mixture (Fig. 2 and 3 *C*). Interestingly, the chirality of the glycerol backbone in these di-14:0-β-GalDAGs does not seem to greatly alter the temperature of the mesophase (L_β/L_α and L_α/H_{II}) transitions or the nature of the lamellar and nonlamellar phases formed. This behavior contrasts with that exhibited by the di-12:0-β-D-GlcDAGs, where the number of Q_{II} phases, as well as the temperature range over which they are stable, was found to depend on the chirality of the glycerol backbone (Mannock et al., 1992). The absence of stable Q_{II} phases in the lipids studied here[2] may be due to a critical difference in the balance of headgroup-hydrocarbon chain contributions to the stability of the fluid phases of the dialkyl β-GalDAGs with chain lengths of 12 and 14 carbon atoms.

Measurements of the 1,2-di-*O*-tetradecyl-3-*O*-(β-D-galactosyl)-*sn*-glycerol L_c phase transitions

On annealing at suitable temperatures and on subsequent reheating, all three β-GalDAGs exhibit a highly endothermic phase transition(s) at temperatures above those of the chain-melting event seen on initial heating. The time required for these stable states to form is dependent on both the annealing temperature and the chirality of the glycerol backbone. At 22°C the conversion processes appear to reach completion after the following times: 1,2-*sn*, <1 h; 1,2-*rac*, 24 h; 2,3-*sn*, 4–5 days (see Fig. 1[3]). The pattern of thermal events and their transition temperatures are different in each di-14:0-β-GalDAG stereoisomer (Figs. 1 and 2). In the 1,2-*sn* lipid

the α and β anomers and so a small proportion of the α anomer (di-14:0-α-D-GalDAG, α_D^{20} = 52.0, c0.74, $CHCl_3$; Ogawa and Beppu, 1982) may remain and can greatly alter the ORD measurement. Also, as a general rule, ORD values decrease with increasing chain length because of the poor solubility of the these lipids (See Mannock et al., 1990a). As a consequence these measurements are limited in their accuracy. ^1H NMR measurements of the peracetate synthesized from the purified lipids used here for DSC measurements indicate the presence of approximately 2% of the α anomer but that in other respects, for example chirality (see Yamagishi and Kakinuma, 1989; Uzawa et al., 1989), they are essentially pure. This problem of anomeric separation is very common in galactose-containing compounds where Koenigs-Knorr condensation procedures are employed.

[2] Shyamsunder et al. (1988) have shown that a cubic lattice emerges in several lipid systems upon repeated cycling across the L_α/H_{II} phase transition. Although this has not been investigated with the lipids studied here, a few faint peaks which did not index as L_α or H_{II} were sometimes observed in the vicinity of the L_α/H_{II} phase transition. It would not be surprising if a cubic phase were obtained upon cycling through the transition.

[3] Note: these samples were equilibrated at the above temperature for up to 3 years. After this time, small increases in transition enthalpy were observed. Those of the 1,2-*rac* compound were the most significant, but were consistently less than 5% (see below). Nevertheless, one can never be certain that a state of equilibrium has been achieved.

TABLE 1 (*A*) Phase transition temperatures (T_m, °C) and enthalpy (ΔH, Kcal/mol) values and (*B*) low-angle and wide-angle x-ray diffraction measurements (nanometers) for the stereoisomers of the di-tetradecyl-(β-D-galactopyranosyl) glycerols dispersed in excess water

		L_c/L_α		L_c/H_{II}		L_β/L_α		L_α/H_{II}	
A) Glycerol chirality		T_m	ΔH	T_m	ΔH	T_m	ΔH	T_m	ΔH
1,2-*sn*	H			69.45	21.16	51.75†	5.88	63.79	1.43
	C	42.0*	-9.6			51.06	-6.12	62.62	-1.56
[‡]	H			68.9	17.85	52.7	0.6		
2,3-*sn*	H	56.13	20.23			52.22	5.76	63.27	1.50
	C					51.0	-6.12	61.43	-1.63
1,2-*rac*	H	54.80	13.71			52.18	6.25	63.28	1.50
		60.44	4.80						
	C					51.13	-6.46	61.5	1.58
1,2-*sn*-DTβGlc[§]	H	51.5	13.2			51.6	5.95	56.4	1.27

B) Glycerol chirality	$L_c^{\parallel}$ *d* (nm)	$L_\beta^{\parallel}$ *d* (nm)	$L_\alpha^{\parallel}$ *d* (nm)	$H_{II}^{\parallel}$ *d* (nm)
1,2-*sn*	5.38	5.56	4.97	6.63
	0.45, 0.39	0.42	0.45	0.45
	0.35¶			
	5.36			
	0.46, 0.44			
	0.40, 0.38			
	0.36**			
2,3-*sn*	5.24	5.58	4.97	6.67
	0.45, 0.39	0.42	0.45	0.45
	0.35¶			
1,2-*rac*	5.34	5.58	4.98	6.66
	0.45, 0.39	0.42	0.45	0.45
	0.35¶			
	5.33			
	0.45, 0.44			
	0.40, 0.38			
	0.36‡‡			

* In the L_β/L_c conversion process, both the Tm and ΔH varied by ±2 units.

† Data were collected at a heating rate of 2°C/min and have been corrected accordingly.

‡ HSDSC (Hinz et al., 1985, 1991).

§ HSDSC (Kuttenreich et al., 1988).

$\parallel$ *d* = basis length for SAXS; $\lambda/2 \sin\theta$ for WAXS peaks where λ = 0.154 nm and 2θ = the total diffraction angle. The x-ray diffraction measurements given are close to the relevant phase transition, except for the following wide-angle measurements which were obtained at the following temperatures: ¶ L_{c1} @ 20°C, ** L_{c2} @ 65°C, ‡‡ L_{c2} @ 58°C.

Wide-angle x-ray diffraction measurements are ±0.01 nm. Low-angle x-ray diffraction measurements are ±0.05 nm.

only a single endotherm at 69.5°C is observed on heating (Fig. 1 *A* and Table 1). X-ray diffraction measurements of a similarly prepared sample confirm this behavior (Fig. 2 *A*) and identify this event as a transition from a highly ordered, lamellar L_c phase (Fig. 3 *D*) to an inverted hexagonal phase (H_{II}, Fig. 3 *C*). In the cooling experiment the L_β phase is rapidly converted to the L_c phase, a transition which is marked by an exothermic event at ~42°C on cooling (Fig. 1 *A*) with a corresponding decrease in the x-ray diffraction first-order spacing from 5.56 to 5.38 nm. This interpretation is supported by the differential scanning densitometry measurements of Hinz et al. (1991), which show two decreases in partial specific volume on cooling samples of the 1,2-*sn* diastereomer.

In the L_c phase region, the low-angle peak positions vary very little with temperature. In contrast, the wide-angle patterns change significantly with increasing temperature. Those obtained at 20°C consist of three peaks at approximately 0.45, 0.39, and 0.35 nm (see Fig. 3 *D* for details), whereas the diffraction pattern measured at 65°C contains five peaks at about 0.45, 0.44, 0.40, 0.38, and 0.36 nm. These observations suggest that there are two L_c phases in the annealed samples of 1,2-*sn*-β-GalDAG (hereafter termed the L_{c1} (low temperature) and L_{c2} (high temperature) phases, respectively), but that there is no thermal event detectable by DSC which marks the L_{c1}/L_{c2} phase transition, in agreement with earlier reports for the same lipid (Kuttenreich et al., 1988; Hinz et al., 1991). However, the similarity in the pattern of low-angle spacings suggests that the structures of the L_{c1} and L_{c2} phases are closely related but differ slightly in their hydrocarbon chain packing.

This pattern of behavior has also been observed in other studies of the same lipid (Kuttenreich et al., 1988) and in 1,2-di-*O*-stearoyl-3-*O*-(β-D-galactosyl)-*sn*-glycerol (Sen et al., 1981, 1983; Lis and Quinn, 1986; Quinn and Lis, 1987). In the earlier studies of 1,2-*sn*-di-tetradecyl-β-D-GalDAG reported by Hinz and co-workers (Kuttenreich et al., 1988; Hinz et al., 1991), both the L_{c1} and L_{c2} phases were correctly identified. However, the metastable gel phase chain melting transition, which our calorimetric and x-ray diffraction results clearly resolve into discrete L_β/L_α and L_α/H_{II} phase transitions (Fig. 1 and 2), was previously identified (Kut-

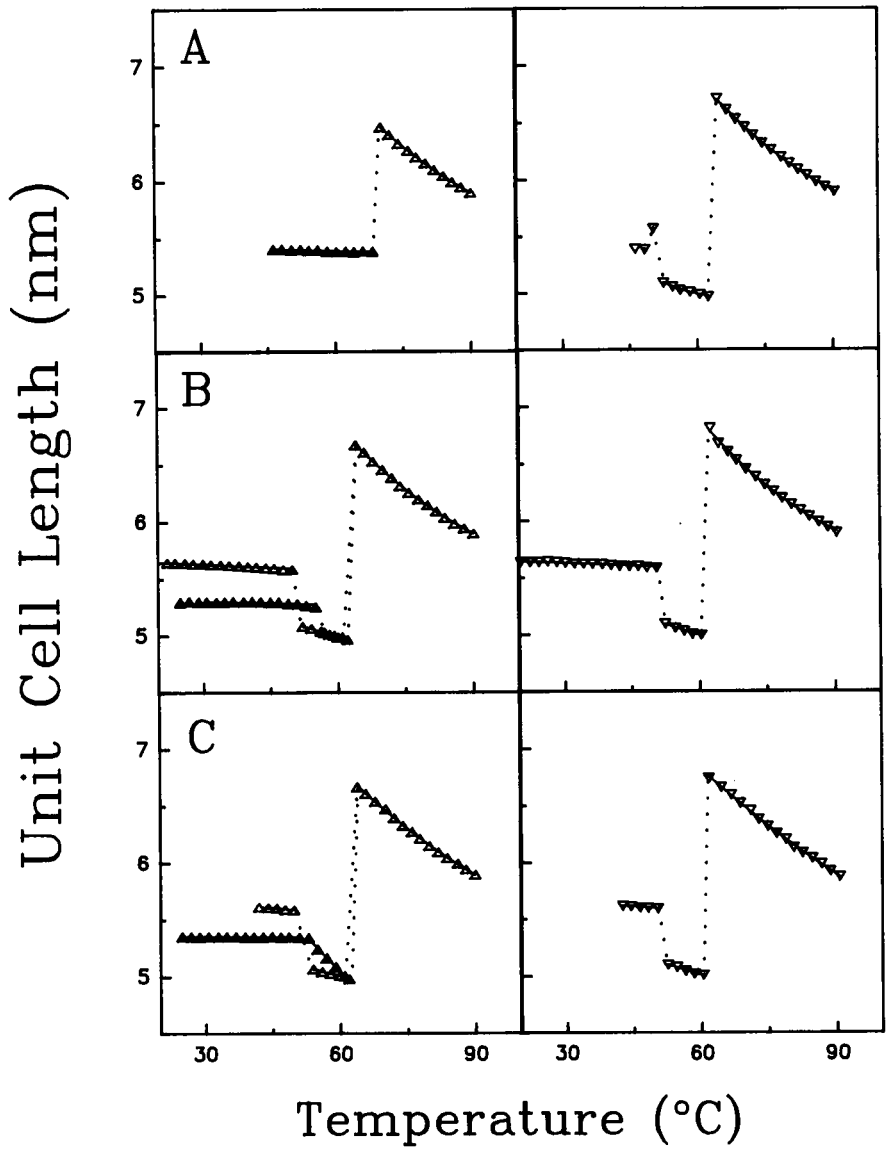

FIGURE 2 Plots of the unit cell length (*d*, in nanometers) as a function of temperature (°C) for (*A*) 1,2-di-*O*-tetradecyl-1-*O*-(β-D-galactopyranosyl)-*sn*-glycerol, (*B*) 2,3-di-*O*-tetradecyl-1-*O*-(β-D-galactopyranosyl)-*sn*-glycerol, (*C*) 1,2-di-*O*-tetradecyl-3-*O*-(β-D-galactopyranosyl)-*rac*-glycerol. The unit cell (or basis vector) length is the distance between adjacent bilayer midplanes in the lamellar phases and the distance between adjacent cylinder centers in the H_{II} phase. In each case the filled triangles represent heating measurements of the annealed samples from the L_c phase. The hollow triangles represent measurements of the unannealed samples. The orientation of the triangles, up or down, corresponds to heating and cooling measurements, respectively. The data points in the H_{II} phase range for the annealed samples (*filled triangles*) follow the unannealed points. The solid lines through the symbols are lines of regression. The dotted lines mark the phase transitions and are merely a guide to the eye.

tenreich et al., 1988; Hinz et al., 1991) as an L_β/H_{II} phase transition. This erroneous assignment arises from the inability of many high sensitivity calorimeters to properly detect transient metastable phenomena because of their slow heating rates. In addition, many of these instruments are, by design, incapable of performing cooling measurements, and such measurements are often required for the isolation and identification of the relevant metastable phases. Although the earlier studies of the 1,2-*sn* diastereomer (Kuttenreich et al., 1988; Hinz et al., 1991) also report x-ray diffraction measurements in both the heating and cooling direction, the temperature interval between data collection points is large, unlike the measurements shown in Fig. 2, where the interval is relatively small (~2°C). Thus, these previous studies resulted in an incomplete characterization of the thermotropic phase behavior of these β-D-GalDAGs (Kuttenreich et al., 1988; Hinz et al., 1991). Indeed, our observations of a homologous series of dialkyl-β-D-galactosyl glycerols (D. Mannock, unpublished observations) suggest that the chain length dependence of their transition temperatures is much like those of the corresponding β-D-GlcDAGs, differing only in the slopes of the L_α/Q_{II} and Q_{II}/H_{II} transition boundaries (Lewis et al., manuscript in preparation; cf. Hinz et al., 1991, Fig. 14).

Measurements of the 2,3-di-*O*-tetradecyl-1-*O*-(β-D-galactosyl)-*sn*-glycerol L_c phase transitions

In the corresponding 2,3-*sn* compound a large endotherm is seen at ~56°C and a smaller endotherm is seen at 63°C (Fig. 1 *B*), as was observed for the L_α/H_{II} phase transition in the unannealed sample. The x-ray measurements clearly show the highly energetic event at ~56°C to be a chain-melting transition from a highly ordered, lamellar L_{c1} phase (Fig. 3 *E*) to an L_α phase and that at 63°C to be an L_α/H_{II} phase transition. Closer examination of the wide-angle diffraction patterns in annealed samples of the 2,3-*sn*-β-GalDAG reveals the existence of minor components

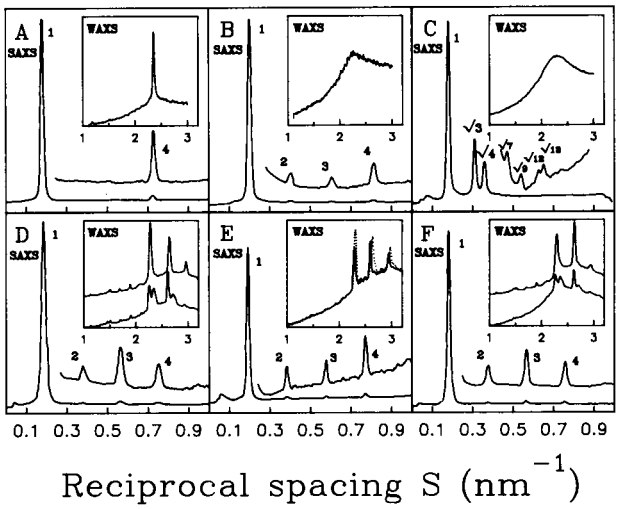

FIGURE 3 Representative low-angle (small-angle x-ray scattering) and wide-angle x-ray (WAXS) diffraction intensity profiles (intensity versus S (nm^{-1}) = $\lambda/2 \sin(\gamma/2)$, where λ is the wavelength of the x-rays (Cu Kα = 0.154 nm) and θ is the angle between the x-ray beam and the diffracted rays) of 1,2-di-*O*-tetradecyl-3-*O*-(β-D-galactopyranosyl)-*sn*-glycerol: (*A*) L_β phase; (*B*) L_α phase; (*C*) H_{II} phase; (*D*) L_c phase; and of 2,3-di-*O*-tetradecyl-1-*O*-(β-D-galactopyranosyl)-*sn*-glycerol in the L_c phase (*E*); and of 1,2-di-*O*-tetradecyl-3-*O*-(β-D-galactopyranosyl)-*rac*-glycerol in the L_c phase (*F*). The inserts in *D* and *F* contain the wide-angle data for the L_{c1} (*upper*) and L_{c2} (*lower*) phases, respectively. In *E* (WAXS) the dotted line is an L_{c1} phase at 20°C; the solid line is an L_{c1} phase at 52°C. The numbers above each peak indicate the position of the peak relative to the first order reflection. See Table 1 for numerical data.

as shoulders on the two major peaks (0.45 and 0.39 nm). These components can be resolved at higher temperatures and suggest that an L_{c2} phase may be formed, but we have been unable to obtain a significant increase in the proportion of this phase to allow a satisfactory identification.

Measurements of the 1,2-di-*O*-tetradecyl-3-*O*-(β-D-galactosyl)-rac-glycerol L_c phase transitions

DSC thermograms of the annealed samples of 1,2-*rac*-di-14:0-β-GalDAG show a complex pattern of peaks at approximately 55, 61, and 63°C (Fig. 1 *C*). Our x-ray data suggest that the highly energetic, lower temperature event corresponds to a transition from an L_{c1} phase (Fig. 3 *F*) to a lamellar, two-phase mixture. One phase has a d-spacing equal to the L_α phase and the other starts with a d-spacing equal to the L_c phase and then shrinks as the temperature is increased (Fig. 2 *C*). Note that the L_α and L_{c1} phases have very similar d-spacings and consequently the first order peaks overlap considerably, thus, it was necessary to use the higher order reflections in order to resolve the two phases. On annealing of the sample at 58°C, the conversion to the L_c phase is complete and the resulting diffraction pattern closely resembles that seen for the L_{c2} phase in the 1,2-*sn*-14:0-β-GalDAG at 65°C. Thus, the endotherm seen at ~61°C can be attributed to an L_{c2}/L_α phase transition and the minor event at 63°C, which is almost obscured by the more energetic components, corresponds to the L_α/H_{II} phase transition seen in unannealed samples.

Modulation of L_c phase structure: the nature of the hydrocarbon chain packing and its regulation by hydrogen-bonding interactions in the headgroup and interfacial regions

Taken together, the x-ray diffraction and the calorimetric data suggest the existence of two extremes of behavior in these glycolipids. In the 1,2-*sn*- and 1,2-*rac*-β-GalDAG there is no calorimetrically detected L_{c1}/L_{c2} phase transition. Moreover, the temperature window over which the L_{c2} phase is stable seems to decrease on going from the 1,2-*sn* to the 1,2-*rac* lipid. In the 2,3-*sn* compound, the L_{c1} phase dominates and the L_{c2} phase may be either totally or almost completely excluded (see our earlier comments and Fig. 3 *E*). This behavior resembles that seen in the corresponding 1,2(2,3)-di-dodecyl-3(1)-*O*-(β-D-glucopyranosyl)-*sn*-glycerol series in which only the 1,2-*sn* lipid formed an L_c phase (Mannock et al., 1992). From Fig. 3, *D–F*, it is clear that all of the L_{c1} phases observed here are similar in structure. Furthermore, the structures of the L_{c2} phases formed by all three compounds may also be similar. In the diffraction patterns of both phases, the small variations in peak intensity in the low-angle region are most likely indicative of differences in long range order rather than basic structure. However, it is not possible to say whether such variations reflect differences in the hydration, or in surface hydrogen-bonding networks of these phases.

The variations in both intensity and peak position in the wide-angle region clearly reflect small differences in hydrocarbon chain packing. Details of the lateral hydrocarbon chain packing modes of these phases can be calculated using Eqs. 1 and 2:

$$s_{hk}^2 = h\mathbf{a}^{*2} + k\mathbf{b}^{*2} - 2hk\mathbf{a}^*\mathbf{b}^* \cos\gamma, \quad (1)$$

where $\mathbf{a}^*$ and $\mathbf{b}^*$ are the magnitudes of the reciprocal lattice vectors and γ is the angle between them, and:

$$A = (\mathbf{a}^*\mathbf{b}^* \sin\gamma)^{-1}, \quad (2)$$

where A is the area of the unit cell in real space. These calculations provide the dimensions of the hydrocarbon chain unit cell and the area per chain measured perpendicular to the chains. For the L_β phase in these β-GalDAGs there is a single peak at 0.42 nm (±0.01 nm) in the wide-angle region, which is indicative of hydrocarbon chains packed on a hexagonal lattice, thus $\mathbf{a}^* = \mathbf{b}^* = 1/(0.42\text{ nm})$, $\gamma = 120°$, and therefore $A = 0.204 \pm 0.01$ nm^2.

For the L_{c1} phase in the 1,2-*sn*, 1,2-*rac*, and 2,3-*sn*-β-GalDAGs, there are three peaks at 0.45, 0.39, and 0.35 nm (all ± 0.01 nm, see Fig. 3). Letting $\mathbf{a}^* = 1/(0.45\text{ nm})$, $\mathbf{b}^* = 1/(0.39\text{ nm})$, and $s_{11} = 1/(0.35\text{ nm})$, then $\gamma = 107°$, and $A = 0.184 \pm 0.01$ nm^2. In the case of the L_{c2} phase, the number of peaks in the diffraction pattern makes calculations very difficult. That three of the five visible reflections in the wide-angle region are common to both L_c phases suggests that the hydrocarbon chain packing is also similar and that differences in the relative peak intensities may reflect distortion of the hydrocarbon chain subcell. Maulik et al. (1990)

have recently demonstrated that, in theory, both the intensity and position of some wide-angle diffraction peaks may vary relative to one another in the monoclinic phase with small changes in γ, the subcell parameter ratio (**a*/b***), and the subcell area. In principle any of these variables could account for the differences in the wide-angle diffraction pattern of the L_{c1} and L_{c2} phases seen in the lipids studied here. Such an interpretation is the most likely explanation of the changes in peak position and intensity seen in the wide-angle patterns of 2,3-*sn*-β-GalDAG at 20 and at 52°C (Fig. 3 *E*). However, the more marked changes in the wide-angle diffraction patterns of the 1,2-*sn*- and 1,2-*rac*-β-GalDAG originate either from a more substantial distortion of the hydrocarbon chain subcell or from the presence of two coexistent L_c-type phases. At the present time it is not possible to make a clear distinction between these two alternatives. Taken overall, the variations in long range order suggested by the low-angle diffraction measurements and the differences in hydrocarbon chain packing seen in the wide-angle diffraction measurements suggest that, while the chain packing in the L_c phases of the diastereomers and their mixture are very similar, they may ultimately be prevented from being truly isostructural by small differences in the properties of the headgroup/interfacial region.

The possible role of water in determining the mesophase metastability and the phase structure in glycoglycerolipids

Our observations show that, whereas the hydrocarbon chain packing of the mesophases and solid states of the diastereomeric 1,2-di-tetradecyl-β-GalDAGs and their mixture are analogous, both their kinetic properties, determined by the rate of the L_β/L_c conversion process, and their thermodynamic properties, determined by the presence or absence of the L_{c2} phase, are very different. The comparable mesophase behavior in these β-Gal diastereomers and their related β-GlcDAGs (Mannock et al., 1992; Kuttenreich et al., 1988; Hinz et al., 1985) may originate from similar headgroup orientations, as has lately been observed for some alkyl glycosides, in spite of variations in their headgroup stereochemistry (Sanders and Prestegard, 1992).

Hinz and co-workers have recently suggested that bilayers composed of β-GalDAGs are more stable than those composed of the corresponding β-GlcDAGs and that this increased stability most likely originates from the lower hydration of the β-D-galactosyl headgroup versus the β-D-glucosyl headgroup. This observation was based on a comparison of the thermodynamic parameters obtained for the metastable chain-melting transition of the β-GlcDAGs with those obtained for the L_c/H_{II} phase transition of the β-GalDAGs. Such a cross comparison is clearly misleading, since lamellar crystalline phases are, almost by definition, less hydrated than the corresponding gel (L_β or L'_β) phases. The similar L_β/L_α phase transition temperatures of the di-tetradecyl-β-D-Glc- and Gal-*sn*-glycerols (Table 1; Kuttenreich et al., 1988; Hinz et al., 1985, 1991; Mannock et al., 1992) argue strongly for the fact that the number of water molecules hydrating the L_β and L_α phases in the β-GlcDAGs and β-GalDAGs is very similar, but that it is either the strength or the number of hydrogen bonds between the headgroup and water which is different. We suggest that it is these latter factors which regulate the kinetics of the L_β/L_c phase conversion process, not the number of hydrating water molecules. Indeed, recent measurements of kinetic medium effects (Galema et al., 1990, 1992) and carbohydrate hydration properties (Hoiland and Holvik, 1978; Galema and Hoiland, 1991) suggest that the free volume which exists in water (Lumry and Rajender, 1970) can itself be changed by the inclusion of small solute molecules. Such measurements demonstrate that the degree to which the ordering of the surrounding water molecules is perturbed and the strength of the solvent/solute hydrogen-bonding interactions is determined by the distribution of the hydrophilic and hydrophobic surfaces on the sugar molecule and thus by the solute stereochemistry.

Lemieux and co-workers (Beierbeck and Lemieux, 1990; Lemieux et al., 1991; Nikrad et al., 1992; Beierbeck et al., 1994) have recently suggested that the complexation of the monodeoxy and other derivatives of the tetrasaccharide α-L-Fuc(1,2)-β-D-Gal(1,3)[α-L-Fuc(1,4)]-β-D-GlcNAc-OMe (Le^b-OMe) with the lectin IV of *Griffonia simplicifolia* involve very similar changes in the conformations of both the lectin and the ligands. Thus, they proposed that the linear enthalpy-entropy compensation observed for this binding process arises because water molecules hydrogen-bonded to the amphiphilic surfaces of the unbound oligosaccharide and the protein are more mobile and less strongly hydrogen-bonded than are the water molecules in bulk solution. Related studies of the binding of the H-type 2 human blood group trisaccharide with an acidic lectin from *Psophocarpus tetragonolobus* endorse this interpretation (Lemieux et al., 1994). Such an explanation would support our earlier hypothesis (Mannock et al., 1992), that it is the degree of ordering of the water molecules and the strength of their hydrogen-bonding interactions within the lipid headgroup/interface, rather than the size of the lipid headgroup per se, which determines both the kinetics of the L_β/L_c conversion process and the lamellar/nonlamellar phase transition temperature, since there must also be an interfacial hydration component to the curvature free energy term which contributes to the spontaneous radius of curvature of each opposing lipid monolayer (Turner et al., 1992; Seddon and Templer, 1993). Thus, we believe it is a combination of these headgroup/interfacial hydration phenomena, together with the position of the *sn*2 hydrocarbon chain relative to chiral centres in the glycerol backbone and the carbohydrate headgroup, which primarily determine the molecular packing of the whole 1,2-di-tetradecyl-β-D-GalDAG molecule.

Note added in proof—The authors would like to make the reader aware of a brief note published by Hinz and co-workers in *Chem. Phys. Lipids. 66;* 55–62 (1993), which extends our original observations published in this journal (vol. *63,* pp. 1355–1368; (1992) and which supports the observations presented here.

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