Physical Properties of Glycosyldiacylglycerols: An Infrared Spectroscopic Study of the Gel-Phase Polymorphism of 1,2-Di-*O*-acyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerols†

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ABSTRACT: The thermotropic and barotropic gel-phase polymorphism of a homologous series of saturated, straight-chain β-D-glucosyldiacylglycerols was studied by Fourier transform infrared spectroscopy. Three spectroscopically distinct lamellar gel phases were detected thermotropically. Upon cooling to temperatures below the gel/liquid-crystalline phase transition temperature, all of these lipids form a metastable L_β gel phase characterized by orientationally disordered all-trans acyl chains. The transformation of the metastable L_β phase to a stable crystalline (L_{c2}) phase first involves the formation of an intermediate which itself is an ordered crystal-like (L_{c1}) phase. In the intermediate L_{c1} phase, the zigzag planes of the polymethylene chains are nearly perpendicular to one another, and one of the ester carbonyl oxygens is engaged in a strong hydrogen bond, probably to the 2-hydroxyl of the sugar headgroup. The transformation of the L_{c1} phase to the L_{c2} phase involves a reorientation of the all-trans hydrocarbon chains and is probably driven by the strengthening of the hydrogen bond between the carbonyl ester oxygen and its proton donors. Since a "solid-state" reorganization of the acyl chains is an integral part of that process, it tends to become more sluggish as the chain length increases and is not observed with the longer chain homologues ($N > 16$). The spectroscopic characteristics of the most stable gel phases of the odd- and even-numbered members of this homologous series of compounds exhibit only minor differences, indicating that the structures of these phases are generally similar. The barotropic phase behavior of the shorter and longer chain β-D-glucosyldiacylglycerols is also different. Compression of the L_β phase of the shorter chain compounds results in immediate conversion to their stable L_c phases, whereas compression of the L_β phase of the longer chains does not. Furthermore, compression of the longer chain compounds may result in the formation of chain-interdigitated bilayers, whereas this is not the case for the shorter chain homologues. We suggest that the gel phase formed by any given homologue at a given temperature or pressure is that which maximizes the sometimes competing requirements for the optimal packing of the sugar headgroups and the hydrocarbon chains.

Glycolipids are now known to be important immunological and structural components of many cell membranes (Quinn & Williams, 1978, 1983; Rogers et al., 1980; Gigg, 1980; Wiegandt, 1985; Slomiany et al., 1987; Curatolo, 1987a,b). Of these lipids, the glycosyldiacylglycerols are especially important since they are major components of the membranes of many plants and microorganisms. At the present time a clear understanding of the way(s) in which the structure of glycolipid molecules is related to their general and unique functional roles in biomembranes is lacking, mainly because little data on this important class of lipids are available in the literature. Clearly, our understanding of the roles of these lipids in biomembranes would be enhanced by a thorough characterization of their physical properties. To date, progress in this area of biomembrane research has been limited by the heterogeneous fatty acid composition of natural glycolipids and by the difficulties encountered in the synthesis of significant quantities of optically active glycosyldiacylglycerols of high anomeric purity (Wickberg, 1958; Gigg, 1980). Two alternative solutions to these problems have been investigated. The first was to isolate the glycolipid from natural sources and modify the hydrocarbon chain structure either by catalytic hydrogenation of a partially purified compound with unsaturated acyl chains (Sen et al., 1981; Gounaris et al., 1983) or by means of a suitable protecting group scheme, creation of semisynthetic compounds with defined acyl chain structures (Heinz, 1971; Heisig & Heinz, 1972). However, neither procedure can be applied to the preparation of glycolipids with a wide range of different headgroups, since sources of large quantities of native material with a range of headgroup structures are scarce and the exchange of one sugar headgroup for another is not presently feasible. While catalytic hydrogenation has produced ample di-16:0- and di-18:0-β-GalDG[1]

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[1] Abbreviations: DSC, differential scanning calorimetry; NMR, nuclear magnetic resonance; FT-IR, Fourier transform infrared; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; β-GlcDG, 1,2-di-*O*-acyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol; β-GalDG, 1,2-di-*O*-acyl-3-*O*-(β-D-galactopyranosyl)-*sn*-glycerol; β-GlcDAG, 1,2-di-*O*-alkyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol; β-GalDAG, 1,2-di-*O*-alkyl-3-*O*-(β-D-galactopyranosyl)-*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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for physical studies, the low yields of the acyl chain substitution procedure make such an exercise very expensive and better suited to the preparation of chromatographic standards. In spite of these limitations, there have been useful studies of both the β-galactosyl- (Sen et al., 1981, 1983; Gounaris et al., 1983; Mannock et al., 1985; Lis & Quinn, 1986; Quinn & Lis, 1986, 1987; Mansourian & Quinn, 1986; Tomoaia-Cotisel et al., 1982, 1983, 1987; Mansourian et al., 1986) and α-glucosyldiacylglycerols (Wieslander et al., 1978, 1981a,b; Khan et al., 1981; Silvius et al., 1980) using a variety of physical techniques, which have shown a pattern of gel-phase and bilayer/nonbilayer-phase polymorphism reminiscent of the diacylphosphatidylethanolamines (Seddon et al., 1983a,b, 1984; Chang & Epand, 1983; Mantsch et al., 1983). Such characteristic behavior is typified by the existence of an L_β or $L_{\beta'}$ phase, which melts at a higher temperature than the corresponding $P_{\beta'}$ phase seen in phosphatidylcholines but which also slowly converts to a poorly hydrated L_c phase under suitable annealing conditions. Furthermore, at higher temperatures the L_α phase is replaced by a nonbilayer phase, which may be either an inverted cubic (Shyamsunder et al., 1988; Mannock et al., 1990) or an inverted hexagonal phase (Seddon et al., 1983a, 1984; Lewis et al., 1989a; Mannock et al., 1988, 1990). Both the L_β/L_c conversion process and the nature of the nonbilayer phase have recently been shown to be chain length dependent in the saturated straight-chain diacyl-α- and diacyl-β-GlcDGs (Mannock et al., 1988, 1990; Sen et al., 1990; Sorokoumova et al., 1987; Lewis et al., 1990, and references cited therein).

The second solution to the problem of glyceroglycolipid synthesis was to avoid some of the difficulties inherent in the synthesis of glycosyldiacylglycerols by preparing the corresponding dialkyl analogues. Several synthetic procedures for the preparation of the β-glucosyl- and β-galactosyldialkylglycerols have been reported (Heinz et al., 1979; Ogawa & Beppu, 1982; Endo et al., 1982; Six et al., 1983). These compounds are more easily synthesized and purified in quantities suitable for physical studies, and there have been several recent investigations of these synthetic compounds using ^{2}H NMR spectroscopy (Jarrell et al., 1986, 1987a,b; Auger & Jarrell, 1990; Auger et al., 1990) and DSC and X-ray diffraction measurements (Hinz et al., 1985; Kuttenreich et al., 1988; Koynova et al., 1988). Hinz and co-workers have shown that the L_β/L_α phase transition temperatures of the di-14:0-, di-16:0-, and di-18:0-β-GlcDAG and -β-GalDAG are very similar (51–52 °C, 63–64 °C, and 71–72 °C, respectively) but that their corresponding L_c/L_α phase transition temperatures are different, for example, di-14:0-β-GlcDAG, 51 °C; di-16:0-β-GlcDAG, 62 °C; di-14:0-β-GalDAG, 69 °C; di-16:0-β-GalDAG, 73 °C (Hinz et al., 1985; Kuttenreich et al., 1988), and have suggested that this may reflect differences in the hydrogen-bonding network of the glucose and galactose headgroup. It is interesting in this respect that in the dialkyl compounds the L_β/L_α phase transition temperatures are higher and the bilayer/nonbilayer phase transition temperatures are lower than those of the corresponding diacyl compounds, in agreement with the earlier observations of diacyl- and dialkyl-PCs and -PEs (Lewis et al., 1987; Laggner et al., 1987; Seddon et al., 1983a,b, 1984).

Jarrell and co-workers have provided information on the orientation of the sugar headgroups in the L_α phases of several ditetradecyl compounds by ^{2}H NMR spectroscopy and have shown that the orientation of the headgroup changes from one in which the sugar moiety is extended away from the bilayer surface in the β-D-glucosyl anomer to one which is almost parallel to the bilayer surface in the corresponding α-anomer (Jarrell et al., 1986, 1987a,b). Moreover, they have also shown that the orientation of the headgroup changes on passing from the L_α phase to the H_{II} phase in both the β-D-glucosyl and the α-D-mannosyl compounds but not in the α-D-glucosyl compound (Jarrell et al., 1987b). However, the range of compounds studied so far has been relatively small, and it is not presently clear whether the headgroup orientations exhibited by the L_α or H_{II} phases in these compounds resemble those in their corresponding L_β and L_c phases. The crystallographic data available for β-D-galactosylceramide (Pasher & Sundell, 1977; Nyholm et al., 1990), as well as two computer-aided conformational analyses (Brasseur et al., 1983; Wynn et al., 1986), suggest that headgroup conformation in the L_c phase may differ significantly from that in the L_α phase determined by ^{2}H NMR.

In view of the importance of hydrogen-bonding interactions both in glycolipids (Pasher & Sundell, 1977; Nyholm et al., 1990; Mannock et al., 1990; Boggs, 1987) and in carbohydrates in general (Franks, 1989), and the obvious differences in the hydrogen-bonding substituents in the interfacial region of ether- and ester-linked phospholipids, which must alter the molecular packing density and consequently the lipid-phase properties, we felt that it was unwise to consider the glycosyldialkylglycerols as analogues of the naturally occurring glycosyldiacylglycerols. Our approach has been to modify the synthetic procedures available for the preparation of glycosyldiacylglycerols to improve their yield and purity, so as to obtain sufficient material for a thorough characterization of their physical properties. In this regard, we have recently developed improved procedures for the synthesis and purification of β-linked glucosyldiacylglycerols (Mannock et al., 1987) and have prepared a highly purified homologous series of such compounds with acyl chains ranging from 12 to 20 carbon atoms. Our DSC and X-ray diffraction studies indicate that these lipids exhibit a rich pattern of gel-phase polymorphism, which is strongly influenced by acyl chain length and by whether the hydrocarbon chains contain an odd or an even number of carbon atoms (Mannock et al., 1988). We now report the results of an FT-IR spectroscopic study aimed at further characterization of the gel-phase polymorphism of these glucolipids. These studies were also designed to probe the structural basis for the calorimetric behavior observed previously.

MATERIALS AND METHODS

The diacylglucolipids used in this study were synthesized and purified by the methods developed by Mannock et al. (1987). Differential scanning calorimetric measurements were performed with a Perkin-Elmer DSC-2C calorimeter equipped with a thermal analysis data station. The lipid samples were prepared for the calorimetric measurements and quantified by the same methods reported by Mannock et al. (1988).

Sample preparation for the infrared spectroscopic measurements differed according to the types of experiments performed. For the thermotropic studies, samples were prepared by thoroughly mixing 2–3 mg of the freeze-dried lipid with 10–20 μL of D_2O and squeezing the paste formed between two CaF_2 plates to form a 12 μm thick film. The plates were mounted on the sample holder, and the sample was heated in situ to temperatures above that of the gel/liquid-crystalline phase transition of the lipid to ensure complete hydration. The samples were then annealed under conditions appropriate for the formation of the lipid phase of interest, either as described in the text or as described by Mannock et al. (1988). Infrared spectra were recorded at a resolution of 2 cm^{-1} on a Digilab

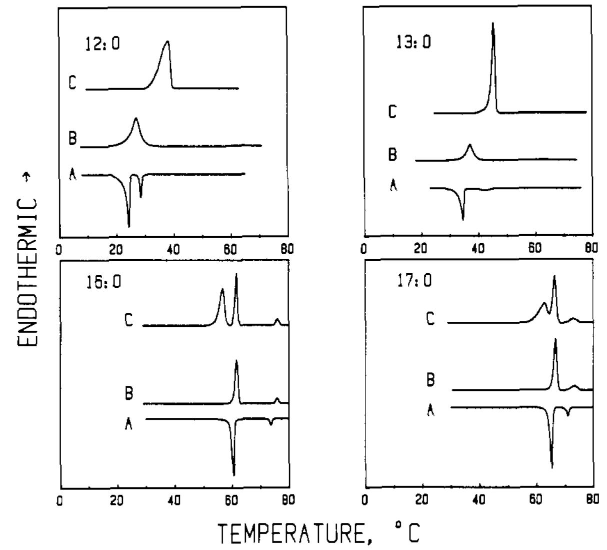

FIGURE 1: DSC thermograms of representative 1,2-di-*O*-acyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerols. The thermograms shown are (A) cooling exotherms, (B) heating endotherms obtained immediately after cooling from high temperature, and (C) heating endotherms obtained after annealing of samples at low temperature. The thermograms labeled B in the 12:0 and 13:0 panels were obtained at a heating rate of 5 °C min^{-1} and scan rate normalized to a heating rate of 1 °C min^{-1}. All other thermograms were obtained at 1 °C min^{-1}. The weakly energetic events occurring at temperatures above those of the main gel/liquid-crystalline phase transitions are the bilayer/nonbilayer transitions of these lipids. The samples shown are representative of the behavior of odd- and even-numbered members of the short- and long-chain homologues. DSC thermograms of other members in this series can be found in Mannock et al. (1988).

FTS-60 spectrometer equipped with a deuterated triglycine sulfide detector by use of data acquisition and data processing parameters similar to those previously reported (Mantsch et al., 1985).

For the barotropic studies, all experiments were performed at room temperature. The samples were first mixed with D_2O in a clean glass tube, heated to temperatures above the gel/liquid-crystalline phase temperature to ensure complete hydration, and then annealed to form the gel phase of interest. The lipid samples were then mounted on a specially designed diamond anvil cell and associated accessories (Wong et al., 1985; Wong & Mantsch, 1985), along with a few quartz crystals which served as an internal pressure calibrant. The spectra were recorded at a nominal resolution of 4 cm^{-1} (with an encoding interval of 2 cm^{-1}) on a Digilab FTS-45 spectrometer equipped with a 0.25-mm mercury–cadmium–telluride detector operated at 77 K. The parameters used for both data acquisition and data processing were similar to those previously reported (Wong & Mantsch, 1988).

RESULTS

The thermotropic phase behavior of the homologous series of saturated, straight-chain β-linked glucosyldiacylglycerols have previously been characterized by DSC and X-ray diffraction techniques (Mannock et al., 1988). In this study we used infrared spectroscopy to examine both the thermotropically and barotropically induced gel phases, so as to obtain additional insights into the structural basis of the gel-phase polymorphism exhibited by these compounds.

Illustrated in Figure 1 are DSC thermograms showing the basic pattern of phase behavior seen in four representative diacyl-β-D-glucosyl-*sn*-glycerols [for a detailed description of the thermotropic phase properties of these lipids, see Mannock et al. (1988)]. The number and structure of the phases present in these compounds are chain length dependent. Upon cooling of any of the β-GlcDGs from high temperatures (see Figure 1, thermogram A in all panels), a nonbilayer (cubic or H_{II}) phase transforms to an L_α phase. Continued cooling of the L_α phase results in a transformation to the metastable L_β-type gel phase. Further cooling of the system to temperatures below some critical nucleation point transforms the L_β phase, in some cases via an intermediate gel phase (L_{c1}), to a stable gel phase (L_{c2}).[2]

Upon heating of the sample, two possible scenarios may occur depending on whether the sample has been nucleated or not. Samples which have been immediately reheated from the L_β phase show a pattern of endothermic events corresponding to those events seen on cooling (compare thermograms A and B in all panels of Figure 1). Note, however, that there is some hysteresis in the higher temperature event, exhibited by the 12:0 and 13:0 compounds. This thermal event has been assigned to a Q_{II}/L_α phase transition by low-angle X-ray diffraction measurements on the di-13:0 compound, which shows a *Pn3m* cubic phase above 70 °C on heating and down to 45 °C on cooling with a cell lattice parameter of 17.44 nm at 45 °C. Thermograms of annealed samples may show one or more endothermic events, one of which corresponds to the conversion of the stable gel phase to a second gel phase, whose structure and transition temperature depend on the acyl chain length.

The pattern of thermal behavior observed in a thermogram of any given lipid is dependent on the kinetics of the conversion process following nucleation, the annealing time, and the structure of the second gel phase. In this respect, the thermograms in Figure 1 (DSC curves C in all panels) represent the end point of this conversion process. However, it is clear that the pattern of thermal events seen in the shorter chain compounds (12:0 and 13:0), which show a single gel/liquid-crystalline phase transition, and those seen in the longer chain compounds (16:0 and 17:0), which show two low-temperature events corresponding to a gel/gel and the gel/liquid-crystalline phase transitions, are completely different. There are two possible reasons for these differences. The first is that the kinetics of the conversion process are much faster in compounds with shorter acyl chains, and the second is that the intermediate phase may become thermodynamically more stable as the chain length increases. From these arguments it follows that because DSC is a dynamic technique, it must be able to follow this conversion process. Whether the resulting thermograms show multiple thermal events indicative of a mixed-phase system or a single thermal event corresponding to a pure intermediate phase is then dependent on the kinetics of the process in that particular lipid.

In this paper we have used FT-IR spectroscopy to investigate both the structure of these gel phases and the nature of the interconversion processes. In the case of the shorter chain compounds, because the conversion from the metastable L_β to the stable L_{c2} phase is so rapid, it was more convenient to perform these studies in the cooling mode in order to exert greater control over the rate of formation of the L_{c1} and L_{c2} phases. The infrared spectra revealed that the structural transformations detected by DSC were accompanied by diagnostic changes in spectroscopic parameters sensitive to

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[2] In the nomenclature adopted by Mannock et al. (1988), the metastable L_β gel phase was designated G_I, and the stable crystal-like gel phases of the short-chain homologues were designated as G_{III}. The intermediate gel phase formed en route to the G_{III} phase of the shorter chain lipids was designated G_{II}, and the stable gel phase of the longer chain homologues was designated as $G_{II'}$. Here, L_β is used in place of G_I, and L_{c1}, $L_{c1'}$, and L_{c2} replace G_{II}, $G_{II'}$, and G_{III}, respectively.

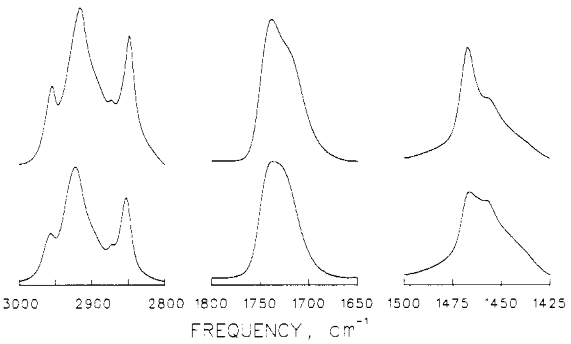

FIGURE 2: Infrared absorbance spectra showing the spectroscopic changes which occur at the transition from the L_α phase (bottom spectra) to the metastable L_β phase (top spectra) of the di-12:0-β-GlcDG. The CH_2 stretching (left panel), $C{=}O$ stretching (middle panel), and CH_2 scissoring (right panel) regions of the spectrum are shown.

changes in acyl chain packing (CH_2 scissoring band), acyl chain conformation (CH_2 stretching band), and the organization of the polar/apolar interfacial region ($C{=}O$ stretching band) of these glucolipid bilayers. The details of the spectroscopic changes observed with the compounds studied are described below.

Illustrated in Figure 2 are the changes in the diagnostic regions of the infrared spectrum of the di-12:0 compound observed upon cooling from the liquid-crystalline phase to the metastable L_β-type gel state. These are typical of the infrared spectroscopic changes observed at the L_α/L_β transition of all the β-linked glucolipids studied. The L_α/L_β transition coincides with a narrowing and a decrease in the frequency of the CH_2 symmetric stretching band (from 2851 to 2849 cm^{-1}). A thermally induced change in the CH_2 symmetric stretching frequency of this magnitude is typical of thermotropic phase transitions in which conformationally disordered (melted) polymethylene chains convert to the ordered (all-trans) state (Snyder, 1967; Casal & Mantsch, 1984). Such changes have been observed at the liquid-crystalline/gel phase transitions of all lipid bilayers so far studied. At the L_α/L_β transition of these glucolipids, there is practically no change in the frequency of the CH_2 scissoring band (~1468 cm^{-1}) though the band becomes considerably narrower. This is not surprising, since CH_2 scissoring frequencies near 1468 cm^{-1} are typical of both melted polymethylene chains (Casal et al., 1982) and solid polymethylene chains with a hexagonal packing, such as that found in the rotator phase of solid *n*-paraffins (Casal et al., 1982) or in L_β-type gel phases of lipid bilayers (Cameron et al., 1980a,b, 1981). In the $C{=}O$ stretching region of the spectrum, the conversion of the liquid-crystalline phase to the L_β gel phase coincides with a change from a broad, flat-topped band contour centered near 1734 cm^{-1} to a narrower peak at 1737 cm^{-1} with a shoulder near 1720 cm^{-1}. After band narrowing by Fourier self-deconvolution (see example, in Figure 3), the broad band contours exhibited by both the L_α and L_β phases of these lipids are shown to be a composite of two bands at 1743 and 1719 cm^{-1}, each of which shows slight changes in intensity and position as the lipid undergoes its liquid-crystalline/gel phase transition. The two $C{=}O$ stretching bands arise from the ester carbonyls (Bush et al., 1980) and are attributable to free and hydrogen-bonded ester carbonyl groups (Blume et al., 1988). As has been the case with many other phospholipids (Mendelsohn & Mantsch, 1986; Mantsch et al., 1985, 1987, 1989; Wong & Mantsch, 1988), relatively small changes in band position and band intensity typify L_α/L_β-type phase transitions in lipid bilayers.

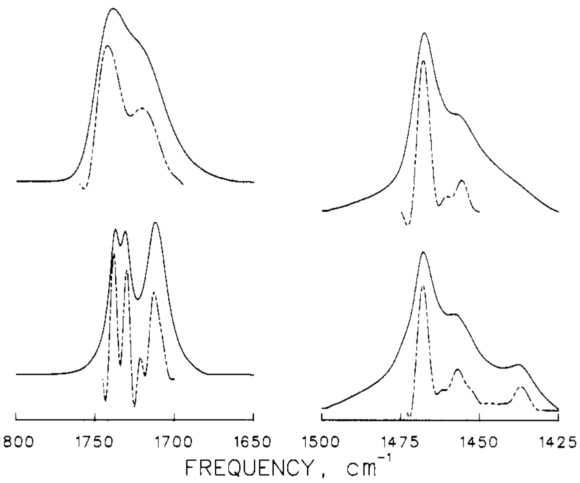

FIGURE 3: Infrared absorbance spectra showing the changes in the $C{=}O$ stretching (left panel) and CH_2 scissoring bands (right panel) of di-13:0-β-GlcDG upon conversion from the metastable L_β gel phase (top) to the stable L_{c2} gel phase (bottom). The solid lines show the original spectra, and the dashed lines show the spectra obtained after Fourier self-deconvolution with deconvolution parameters that resulted in band narrowing by a factor of 2.

The absolute frequencies of the symmetric CH_2 stretching band exhibited by various phospholipids, both below and above their gel to L_α phase transition temperatures, are known to vary with the structure of the hydrocarbon chains and the polar headgroup, and thus with the nature of the gel and L_α phases formed by these compounds. For DPPC, the relatively disordered $P_{\beta'}$ phase which exists just below T_m has a band frequency of ~2851 cm^{-1}, whereas the more ordered L_β phase of DPPE exhibits a band frequency of 2848.5 cm^{-1} just below its T_m (Casal & Mantsch, 1984). The dipalmitoyl-β-GlcDG studied here exhibits a band frequency of 2849 cm^{-1} at a temperature just below its T_m. This relatively low frequency is consistent with the formation of the relatively high-melting, well-ordered $L_{\beta'}$ phase formed by this compound, which would be expected to more closely resemble the organization of the gel phase formed by DPPE than that formed by DPPC. It is interesting, however, that the symmetric CH_2 stretching frequency in the L_α phase of this glycolipid just above its T_m is 2851 cm^{-1}, whereas DPPE and DPPC both exhibit frequencies of ~2853.5 cm^{-1} under similar conditions. Furthermore, the relatively low frequency observed for the β-GlcDG in its L_α phase implies that this glycolipid forms a liquid-crystalline bilayer in which the hydrocarbon chains contain a smaller number of gauche conformers than is found in the L_α states of the corresponding phospholipids. Frequencies in the range 2850–2851 cm^{-1} have also been observed for the liquid-crystalline phase of PCs containing methyl isobranched (Mantsch et al., 1985), methyl anteisobranched (Mantsch et al., 1987), and ω-cyclohexyl fatty acids (Mantsch et al., 1989). It is noteworthy in this regard that PCs containing these branched chain or alicyclic fatty acids form more closely packed monolayers (Rice et al., 1987; Balthasar et al., 1988; Asgharian et al., 1989) and more highly ordered bilayers (Macdonald et al., 1983, 1984) at a temperature just above their T_m's than does DPPC.

While the L_α/L_β phase transition of these lipids is reflected by relatively small changes in the $C{=}O$ bands, the conversion from the metastable L_β phase to a stable L_c-type gel phase is accompanied by major changes in this region of the infrared spectrum. The spectral changes observed after suitable annealing of these lipids are exemplified by the data acquired for di-13:0-β-GlcDG (Figure 3). With the formation of the stable gel phase, the relatively broad ester $C{=}O$ bands of the

L_β phase at 1743 and 1719 cm^{-1} are replaced by considerably narrower bands near 1737, 1731, and 1713 cm^{-1}. Moreover, the use of band narrowing procedures reveals that the low-frequency band at 1713 cm^{-1} is itself a composite of two bands with maxima at 1713 and 1709 cm^{-1}. The appearance of low-frequency components in the C=O stretching band contour has been observed in the infrared spectra of the subgel-like phases formed by some branched-chain PCs (Mantsch et al., 1985, 1987; Lewis et al., 1989b), ω-cyclohexyl PCs (Mantsch et al., 1989), and a thio analogue of DPPC (Mantsch et al., 1982) and is believed to be the result of hydrogen bonding to the ester carbonyls (Wong & Mantsch, 1988). For these glucolipids, the frequencies of the "hydrogen-bonded components" (1713 and 1709 cm^{-1}) are fairly low, and all of the carbonyl bands are very sharp. This is consistent with the formation of a crystalline structure in which the mobility of the ester carbonyls has been substantially reduced and in which one of those groups is very strongly hydrogen bonded. Moreover, the splitting of the C=O stretching bands may also be indicative of the formation of a crystalline structure in which there is interaction between C=O groups and/or in which the ester carbonyls of the lipid molecules in the unit cell are vibrationally inequivalent.

The formation of the stable gel phases of these compounds is also accompanied by other, though less drastic, changes in the region of the infrared spectrum which encompasses the methyl and methylene deformation bands. The peculiarity here is that there is a single sharp methylene scissoring band at 1468 cm^{-1}. With most crystalline aliphatic hydrocarbons or the subgel-like phases of other lipid bilayers, one finds either a single CH_2 scissoring band at frequencies near 1472 cm^{-1} [usually associated with parallel packing of the polymethylene chains; see Mantsch et al. (1985, 1987, 1989) and Lewis et al. (1989b)] or factor group splitting of the CH_2 scissoring band [usually associated with a nonparallel packing of the polymethylene chains; see Casal et al. (1983) and Mantsch et al. (1989)]. In a system containing all-trans polymethylene chains, a single CH_2 scissoring band near 1468 cm^{-1} has been associated with loose hexagonal packing, which typifies structures such as the rotator phases of solid *n*-alkanes (Casal et al., 1983) and the L_β-type gel phases of lipid bilayers (Mendelsohn & Mantsch, 1986). Here, the CH_2 scissoring frequency in the stable gel phase is also 1468 cm^{-1}, but despite this the structure of the stable gel phase of these glucolipid bilayers differs from that of the phases mentioned above. Our data suggest that the hydrocarbon chains are relatively immobile, in contrast to the chain mobility of both the rotator phases of solid hydrocarbons and the L_β-type gel phases of lipid bilayers. Upon formation of the stable gel phases of these glucolipid bilayers, there is a general narrowing of the methyl and methylene deformation bands in the 1300–1500-cm^{-1} region of the infrared spectrum (data not shown). The sharpening of these bands has been observed in solid paraffinic hydrocarbons at low temperatures (Steim & Sutherland, 1954; Ewen et al., 1974) and typifies the formation of crystalline structures in which there is an effective "immobilization" of the hydrocarbon chains.

The fact that the metastable and the stable gel phases of these glucolipids have such distinct infrared spectral characteristics made possible the use of this spectroscopic technique to search for and characterize the intermediate L_{c1} phases which are formed as the L_β phase converts to the stable L_{c2} phase. The formation of such intermediates was inferred in the previously published DSC and X-ray studies of Mannock et al. (1988) and can be demonstrated by DSC of samples of di-12:0-β-GlcDG which had been incubated for various times at relatively low temperatures and subsequently heated at very fast scan rates. As shown in Figure 4, fast heating of an unannealed sample of di-12:0-β-GlcDG results in a single endothermic peak which is attributable to the melting of the L_β phase. It is also apparent that a single endothermic peak is observed after prolonged incubation of the sample at low temperatures. This endothermic event, which corresponds to an L_{c2}/L_α phase transition, occurs at a higher temperature than the L_β/L_α phase transition observed with the unannealed sample and has a much larger enthalpy. At modest incubation times, however, three endothermic peaks are observed, of which two clearly correspond with the melting transitions of the unannealed and fully annealed samples, respectively. The third peak is observed at a temperature in between those of the melting transitions of the L_β and L_{c2} phases, respectively. The time-dependent changes in the relative areas of these peaks are consistent with the assignment of the third peak to the melting of an intermediate phase formed en route to the stable gel phase. This assignment is further substantiated by the

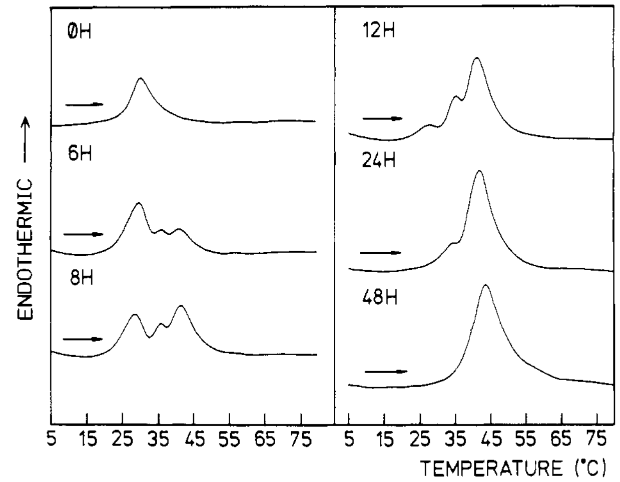

FIGURE 4: DSC thermograms of di-12:0-β-GlcDG acquired as a function of incubation time at −3 °C. The thermograms were obtained after the incubation times shown and were recorded at a very fast heating rate (20 °C min^{-1}) to avoid conversion to the stable gel phase during the DSC run. The low-temperature peak corresponds to the L_β/L_α phase transition, the middle peak can be tentatively assigned to the L_{c1}/L_α phase transition, and the high-temperature peak corresponds to the L_{c2}/L_α phase transition.

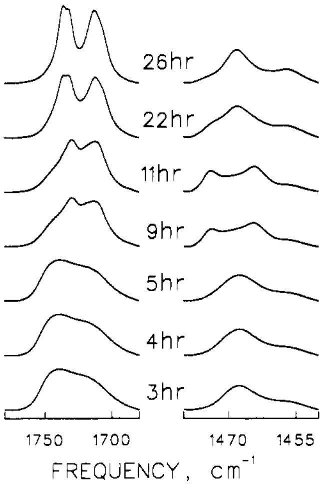

FIGURE 5: Infrared spectra, in the C=O stretching (left panel) and CH_2 scissoring (right panel) regions, of di-12:0-β-GlcDG, acquired as a function of incubation time at −5 °C, showing the gradual conversion of the L_β phase to the L_{c2} phase. The components attributable the the L_{c1} phase dominate in the 9–11-h spectra.

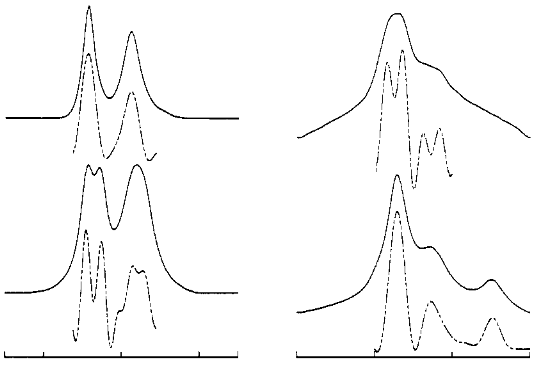

FIGURE 6: Infrared spectra in the regions of the C=O stretching (left panel) and CH_2 scissoring bands (right panel) of di-14:0-β-GlcDG. The top spectra are those of the intermediate L_{c1} gel phase; the bottom spectra are those of the stable L_{c2} gel phase. In order to obtain spectra of the intermediate L_{c1} phase, the sample was first nucleated at -5 °C, monitored in the infrared spectrum at room temperature, and then annealed at 4 °C for 12 h. The infrared spectrum of the L_β phase of this lipid was similar to that of 12:0- and 13:0-β-GlcDGs. Original spectra are shown by the solid lines, and those obtained after band narrowing by a factor of 2 are shown by the dashed lines.

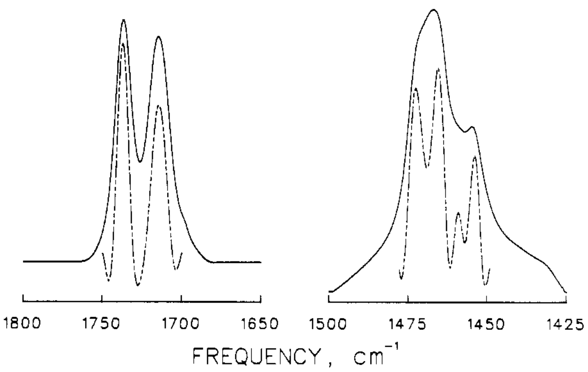

FIGURE 7: Infrared spectra showing the C=O stretching (left panel) and CH_2 scissoring bands (right panel) of the stable $L_{c1'}$ gel phase of di-17:0-β-GlcDG. Original spectra are shown by the solid lines, and spectra after Fourier self-deconvolution (with a band narrowing factor of 2) are shown by the dashed lines.

spectroscopic data illustrated in Figure 5, which show the changes in the C=O stretching and CH_2 scissoring bands of di-12:0-β-GlcDG as a function of incubation time near -5 °C. The formation of an intermediate phase with distinct spectral characteristics is clearly demonstrated by the time-dependent changes in the CH_2 scissoring band. Initially, one observes a single CH_2 scissoring band centered at 1468 cm^{-1}, which is typical of the L_β gel phase. However, incubation at such low temperatures results in the disappearance of the band at 1468 cm^{-1} and the concomitant appearance of two bands at 1464 and 1472 cm^{-1}. Further incubation results in the eventual disappearance of these two bands and the reappearance of a considerably sharper peak at 1468 cm^{-1}. Thus, the hydrocarbon chain packing in the intermediate L_{c1} phase is different from that of the stable L_{c2} phase which is eventually formed. In the intermediate phase there is factor group splitting of the methylene scissoring band, and this is indicative of the formation of an ordered crystal-like phase in which there is interchain coupling of the methylene bending vibrations, as is observed in crystalline *n*-alkanes with orthorhombically packed all-trans polymethylene chains (Casal et al., 1983).

The spectra shown in Figure 5 indicate that in the C=O stretching region there are further spectroscopic differences between the intermediate and stable gel phases of these β-GlcDGs. With the di-12:0 species these differences were obscured because the rapid rate of conversion to the stable L_{c2} phase, which often resulted in a mixed-phase system, made it difficult to obtain spectra of the pure intermediate phase. However, the spectroscopic differences between these phases are easily illustrated with the di-14:0 sample (see Figure 6), because of the slower rate of conversion and the fact that the L_{c1}/L_α phase transition occurs above the L_{c2}/L_α phase transition; this is not the case in the di-12:0 compound where it occurs at a lower temperature [see Mannock et al. (1988) for details]. However, spectroscopically the L_{c1} phases in both of these compounds appear to be very similar and are most likely identical in structure, which makes it possible to obtain spectra of the intermediate L_{c1} and the stable L_{c2} gel phases in their pure forms. In addition to the obvious differences in the CH_2 scissoring bands, the differences in the C=O stretching bands clearly indicate that the organization of the polar/apolar interfacial regions of the intermediate and stable phases are different. The carbonyl band contour of the L_{c1} phase resolves into two fairly broad bands (at 1737 and 1713 cm^{-1}), each of which splits into two bands when the L_{c2} phase forms. This suggests that in the intermediate L_{c1} phase the lipid molecules have "crystallized" into a structure in which the intermolecular or intramolecular interactions which cause the splitting of the C=O stretching vibrations are either nonexistent or considerably weaker than is the case with the L_{c2} phase. It should be noted that in the intermediate gel phase the low-frequency ester C=O stretching band at 1713 cm^{-1} is already present. Thus, the strong hydrogen bond between one of the ester carbonyls and its proton donor also exists in the intermediate gel phase.

We also found that the methyl and methylene deformation bands sharpened considerably when the L_β phase converted to the L_{c1} phase. The 1300–1500-cm^{-1} region of the infrared spectrum is remarkably similar to that of the stable gel phase, and for the most part, there were only minor differences in intensity and band position. Thus, in both the L_{c1} and L_{c2} phases we expect that the hydrocarbon chains are "relatively" immobile. Yet, in the L_{c2} phase there is a fairly strong band at 1436 cm^{-1} which does not appear in the L_{c1} phase. At this time we are uncertain as to the origin of this band. However, since a similar band has not been observed in our previous studies of phospholipids (both aqueous dispersions and solids), one can speculate that it may originate from the sugar headgroup of these glucolipids. If this is true, then the fact that it only appears in the infrared spectrum of the L_{c2} phase would suggest that the sugar headgroup becomes effectively immobilized when that phase forms.

The demonstration that the three gel phases of these compounds can be unambiguously identified by their infrared spectroscopic characteristics also made possible the use of this technique to identify the stable gel phases of the longer chain homologues (N = 16, 17, 18, and 20). Unlike the shorter chain compounds, which upon heating undergo direct transitions from the stable phase to the liquid-crystalline phase, the stable gel phase of these longer chain lipids [designated $G_{II'}$ by Mannock et al. (1988)][2] first transforms to the L_β phase, which then melts to the L_α phase at higher temperatures. Moreover, a discontinuity in the thermodynamic properties of the stable gel phases [see Mannock et al. (1988)] suggests that the stable gel phases of the longer chain homologues may be structurally different from those of their shorter chain counterparts. This was supported by a comparison of their FT-IR spectra, which revealed that the stable gel phases of the long-chain homologues exhibit factor group splitting of the CH_2 scissoring mode into bands at 1472 and 1464 cm^{-1} and that the C=O

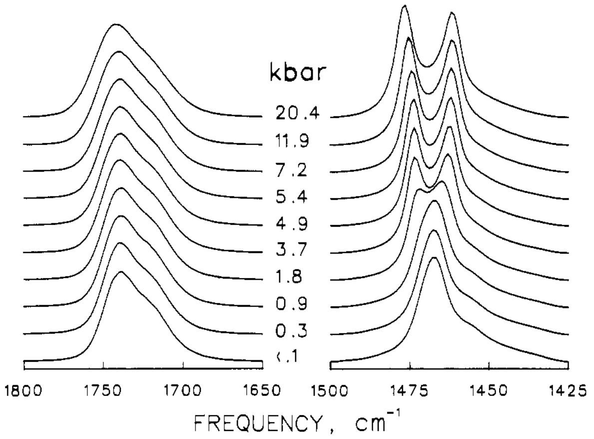

FIGURE 8: Effect of pressure on the C═O stretching (left panel) and CH_2 scissoring bands (right panel) of the L_β phase of di-19:0-β-GlcDG. The spectra were obtained at the indicated pressures (in kbar) and were recorded at increasing pressures.

stretching band contour resolves into two bands (1737 and 1713 cm^{-1}) which are not resolvable into other components (see Figure 7). These spectral features are unlike those seen in the stable gel phases of the shorter chain homologues, but they do closely resemble those of the transient intermediate gel phase formed by di-12:0- and di-14:0-β-GlcDG (see Figures 5 and 6). With these longer chain lipids we found no evidence for the formation of a stable gel phase that is spectroscopically similar to the stable gel phases of the short-chain lipids, even after annealing under exacting conditions and for prolonged periods. Given this, it seems reasonable to suggest that the stable gel phases of these longer chain homologues (here designated as $L_{c1'}$)[2] are similar in structure to the transient intermediate gel phase observed in the shorter chain homologues. Moreover, the formation of stable gel phases similar to those exhibited by the short-chain homologues may be thermodynamically or kinetically unfavorable under our experimental conditions.

The polymorphism exhibited by these β-D-glucosyldiacylglycerols was further investigated by an examination of the spectroscopic changes that occur upon compression of these lipids. In particular, we concentrated on the long-chain homologues to determine whether the application of high pressure can induce the type of polymorphism exhibited by their short-chain homologues. The spectroscopic changes that occur upon compression of the L_β-like gel phase of di-19:0-β-GlcDG are illustrated in Figure 8. This compound is the only member of the homologous series studied which has not yet formed a crystal-like phase under our conditions [see Mannock et al. (1988)]. Figure 8 shows that the compression of the L_β phase of this compound does not result in the changes in the C═O ester stretching bands observed when the stable gel phases of any of the other glycerolipids were formed. This is in marked contrast to the barotropic behavior of the short-chain homologues, which spontaneously transform to their stable (L_{c2}) gel phases upon application of modest pressures (data not shown). It is clear, however, that when the di-19:0 lipid is compressed, there is a large factor group splitting of the CH_2 scissoring bands. The splitting of the CH_2 scissoring bands is barotropically reversible and is undoubtedly the result of the damping of reorientational fluctuations of the acyl chains upon application of high pressure. The fact that these bands split into components of equal intensity under a relatively modest pressure suggests that, once the chain fluctuations are damped, the hydrocarbon chains spontaneously tend to pack with the zigzag planes perpendicular to each other [see Wong et al. (1988) and references cited therein]. A similar pattern of barotropically induced factor group splitting is also observed with the CH_2 rocking bands (data not shown). Both of these effects closely resemble those obtained by compression of the gel phases of lyso-PC, dihexadecyl-PC, and 1,3-DPPC (Siminovitch et al., 1987) and of mixed disaturated PC's of markedly different hydrocarbon chain lengths (Wong & Huang, 1989); all of these compounds are known to form interdigitated gel phases (Serrallach et al., 1983; Ruocco et al., 1985; Kim et al., 1987). However, a different pattern of barotropically induced factor group splitting is observed in the L_β gel phase of DPPC and in former phospho- and glycolipids which do not form interdigitated gel phases (Siminovitch et al., 1987). This similarity between the barotropic behaviors of di-19:0-β-GlcDG and the other lipids thus suggests the possibility that this lipid (and possibly other homologues) can adopt an interdigitated structure under certain (though as yet undefined) conditions. Furthermore, since the shorter chain compounds do not exhibit comparable behavior when compressed, it also seems reasonable to suggest that if these compounds can form interdigitated gel phases, their tendency do so must also be chain length dependent. However, barotropic X-ray diffraction studies of these compounds will be necessary to confirm this suggestion.

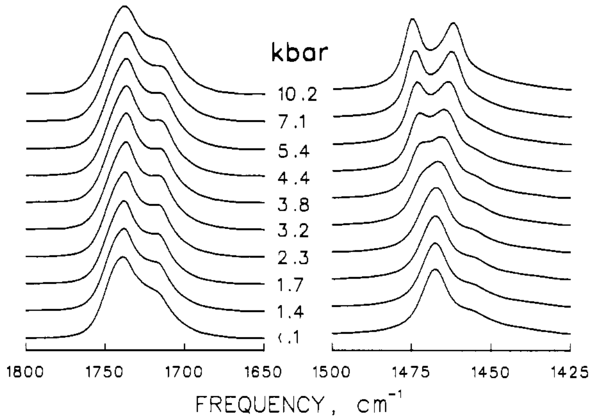

FIGURE 9: Effect of pressure on the C═O stretching (left panel) and CH_2 scissoring bands (right panel) of the L_β phase of di-16:0-β-GlcDG. The spectra were obtained at increasing pressures.

The effect of pressure on the gel phases of the di-16:0 homologue was also investigated. As shown in Figure 9, the application of high pressure does not induce the formation of any of the highly ordered gel phases (L_{c1}, $L_{c1'}$, or L_{c2}). With this longer chain lipid, the relatively narrow C═O stretching bands characteristic of the stable gel phases are not formed at the pressures examined. The principal change observed here is that the increase in pressure results in a shift of the low-frequency component of the C═O ester band contour from 1718 to 1711 cm^{-1}. This pressure-induced decrease in the frequency of a C═O stretching band is characteristic of hydrogen-bonded ester carbonyl groups (Wong & Mantsch, 1988). The barotropically induced changes in the CH_2 scissoring bands of the L_β phase of di-16:0-β-GlcDG are in some respects similar to those which occur when the L_{c1} or $L_{c1'}$ phases are thermotropically formed. As shown in Figure 9, an increase in applied pressure eventually results in a pronounced splitting of the CH_2 scissoring band. However, the splitting of this band is only superficially similar to that observed when the L_{c1} or $L_{c1'}$ phases are thermotropically induced at atmospheric pressure, since it is more pronounced and reversible. This is also in marked contrast to the short-chain homologues for which the pressure-induced transformation of the L_β phase to the crystalline L_{c2} phase is barotropically irreversible. Thus, given the possibility of pressure-induced

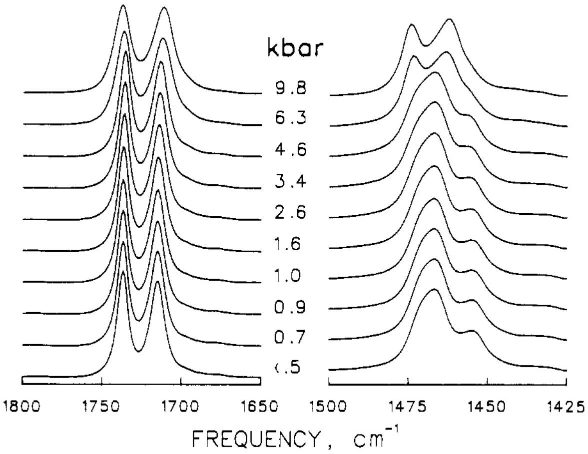

FIGURE 10: Effect of pressure on the C=O stretching (left panel) and CH_2 scissoring bands (right panel) of the stable $L_{c1'}$ gel phase of di-16:0-β-GlcDG. The spectra were obtained at increasing pressures.

interdigitation with di-19:0-β-GlcDG, these data further suggest that such interdigitation may also occur with the di-16:0-β-GlcDG.

The barotropic studies on the $L_{c1'}$ gel phase of di-16:0-β-GlcDG also failed to detect any spectroscopic evidence for the formation of L_{c2}-type phases comparable to those formed by their shorter chain homologues (see Figure 10). However, at the higher pressures there is a discontinuous increase in the splitting of the CH_2 scissoring band, and this property is barotropically reversible. In this respect the spectroscopic changes observed at higher pressures are very similar to those observed upon compression of the L_β phases of both the di-19:0 and di-16:0 compounds. This also suggests the possibility that an interdigitated phase was formed. If such is the case, however, our data suggest that this interdigitated phase is not the same as that formed by compression of the L_β phase, since the pressure dependences of the C=O stretching bands are markedly different.

DISCUSSION

The results presented here indicate that the thermotropic gel-phase polymorphism exhibited by the saturated, straight-chain β-D-glucosyldiacylglycerols is attributable to interconversions between three spectroscopically distinct gel phases. Consequently, the picture that emerged from the DSC study (Mannock et al., 1988) has been clarified as regards the molecular basis of the differences in thermotropic behavior between the short-chain lipids and their longer chain homologues. The infrared data indicate that the metastable L_β gel phases of the short-chain compounds convert to a very stable gel phase and that this process involves the formation of an intermediate phase with distinct spectroscopic characteristics. Furthermore, the intermediate (designated here as L_{c1}) and the stable (designated here as L_{c2}) gel phases of these lipids are both crystal-like phases. Thus, with these glucolipids the formation of stable crystal-like phases is a complex, multistage process as has been observed with saturated straight-chain PCs and PEs (Seddon et al., 1983a,b; Finegold & Singer, 1986; Tristram-Nagle et al., 1987; Lewis et al., 1987; Slater & Huang, 1987). With the longer chain β-GlcDGs, however, our results suggest that the process does not go to completion, since their stable gel phases (designated here as $L_{c1'}$) are spectroscopically similar to the intermediate L_{c1} gel phases formed by their short-chain homologues. Thus, it is possible that with the longer chain homologues there may be sizable kinetic and/or thermodynamic barriers against completion of that process. In our studies on the long-chain β-GlcDGs we were unable to induce the conversion to the L_{c2}-type gel phase, by using either high pressure or the more traditional thermotropic methods. On the other hand, the high-pressure studies on the longer chain β-GlcDGs suggest that it may be thermodynamically feasible for these lipids to form interdigitated gel phases. However, because the evidence suggesting interdigitation was only obtained with the long-chain homologues, any tendency toward the formation of chain-interdigitated bilayers in these lipids must be strongly chain length dependent. With the longer chain homologues, the data even suggest that there may be conditions under which formation of a chain-interdigitated phase is favored over the formation of any of the crystalline gel phases which can be thermotropically induced.

The infrared spectroscopic data presented here, however, failed to identify the molecular basis of the odd/even discontinuities detected calorimetrically; we found no evidence of major structural differences between the stable gel phases of odd- and even-numbered members of the homologous series. Instead, the FT-IR data suggest that the crystal-like phases of the odd- and even-numbered homologues are generally similar, though there are subtle differences between them. Thus, for example, we found that the methyl and methylene deformation bands of the stable gel phase of the odd-numbered homologues were more pressure sensitive than those of their even-numbered counterparts (data not shown). Evidently, there are differences in the compressibility of their stable gel phases. Nevertheless, it is clear that the structural differences between odd- and even-numbered homologues are minor, and the odd/even discontinuities detected in DSC studies (Mannock et al., 1988) are probably the result of small alternations in the geometry of a basic structure common to both the odd- and even-numbered homologues.

The differences in the spectroscopic properties of the gel phases formed by these lipids provide valuable structural information about the phases formed and give some insight into the mechanisms by which transformations between these phases take place. We demonstrated here that the conversion from the L_β gel to either the intermediate or stable gel phases involves the formation of a strongly hydrogen-bonded ester carbonyl group. In principle, this could arise as a result of hydrogen bonding to a tightly bound interfacial "water of crystallization" or of the formation of a hydrogen bond with a suitably oriented hydroxyl group on the sugar headgroup. We suspect that the latter possibility is the more likely, since a change in the anomeric configuration of the glucosidic linkage (to form the α-linked glucosyldiacylglycerol) suppresses any evidence for such a strongly hydrogen bonded ester carbonyl group (unpublished experiments from this laboratory). Moreover, our studies using molecular models and molecular graphics suggest that, with a sugar headgroup conformation similar to that proposed by Jarrell et al. (1986, 1987b), the 2-hydroxyl group of the glucose moiety would point downward into the interfacial region of the lipid bilayer and can approach within the hydrogen-bonding distance of the *sn*-2 ester carbonyl group. Assuming such a contact can occur, it is possible that the sugar headgroup may tilt, in such a way that the molecule may adopt a "shovel" shape in the L_c phase similar to that calculated for the corresponding β-GalDG (Brasseur et al., 1983) and found in the crystallographic studies of β-D-galactosylceramide (Pasher & Sundell, 1977; Nyholm et al., 1990). The tendency for the 2-hydroxyl on the β-glucosyl moiety of amphipathic glucosidic molecules to penetrate the polar/apolar interfacial regions of micellar assemblies of such molecules has been noted in previous studies on glycoside detergents (Brown et al., 1970).

It is also apparent that the transformation of the metastable L_β gel phase to any of the stable L_c-type gel phases involves discrete molecular events, some of which must be influenced by the length of the acyl chain. Our data suggest that, at the very least, a partial dehydration of the polar/apolar interfacial region of the lipid bilayer must occur when the L_β phase converts to any of the L_c phases. Such a process would be a necessary prerequisite for the formation of the strong hydrogen bond which probably occurs between the *sn*-2 ester carbonyl oxygen and 2-hydroxyl hydrogen of the sugar molecule. Indeed, with hydrated lipid bilayer assemblies in general, expulsion of some water from the polar/apolar interface is believed to be an integral part of the formation of stable crystal-like phases. Our results also suggest that, during the formation of the intermediate L_{c1} gel phase, the establishment of a hydrogen-bonded ester carbonyl at the polar/apolar interface is accompanied by the formation of an ordered crystal-like structure in which the all-trans polymethylene chains are packed with their zigzag planes perpendicular to each other, thus allowing an interchain coupling of the scissoring vibrations of the methylene groups. This leads to the splitting of the CH_2 scissoring and CH_2 rocking bands, once chain reorientational fluctuations are damped. This process must be chain length dependent, since previously published DSC studies (Mannock et al., 1988) have shown that the rate of formation of all of the stable gel phases decreases when the acyl chain length increases. However, in our studies with the long-chain homologues we found that during the low-temperature nucleation processes, leading to the formation of the stable $L_{c1'}$ gel phase, there is evidence of splitting of the CH_2 scissoring band long before any spectroscopic changes occur in the C=O stretching region of the infrared spectrum. This observation suggests that the ordering of the acyl chains may not be the rate-limiting step leading to the intermediate L_{c1} and stable $L_{c1'}$ and L_{c2} phases of these lipids. In principle, this observation could be rationalized by suggesting that the structural transformations at the headgroup and/or polar/apolar interfacial regions of the lipid bilayer are the critical rate-limiting events. However, if such were the case, it would be difficult to envisage how the overall process can still be as acyl chain length dependent as it obviously is. Consequently, we are led to the conclusion that the conversion of the L_β phase to the intermediate gel phases formed by these glucosyldiacylglycerols must also involve some other chain length dependent process(es) which may be transparent to infrared spectroscopy.

Finally, the spectroscopic changes coincident with the conversion of the L_{c1} phase to the L_{c2} phase are themselves indicative of some structural reorganization. These spectroscopic changes also provide clues to the differences in thermotropic phase behavior between the short-chain compounds and their longer chain counterparts, as well as the differences in behavior between the odd- and even-numbered homologues. We observed that the C=O stretching bands become narrower and shift to lower frequencies when the L_{c2} phase forms, suggesting a further dehydration of the polar/apolar interface of the bilayer and leading to a strengthening of the hydrogen bond between the ester carbonyl group and its proton donor. In addition, the collapse of the factor group splitting of the CH_2 scissoring band and the splitting of both C=O stretching bands also suggest that there must be some net reorientation of the acyl chains such that interchain coupling of the CH_2 scissoring vibrations becomes weaker, while interactions between carbonyl ester groups become stronger. The fact that a "solid-state" reorientation of the acyl chains is an integral part of the conversion of the L_{c1} to the L_{c2} phase would provide a rationale for the increasingly sluggish nature of the process as hydrocarbon chain length increases and may also explain why the conversion to the L_{c2} phase has not yet been observed with the long-chain homologues. Our data also suggest that the formation of the L_{c2} phase probably involves considerable distortion of the hydrocarbon chain packing of the L_{c1} phase. Given that this process seems to involve some weakening of the coupled interactions between the acyl chains, it seems reasonable to suggest that the driving force may be provided by the strengthening of hydrogen-bonding interactions at the bilayer polar/apolar interface and possibly the formation of an extended hydrogen-bonding network involving the sugar headgroups. The suggestion that hydrogen-bond formation in the polar headgroup region of the bilayer may be driving the "crystallization" of these lipids into highly ordered structures could, at least in part, explain why there are such small structural differences between the stable gel phases of the odd- and even-numbered homologues. This suggestion may also explain why the kinetics of formation decrease with increases in acyl chain length. Since our results indicate that the formation of the stable gel phase distorts (and possibly weakens) acyl chain packing interactions, it is logical to expect that this process would be considerably less favorable as acyl chain length increases, because the strength of the van der Waals interactions between the acyl chains increases with increasing hydrocarbon chain length. In addition, it is well-known that odd- and even-numbered paraffinic compounds tend to crystallize into different forms (Broadhurst, 1962). Thus, it is also possible that the barriers against the assembly of both the odd- and even-numbered homologues into a single crystalline form would be different. This may well explain the difference in the kinetics of formation of the stable gel phases of the odd- and even-numbered homologues, despite the fact that both homologues appear to be structurally similar.

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Registry No. Di-13:0-β-GlcDG, 114219-08-8; di-12:0-β-GlcDG, 114219-07-7; di-14:0-β-GlcDG, 114219-09-9; di-17:0-β-GlcDG, 114219-11-3; di-19:0-β-GlcDG, 114219-12-4; di-16:0-β-GlcDG, 42869-22-7.

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Affinity Chromatography Purification of Angiotensin II Receptor Using Photoactivable Biotinylated Probes[†]

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ABSTRACT: We have developed biotinylated photoactivable probes that are suitable for covalent labeling of angiotensin II (AII) receptors and the subsequent purification of covalent complexes through immobilized avidin or streptavidin. One of these probes, biotin-NH(CH$_2$)$_2$SS(CH$_2$)$_2$CO-[Ala^1,Phe(4N$_3$)^8]AII, which contains a cleavable disulfide bridge in its spacer arm and which displays, in its radioiodinated form, very high affinity for AII receptors ($K_d \sim 1$ nM), proved to be suitable for indirect affinity chromatography of rat liver receptor with facilitated recovery from avidin gels by use of reducing agents. This consistuted the central step of an efficient partial purification scheme involving hydroxylapatite chromatography, streptavidin chromatography, and thiopropyl-Sepharose chromatography. SDS–PAGE analysis and autoradiography established the identity of the purified entity (molecular weight 65K) as the AII receptor. Possible ways of completing purification to homogeneity and extrapolation of the protocols to a preparative scale are discussed, as well as the potential contribution of our new probes to the study of the structural properties of angiotensin receptors.

The inability of solubilized angiotensin II receptors to bind angiotensin II (AII:[1] Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) has considerably hampered the development of a simple purification by classical affinity techniques and hence progress in the elucidation of their structural and molecular properties. In spite of the data from Soffer's (Sen et al., 1984; Bandhyopadhyay et al., 1988; Kiron & Soffer, 1989) and Blalock's (Elton et al., 1988) groups, purification protocols providing sequencable amounts of receptors are not yet available. The ability to efficiently covalently label the receptor with AII derivatives bearing an azidophenylalanine at their C-termini (Escher et al., 1978; Guillemette et al., 1985, 1986) and the previous demonstration that biotinylation through N-terminal modifications of the AII molecule preserved affinity for the

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[1] Abbreviations: AII, angiotensin II; [Sar^1]AII, [1-sarcosine]angiotensin II; HPLC, high-performance liquid chromatography; NMR, nuclear magnetic resonance; NEM, *N*-ethylmaleimide; BSA, bovine serum albumin; EDTA, ethylenediaminetetraacetate; PMSF, phenylmethanesulfonyl fluoride; TFMS, trifluoromethanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; DTT, dithiothreitol; Ahx, aminohexanoic acid; Bio-AII-N$_3$, biotin-Ahx-[Ala^1,Phe(4N$_3$)^8]AII; Bio-S-S-AII(N$_3$), biotin-NH(CH$_2$)$_2$SS(CH$_2$)$_2$CO-[Ala^1,Phe(4N$_3$)^8]AII.

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