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# Structure and Dynamics of a Glyceroglycolipid: A $^2H$ NMR Study of Head Group Orientation, Ordering, and Effect on Lipid Aggregate Structure†

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ABSTRACT: The head group orientation and the motional characteristics of 1,2-di-*O*-tetradecyl-3-*O*-β-D-glucopyranosylglycerol selectively $^2H$-labeled on the glucose moiety have been investigated by differential scanning calorimetry and $^2H$ NMR. The glycolipid undergoes a major endothermic transition at 52 °C, which is attributed to the gel to liquid-crystal phase transition. The nature of a less energetic endothermic transition at 58 °C, determined to be a lamellar to hexagonal mesophase transition by $^2H$ NMR, is confirmed by X-ray diffraction. In the lamellar phase, the glycolipid head group undergoes axially symmetric motion and has an orientational order parameter $S_{mol}$ of 0.45, which is significantly larger than that (0.31) reported for an analogous glucosylcerebroside. The head group is extended away from the bilayer surface. On entering the hexagonal mesophase, the orientational order parameter for the sugar ring is reduced slightly to 0.38, but the local rotation axis undergoes a large reorientation with respect to the carbohydrate ring. In a phospholipid matrix, the orientation of the carbohydrate head group of the glycolipid is affected by the greater extension of the surface residues of the host lipid. Two orientations of the exocyclic hydroxyl group of the carbohydrate moiety were detected by $^2H$ NMR and are shown to have unequal populations.

Glycolipids constitute a class of lipid that occurs in plants, microorganisms, and animals (Gigg, 1980). Glycolipids are most frequently composed of a carbohydrate head group anchored to the membrane through a diacyl- (or dialkyl-) glycerol or a sphingosine residue. The carbohydrate head group can be relatively simple (e.g., a single sugar residue) or very complex and may be neutral or charged (Gigg, 1980). Carbohydrates at cell surfaces have been implicated in important cellular events such as cell–cell recognition, ligand–receptor interaction [e.g., cholera toxin receptor (Critchley, 1979)], and ion transport (Karlsson, 1977). The involvement of carbohydrates in such biologically important functions is dependent upon the primary sequence of the surface component but most certainly is also dependent upon the spatial relationship of the constituent residues. Glycolipids may also be the major constituent lipid of membranes such as in *Acholeplasma laidlawii* (Rottem, 1980; Razin, 1978) and in such cases must play a major role in defining the physical properties of the cellular membrane. In view of the major but diverse roles that glycolipids can assume, the elucidation of the orientation of the carbohydrate residues relative to the membrane surface and the dynamical behavior of the head group are of considerable interest. In addition, the response of the head group orientation and motion to perturbations such as ion binding, ligand–receptor interactions, and other surface components is of fundamental importance to the understanding of cell surface phenomena.

Deuterium ($^2H$) NMR[1] is a powerful technique for the elucidation of orientational and motional properties of mole-

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[1] Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; DTGL, 1,2-di-*O*-tetradecyl-3-*O*-β-D-glucopyranosyl-*rac*-glycerol; GC, glucocerebroside; DSC, differential scanning calorimetry; NMR, nuclear magnetic resonance; TLC, thin-layer chromatography.

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cules in an anisotropic environment such as that found for lipid molecules in a membrane (Seelig, 1977; Davis, 1983; Smith, 1984). To date, only one detailed $^2H$ NMR study of the head group properties of a glycolipid, *N*-palmitoylglucosylceramide (GC), in aqueous multilamellar dispersions has been reported (Skarjune & Oldfield, 1982). This study investigates the head group region of a glyceroglycolipid, namely, 1,2-di-*O*-tetradecyl-3-*O*-β-D-glucopyranosylglycerol. A dialkylglycerol lipid was selected for several reasons. First, the attachment of the long lipophilic chains to glycerol by an ether linkage is stable compared to the usual ester bond, and thus, further chemical elaboration of the head group to oligosaccharide moieties is facilitated. The dialkylglycerol moiety would therefore serve as a stable lipophilic group, which could be used to anchor more complex head groups to the membrane. Second, the glycolipid provides a model of the dialkylglycolipids of *Halobacterium cutirubrum* (Kates, 1978). Third, although the replacement of fatty acyl chains with the corresponding alkyl chains in phospholipids does lead to some differences in the physical properties (e.g., $T_c$) of the lipid, these changes do not appear to be dramatic (Dorset & Pangborn, 1982; Harlos & Eibl, 1980; Eibl & Blume, 1979). This study establishes that the head group of the glycolipid 1,2-di-*O*-tetradecyl-3-*O*-β-D-glucopyranosylglycerol in aqueous multilamellar dispersions undergoes rapid axially symmetric motion, exhibits a lamellar–hexagonal structural transition, and is extended away from the bilayer surface. In addition, two orientations of the exocyclic hydroxyl group, which are in slow exchange on the $^2H$ NMR time scale, are detected.

## Materials and Methods

[1-$^2H_1$]- and [6,6-$^2H_2$]-D-glucose were obtained from MSD Isotopes (Montreal, Canada). Dimyristoylphosphatidylcholine (DMPC) was purchased from Sigma Chemical Co. and was homogeneous on TLC ($CHCl_3$–MeOH–$H_2O$, 65/24/4 v/v/v).

[2,3,4,6,6-$^2H_5$]-D-Glucose was prepared according to the method of Koch and Stuart (1977) by catalytic exchange with Raney nickel in $D_2O$. The level of exchange was controlled to give incomplete deuteration at C-3. The degree of deuteration was determined by $^{13}C$ NMR to be 100% at C2, C4, and C6 and approximately 50% at C3. No isomerization of D-glucose during the exchange reaction was detectable by $^{13}C$ NMR.

1,2-Di-*O*-tetradecyl-*rac*-glycerol and 1,2-di-*O*-tetradecyl-*sn*-glycerol were prepared by a reported procedure (Ogawa & Beppu, 1982) and had mp 43–45 °C and 41–42 °C, respectively. For the *sn*-glycerol derivative, mp 42–43 °C was reported (Ogawa & Beppu, 1982). Deuterium-labeled 1,2-di-*O*-tetradecyl-3-*O*-β-D-glucopyranosyl-*rac*-glycerol (DTGL) and 1,2-di-*O*-tetradecyl-3-*O*-β-D-glucopyranosyl-*sn*-glycerol (*sn*-DTGL) were prepared according to published procedures (Ogawa & Beppu, 1982) with the specifically deuterated glycosyl halides. The glycolipids were homogeneous on TLC ($CHCl_3$–MeOH, 10/1), had mp 116–118 °C (lit. mp 118–119 °C; Ogawa & Beppu, 1982), and gave satisfactory elemental analyses and $^{13}C$ NMR spectra.

Calorimetry was performed on a Microcal MC-1 differential scanning calorimeter with a temperature scanning rate of 1.0 °C/min. Typically, samples consisted of 2 mg of lipid dispersed in 1.5 mL of water. The temperature scale was calibrated with the gel to liquid-crystal transition of dipalmitoylphosphatidylcholine.

Samples for $^2H$ NMR consisted of 50–100 mg of dry lipid hydrated with a 10-fold excess of deuterium-depleted water (Aldrich Chemical Co., Milwaukee, WI) in a 10-mm (o.d.) sample tube sealed under vacuum. The lipid mixture was prepared by dissolving DMPC and DTGL in chloroform–methanol (1:1), removing the organic solvent with nitrogen gas, and lyophilizing the residue from water. Hydrated samples were cyclically heated to 60 °C with vortex mixing and freeze-thawed to homogeneity (four to five cycles).

$^2H$ NMR spectra were obtained at 30.7 MHz on a "home-built" Fourier-transform spectrometer. Experiment control and data collection were performed with a Nicolet 1280 computer, a 293B pulse programmer, and an NIC-2090 digital oscilloscope (Nicolet Instrument Corp., Madison, WI). The observe frequency was generated with a PTS 160 frequency synthesizer and by a Varian 4420 amplifier driven by the output of an ENI-3100L amplifier. Data were stored on a Control Data 9427 H disc system. Spectra were acquired by quadrature detection and the quadrupolar echo sequence (Davis et al., 1976) with full-phase cycling of the radio-frequency pulses. Typically, spectra were obtained with a 90° pulse width of 4–5 μs (10-mm solenoid coil), a 60-μs delay between the $\pi/2$ pulses, and a recycle time of 100 ms. The frequency of the spectrometer was carefully set at the center of the quadrupolar powder pattern. Relaxation times, $T_{1z}$, were measured by the inversion–recovery procedure in combination with the quadrupolar echo sequence as described elsewhere (Perly et al., 1984) and were <10 ms for all labeled positions. Samples were enclosed in a glass dewar, in the NMR probe, where the temperature was electronically regulated to within ±0.5 °C. Spectra of lipid in the liquid-crystal phase ($T > 52$ °C) were dePaked to give the 90° oriented sample spectrum, according to the procedure of Bloom et al. (1981). The spectral deconvolution was performed with 500–900 data points of the frequency spectrum. The shapes of the dePaked lines were approximated when required, by use of the curve analysis routine in the Nicolet 1280 NMR software and a Gaussian line shape.

*Computational Methods.* When the motion of a rigid structure possesses axial symmetry, the quadrupolar splitting $\Delta\nu_Q$ is given by (Peterson & Chan, 1977; Taylor et al., 1981; Dufourc et al., 1983)

$$\Delta\nu_Q = \frac{3}{4}A_Q\frac{\langle 3\cos^2\theta - 1\rangle}{2}\frac{3\cos^2\alpha - 1}{2} \qquad (1)$$

where $A_Q$ is the deuterium quadrupolar coupling constant ($e^2Qq/h$), $\alpha$ is the angle between the C–$^2H$ bond vector and the axis about which the molecular segment undergoes rotation, and the term in angular brackets describes the motion of the rotation axis. The latter term is referred to as the molecular order parameter $S_{mol}$ and describes the anisotropic motion of the entire rigid structure. The quadrupole coupling constants, measured from the $^2H$ NMR spectra of solid methyl [2,3,4,6,6′-$^2H_5$]-α-D-glucopyranoside, [6,6′-$^2H_2$]-D-glucose, and [1-$^2H_1$]-D-glucose, were 157 kHz for deuterons attached to the pyranose ring and 168 kHz for those at C6; the electric field gradient tensor appeared to have an asymmetry parameter <0.1.

Equation 1 has two parameters that are to be determined by experiment, namely, $S_{mol}$ and $\alpha$. The value of $S_{mol}$ and the orientation of the molecule with respect to the rotation axis may be calculated as has been described in detail elsewhere (Taylor et al., 1981; Dufourc et al., 1983). Briefly, ratios of the observed quadrupolar splittings were calculated for the deuterons attached to the pyranose ring:

$$R_k = \frac{\Delta\nu_Q{}^i}{\Delta\nu_Q{}^j} = \frac{3\cos^2\alpha_i - 1}{3\cos^2\alpha_j - 1} \qquad (2)$$

Equation 2 is dependent only upon the values of the $\alpha_i$ that

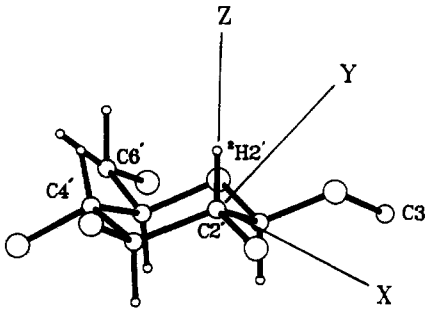


FIGURE 1: Molecule-fixed coordinate system for the head group of 1,2-di-*O*-tetradecyl-3-*O*-β-D-glucopyranosylglycerol. The *Z* axis is taken to lie along the C2′–$^2$H bond, and the plane defined by $^2$H–C2′–O2′ contains the *X* axis. The *Y* axis was chosen to complete a right-handed coordinate system.

are to be determined. The orientations of the carbon–hydrogen bonds were determined with the X-ray crystallographic data reported for methyl β-D-glucopyranoside (Jeffrey & Takagi, 1977). The latter compound was taken to be a good model for the spatial coordinates of the ring atoms of the glycolipid head group and has been shown to be appropriate for these calculations in a similar study of a glucosylceramide derivative (GC) (Skarjune & Oldfield, 1982). Fractional coordinates of the atomic positions were converted to ångström units, and the crystal axis system was translated and rotated to produce a right-handed molecule-fixed coordinate system (Figure 1) having (1) C-2′ at (0, 0, 0), (2) the positive *Z* axis along the C2′–$^2$H bond, and (3) the positive *X* axis such that the *Z*–*X* plane contained the C2′–O2′ bond. In this coordinate system, the rotation axis may be represented as a unit vector with origin at C2′ and direction cosines (sin β cos γ, sin γ sin β, cos β). The direction cosines for each C–$^2$H bond in the molecular axis system are calculated from its atomic coordinates for carbon ($X_c$, $Y_c$, $Z_c$) and deuterium ($X_d$, $Y_d$, $Z_d$):

$$a_x = \frac{X_d - X_c}{b}; \quad a_y = \frac{Y_d - Y_c}{b}; \quad a_z = \frac{Z_d - Z_c}{b} \qquad (3)$$

where *b* is the bond length. The angle between the rotation axis and each C–$^2$H bond vector is then given by

$$\cos \alpha_i = a_x^i \sin \beta \cos \gamma + a_y^i \sin \beta \sin \gamma + a_z^i \cos \beta \qquad (4)$$

By use of this value of $\alpha_i$, quadrupolar splitting ratios $R_k$ were calculated for pairs of C–$^2$H bonds and a particular orientation (γ, β) of the rotation axis. The deviation of the calculated ratios from the experimental values was evaluated as described previously (Taylor et al., 1981; Dufourc et al., 1983). The γ, β pairs that gave the smallest deviation were considered as possible solutions. The assignment of quadrupolar splittings in spectra of 2′,3′,4′,6′,6″-$^2H_5$-labeled glycolipid was based upon the known level of deuteration at each of the carbon positions and spectra obtained for 6′,6″-$^2H_2$-labeled glycolipid.

Once the orientation of the glucopyranose ring with respect to the axis of motional averaging was calculated, the value of $S_{mol}$ was determined according to eq 1. The atomic coordinates of the β-D-glucopyranosyl unit were expressed in the rotation axis ($\bar{n}$) system (C2′[0, 0, 0], $\bar{n}$[0, 0, 1]) and used to generate perspective plots with the PLUTO-78 program (S. Motherwell, University Chemical Laboratories, Cambridge). All calculations were performed on an IBM 370/TSS system. For the analysis of $^2$H NMR data obtained for deuterons at C6′, the molecule-fixed coordinate system (Figure 1) was translated and rotated so that in the new axis system the origin was at C5′, the C5′–C6′ bond lay along the positive *Z* axis, and the C5′–O5′ bond was in the positive *Z*–*X* plane. The hydroxymethyl group was rotated about the *Z* axis to generate a rotamer, and the new deuteron positions were expressed in the original molecule-fixed axis system (Figure 1). Direction cosines for each of the two C6′–H bond vectors were calculated according to eq 2. Since the orientation (γ, β) of the rotation axis with respect to the rigid ring was known, eq 4 and 2 were used to calculate values of $\Delta\nu_Q$ for each deuteron at C6. Rotation about the C5′–C6′ bond was performed in 1° steps, and the corresponding quadrupolar splittings were calculated. Results were considered possible solutions if the calculated value of $\Delta\nu_Q$ was in the range 35.6 ± 0.5 kHz or 2.0 ± 0.3 kHz (Table II).

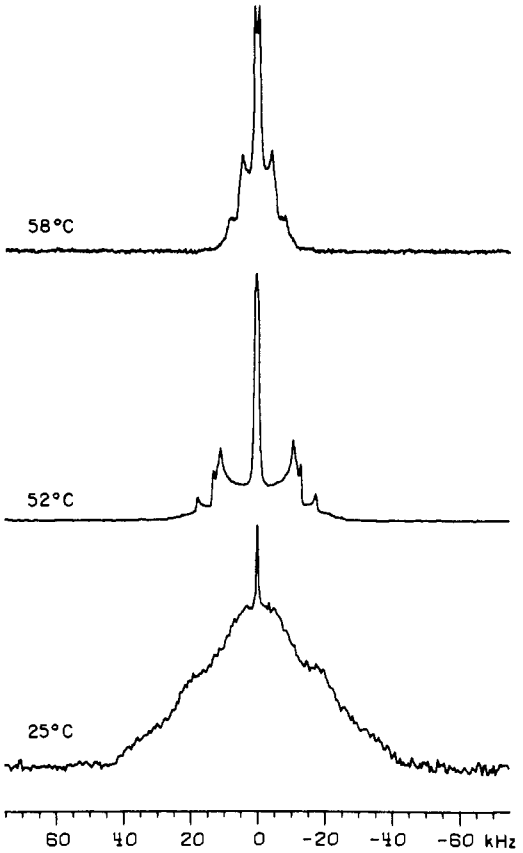


FIGURE 2: Temperature dependence of $^2$H NMR spectra of [2′,3′,4′,6′,6″-$^2H_5$]DTGL. Spectra were obtained at 30.7 MHz with a spectral width of 250 kHz, 36 000 accumulations, 90° pulse width of 5.25 μs, pulse spacing of 60 μs, and recycle time of 100 ms.

## Results and Discussion

*Phase Transition Temperatures.* Several studies have demonstrated that monoglycosyldiglycerides can exhibit polymorphic phase behavior in addition to a gel to liquid-crystal phase transition (Shipley et al., 1973; Silvius et al., 1980; Wieslander et al., 1978). In order to facilitate the interpretation of $^2$H NMR data, the thermal behavior of 1,2-di-*O*-tetradecyl-3-*O*-β-D-glucopyranosyl-*rac*-glycerol (DTGL) was examined. Differential scanning calorimetry (DSC) thermograms exhibited two endothermic transitions. The main transition was centered at 52 °C and is attributed to the gel to liquid-crystal phase transition. A secondary endothermic transition occurs at 58 °C. Typical $^2$H NMR spectra, obtained at several temperatures above and below the main endothermic transition, are shown in Figure 2. Below 52 °C, the spectrum is broad and relatively featureless and is reminiscent of the type of spectrum observed for the acyl chains of lipid in the gel state (Davis, 1983; Smith, 1984). Above 52 °C, the spectra are axially symmetric, reflecting the onset of rapid axially symmetric motion and the transition from gel to liquid-crystal

phase. At and above 58 °C, the $^2H$ spectra retain the axially symmetric shape, but the quadrupolar splitting is reduced by more than a factor of 2. Lipid molecules in hexagonal structures can undergo rapid diffusion about the cylinder axis which, if all other geometrical and motional parameters remain invariant, gives rise to a reduction of the residual quadrupolar splitting by a factor of 2 (Seelig, 1977). The results of the present study suggest that DTGL undergoes a transition from a lamellar to a hexagonal structure. Low-angle X-ray studies confirm that DTGL is organized into lamellae at 55 °C and is in hexagonal structures at 59 °C (B. Perly, personal communication). After this study was completed, 1,2-di-*O*-tetradecyl-3-*O*-β-D-glucopyranosyl-*sn*-glycerol was reported to have a main thermal transition at 50.8 ± 0.4 °C and a secondary transition at 56.9 ± 0.5 °C (Hinz et al., 1985).

Since the glycolipid under study has a racemic glycerol moiety, it is important that the lipid behave as a homogeneous system and not give rise to thermal or spectroscopic artifacts due to its diastereomeric components. 1,2-Di-*O*-tetradecyl-3-*O*-[2′,3′,4′,6′,6″-$^2H_5$]-β-D-glucopyranosyl-*sn*-glycerol ([$^2H_5$]-*sn*-DTGL) was prepared, and its physical properties were compared with DTGL. *sn*-DTGL exhibited a DSC thermogram that was nearly identical with that of DTGL and yielded the same temperature-dependent $^2H$ NMR spectra. The data demonstrate that the two diastereomeric lipids are fully miscible. The latter result compares favorably with recent studies on phosphatidylcholine (PC) (Arnett & Gold, 1982) and phosphatidylglycerol (Borle & Seelig, 1983), which demonstrated that no differences in the phase-transition temperatures could be detected for racemic or diastereomeric lipid as compared with a single chiral isomer.

The propensity of DTGL to enter the hexagonal structure is not surprising since other glycolipids readily show this transition. Monoglucosyldiacylglycerols have been noted to resemble closely the corresponding phosphatidylethanolamines in that they have similar gel to liquid-crystal transition temperatures and have the tendency to form the hexagonal ($H_{II}$) phase (Wieslander et al., 1978). 1,2-Di-*O*-tetradecyl-*sn*-glycero-3-phosphoethanolamine (DTPE) has a $T_c$ of 55.5 °C and a bilayer to hexagonal mesophase transition at 96 °C (Seddon et al., 1983). Interestingly, although DTGL has a similar value of $T_c$ (52 °C), the stability of the lamellar phase is strikingly less than that of DTPE. The replacement of an acyl chain with an alkyl chain has been reported to give rise to a slightly higher $T_c$ and a dramatically lower lamellar–hexagonal transition temperature (Boggs et al., 1981; Seddon et al., 1983). The small temperature region in which the liquid-crystalline lamellar phase is stable for DTGL as compared to DTPE is not associated with the presence of alkyl groups. A monoglucosyldiacylglycerol containing palmitoyl and oleoyl chains (1/1 ratio) was reported to have a $T_c$ of 20–25 °C and to be in the hexagonal mesophase at 30 °C (Wieslander et al., 1978). Its corresponding PE, POPE, and $T_c$ at 27 °C and enters the hexagonal phase at 55 °C (Perly et al., 1984). The low stability of the lamellar phase of these glycolipids relative to the corresponding PE appears to be related to the lipid head group.

*Head Group Motion and Orientation. (A) Lamellar Phase.* Typical $^2H$ NMR spectra of specifically deuterium-labeled DTGL in the liquid-crystalline and hexagonal mesophases are presented in Figure 3. Inspection of the spectra indicate that the head group is undergoing rapid axially symmetric motion in both the lamellar and the hexagonal structures. In order to describe the motion and orientation of the glucose residue in a more quantitative manner, the following approach was

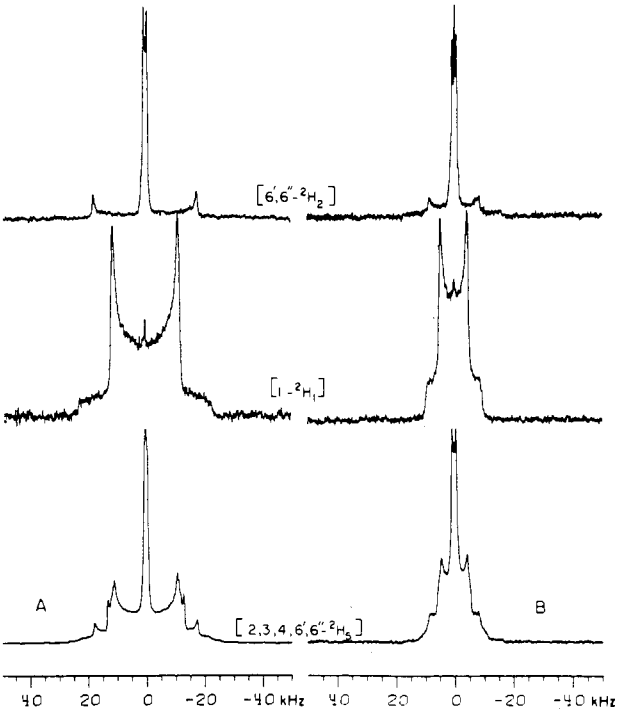


FIGURE 3: $^2H$ NMR spectra of aqueous dispersions of variously labeled DTGL (A) in the liquid-crystalline phase (52 °C) and (B) in the hexagonal phase (58 °C). Spectra were acquired as described in Figure 2.

adopted. The deuteron positions with respect to the rigid pyranose ring are fixed, and as a result, the motion of the pyranose ring describes the motion of the deuterons. The motion of the head group is represented by a rapid rotation of the ring about an axis that is fluctuating about a preferred orientation. A similar motional description has been used to analyze $^2H$ NMR data obtained for a glucosylceramide (GC) (Skarjune et al., 1982). The observed quadrupolar splittings are given by eq 2 in which the orientation of each deuterium atom with respect to the rotation axis, $\alpha_i$, and the angular fluctuations of the rotation axis, as given by $S_{mol}$, are the parameters that must be determined. The value of $S_{mol}$ describes the motion of the rigid ring and is therefore common for each of the deuterium atoms so that differences in values of $\Delta\nu_Q$ reflect differences in the deuteron orientation with respect to the motional axis. Since the positions of the deuterons with respect to the ring are fixed, it is therefore only necessary to determine the orientation of the rigid ring relative to the rotation axis. Details of the calculations are given under Materials and Methods. Equation 2 is symmetric about 90 and 180° so that without knowledge of the sign of the quadrupolar splitting four possible solutions are obtained. In addition, although the assignment of splittings to specific deuterons in spectra of [$^2H_5$]DTGL-$d_5$ was facilitated by comparison with spectra of 6′,6″-$^2H_2$-labeled DTGL and by knowledge of the level of labeling at the ring carbon positions, the relative assignment of the C2′ and C4′ splittings is ambiguous. In the fitting of $^2H$ NMR data, the resonance assignments of deuterons at C2′ and C4′ were interchanged and solutions examined. Accurate values for the $\Delta\nu_Q$ were obtained from dePaked spectra (Figure 4). The assignment of splittings to deuterons at C2′ and C4′ could be made from the calculations of $S_{mol}$ and the rotation axis orientation since only one of the two possible assignments gave satisfactory results; the assignments were confirmed by results obtained with *sn*-DTGL (vide infra). Two of the four calculated orientations of the rotation axis with respect to the pyranose ring could be dis-

Table I: Calculated Head Group Orientation and Quadrupolar Splittings[a]

| lipid | phase | orientation (deg) $\beta$ | orientation (deg) $\gamma$ | $^2$H1′ | $^2$H2′ | $^2$H3′ | $^2$H4′ | $^2$H5′ | $S_{mol}$ |
|---|---|---|---|---|---|---|---|---|---|
| [$^2H_5$]DTGL[b] | lamellar (52 °C) | 103 | 235 | 22.9 (23.1) | 22.7 (22.8) | 23.9 (24.0) | 26.4 (26.6) | | 0.45 |
| | | 257 | 55 | | | | | | |
| | hexagonal (58 °C) | 97 | 114 | 9.6 (9.6) | 10.8 (11.3) | 11.0 (10.5) | 9.4 (9.4) | | 0.38 |
| | | 263 | 294 | | | | | | |
| GC[c] | lamellar (90 °C) | 92 | 70 | 18.2 (19.2) | 18.5 (18.5) | 18.4 (18.5) | 16.0 (16.0) | 17.3 (16.0) | 0.32 |
| | | 268 | 252 | | | | | | |
| DMPC–[$^2H_5$]DTGL (10:1 w/w) | lamellar (35 °C) | 108 | 308 | | 18.9 (18.9) | 17.2 (17.2) | 22.4 (22.4) | | 0.45 |
| | | 252 | 128 | | | | | | |

[a] Values in parentheses are the observed quadrupolar splittings. [b] [2′,3′,4′,6′,6″-$^2H_5$]DTGL. [c] Experimental data from Skarjune and Oldfield (1982).

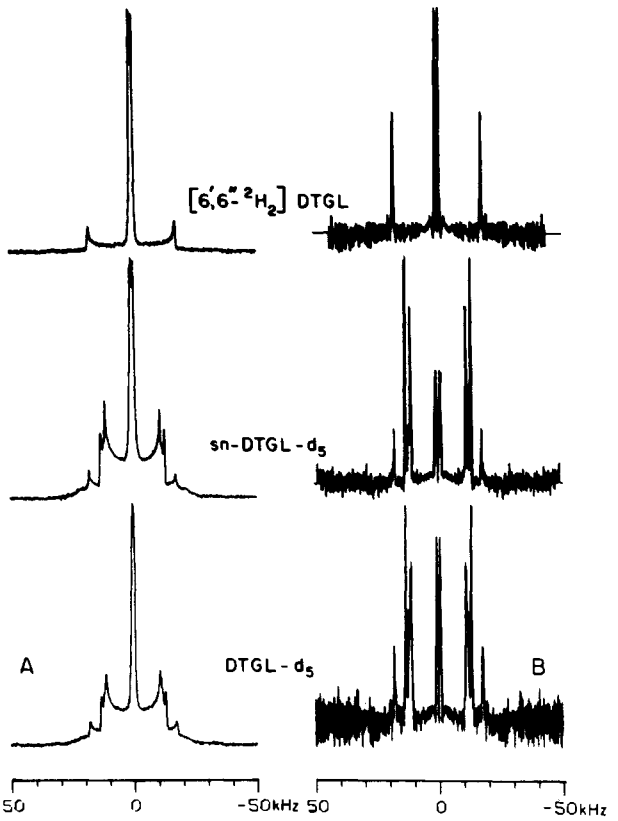


FIGURE 4: (A) $^2$H NMR spectra of [6′,6″-$^2H_2$]DTGL, [$^2H_5$]DTGL, and *sn*-[$^2H_5$]DTGL at 52 °C; (B) dePaked spectra calculated from (A).

carded in the following way. The coordinate system of Figure 1 was rotated ($\beta$, $\gamma$) so that the rotation axis was along the $Z$ axis. The coordinates of the methyl group of the model compound methyl $\beta$-D-glucopyranoside were taken to approximate those of the C3 of glycerol in DTGL. If the atomic positions of the glucose group had $Z$ coordinates that were less than those of the glycerol C3, the head group would penetrate the surface of the bilayer. This was deemed to be unlikely and was discarded as a solution. By this criterion, only two orientations of the rotation axis with respect to the ring were obtained; they differ by only a 180° rotation about the $Z$ axis (i.e., the rotation axis). The results are given by Table I along with the pertinent experimental and calculated values of $\Delta\nu_Q$. The calculated orientation of the pyranose ring and the value of $S_{mol}$ are independent of the initial choice of the molecule-fixed coordinate system since identical results were obtained when the initial $Z$ axis was taken to be along the C2′–C3′ bond or $CH_3$–C1′ direction. The value of $S_{mol}$ was found to be 0.45, which is substantially greater than the value of 0.32 reported for a glucocerebroside (GC) (Skarjune & Oldfield, 1982) and less than that of the glycerol backbone, 0.66 (Seelig & Gally, 1976). In order to compare the head group orientation of DTGL with that of the glucocerebroside derivative, the data reported for GC were analyzed as described for DTGL; the results are given in Table I. Since there was some uncertainty in the resonance assignments of GC, solutions were sought that gave the reported $S_{mol}$ value of 0.32. In order to visualize more readily the head group orientation, the atomic positions were expressed in the coordinate system with the rotation axis as the positive $Z$ direction. Perspective plots of the head group orientation are shown in Figure 5. If the direction of motional averaging is coincident with the bilayer normal for an $S_{mol}$ of 1.0, Figure 5B would depict the orientation of the sugar ring relative to the bilayer surface. In general, the orientation of the glucocerebroside agrees with that reported (Skarjune & Oldfield, 1982). Inspection of Figure 5B,C reveals that DTGL and GC have similar head group orientations. In the following discussion, the head group will be referred to as fully extended along the $Z$ axis (the director $\bar{n}$) if the $\beta$ angle is 109°, which would make the rotation axis coincident with the plane of the pyranose ring. In addition, the sugar ring is fully extended with respect to rotation about the C2′–$^2$H bond if the director is coincident with the C2′–C3′ bond direction when the molecule is viewed from above the plane of the ring ($\gamma$ = 240°); the fully extended orientation is shown in Figure 5A. DTGL is essentially fully extended with respect to the $Z$ axis and with respect to rotation about the C2′–$^2$H bond. GC however appears to be less extended with respect to rotation about both the C2′–$^2$H bond and the rotation axis. Interpretation of the molecular order parameter is not unambiguous at this point. $S_{mol}$ describes the angular fluctuations of the rotation axis with respect to some preferred orientation. In general, for lipid systems this preferred orientation is assumed to be the normal to the bilayer. In a few cases this has been confirmed for phospholipid head groups (Stockton et al., 1974; Seelig & Gally, 1976; Hemminga & Cullis, 1982) and acyl chains (Seelig & Seelig, 1974; P.-Å. Joval, I. C. P. Smith, and H. C. Jarrell, unpublished results). In one $^2$H NMR study of a diglucosyldiacylglycerol, the bilayer normal was established to be the director of motional averaging for the head group (Wieslander et al., 1978).

The C2–C3 bond of the glycerol backbone of phosphatidylethanolamine was assumed to be coincident with the bilayer normal so that a value of $S_{mol}$ of 0.66 was calculated from the quadrupolar splittings associated with deuterons attached to C3 (Seelig & Galley, 1976). However, if the C2–C3 bond were inclined by angle of 19.5° (the director is at 90° with respect to the plane containing the two C–$^2$H bonds), $S_{mol}$ would have a value of 0.44. In the case of DTGL, the orientation of the director of motional averaging with respect to the bilayer normal is unknown. However, it seems most

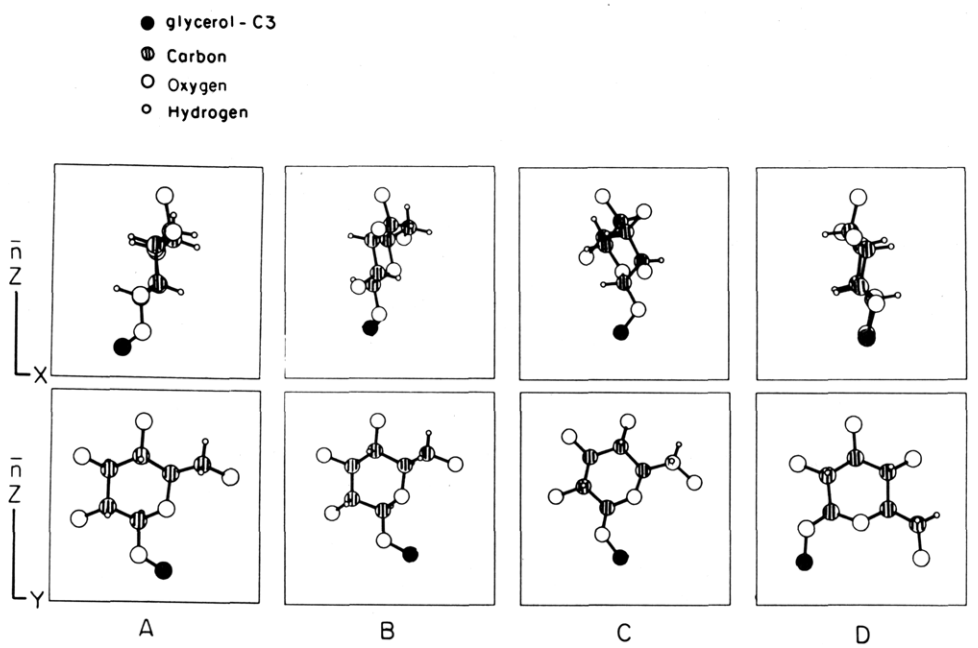


FIGURE 5: Orientation, as calculated from the $^2H$ NMR data (Table I), of the glucose moiety of (A) fully extended DTGL in the lamellar structure, with $\bar{n}$ coincident with the C2′–C3′ bond, (B) DTGL in the lamellar structure at 52 °C, (C) GC in the lamellar structure at 92 °C, and (D) DTGL in the hexagonal phase at 58 °C. (Top) View of the *XZ* plane of the rotation axis ($\bar{n}$) coordinate system, $\bar{n}$ coincident with the *Z* axis; (bottom) top view rotated about $\bar{n}$ by 90°. The positions of the hydroxyl group at C6′ and the carbon atoms at O1′ are undefined and are shown only for completeness. The carbon at O1′ (dark circle) is shown to indicate the point of attachment of the head group to the bilayer surface.

probable that the motions of the pyranose ring and the C2–C3 bond of glycerol are not independent but are coupled. For the $\beta$ anomer of glycopyranosides, the aglycon has been established to adopt a preferred orientation (rotation about the C1′–O1′ bond is not free), which is determined by the exoanomeric effect (Lemieux et al., 1979). In DTGL, the orientation of C3 of the glycerol backbone may therefore be expected to be restricted in its possible orientations with respect to the sugar ring. Thus, it seems reasonable that the glycerol C2–C3 bond and the pyranose ring undergo fluctuations of similar amplitude and about the same director, which would be reflected in similar values for $S_{mol}$. As mentioned previously, C3 of glycerol in phospholipids in the lamellar liquid-crystal phase has been reported to have an $S_{mol}$ of 0.66 (Seelig & Galley, 1976), although a value of 0.44 cannot be discounted. The sugar ring has a molecular order parameter of 0.45, which is close to the latter value for the glycerol backbone and suggests that the surface residues are moving in concert. Oriented sample spectra are required to establish the spatial relationship of the head group director with that of the bilayer normal, the director of motion for the rest of the lipid molecule.

Inspection of liquid-crystal phase spectra of [$^2H_5$]DTGL and the corresponding dePaked spectra (Figure 4) revealed that in addition to the quadrupolar splittings attributable to the C-6′ deuterons there are more than the expected three splittings associated with the deuterons attached to the sugar ring. One possible explanation was that since the lipid was a diastereomeric mixture, there was a slight different in the spatial anisotropy of the two isomers. A small difference in $\Delta\nu_Q$ values has been noted for a diastereomeric mixture of phosphatidylglycerol in which the head group was racemic (Borle & Seelig, 1983). Comparison with spectra of [2′,3′,4′,6′,6″-$^2H_5$]-*sn*-DTGL (Figure 4) reveals that both systems have similar features. However, the dePaked spectrum of the latter compound reveals that there are only three splittings for deuterons at C2′, C3′, and C4′. In addition, the results confirm the previous resonance assignments for deuterons at C2′ and C4′. The additional quadrupolar splittings observed for DTGL appear to be attributable to the presence of the racemic glycerol moiety. It should be emphasized that the differences in the values of $\Delta\nu_Q$ represented by these spectral differences are very small and would correspond to differences in the head group orientation of at most only one or two degrees.

(*B*) *Hexagonal Phase.* The hexagonal phase consists of long lipid cylinders about which lipid molecules are rapidly diffusing. If all other motional and spatial parameters remain the same as those in the lamellar phase, a reduction by a factor of 2 in $\Delta\nu_Q$ is expected for the hexagonal-phase lipid relative to that of the bilayer lipid. Inspection of Table I reveals that the reduction in $\Delta\nu_Q$ is greater than 2, suggesting that the head group orientation and/or the amplitude of the angular fluctuations of the sugar ring is greater than that in the lamellar phase. It is assumed that the lipid molecule undergoes rotation about a local axis and that the local axis diffuses rapidly about the cylinder axis. In addition, the local rotational axis maybe executing angular fluctuations about the normal to the cylinder long axis, leading to a diminished $S_{mol}$. With this model and the quadrupolar splittings of the ring deuterons, the orientation of the local rotation axis relative to the pyranose ring was calculated; the value of $S_{mol}$ is given in Table I. Inspection of the orientational parameters listed in Table I and the perspective plot of the calculated head group orientation, Figure 5C, reveals that as the lipid enters the hexagonal structure there is a reorientation of the ring with respect to its local rotation axis. The head group is less tilted with respect to the rotation axis and is less extended with respect to rotation about the C2′–$^2H$ bond than is DTGL in the lamellar phase. The value of $S_{mol}$, 0.38, is only slightly less than that of DTGL in a bilayer, indicating that there is not a large increase in the amplitude of head group motion on entering the hexagonal phase.

*Lipid Mixture.* The spatial arrangement and motions of surface residues are expected to be sensitive to the presence of other components. A $^2H$ NMR study of a glucosylcerebroside (GC) indicated a disordering of the GC head group

on dispersal in a phospholipid matrix (Skarjune & Oldfield, 1982). [$^2H_5$]DTGL was dispersed in a DMPC matrix at 9 wt % (10 mol %). An aqueous dispersion of the lipid mixture had a main endothermic transition at ca. 26 °C and a pretransition at 17 °C; no endothermic transition at 52 °C was detected. Above 25 °C, $^2H$ spectra were axially symmetric. The results indicate that the glycolipid is homogeneously dispersed in the lipid matrix and is in a lamellar liquid-crystalline phase above 25 °C. Comparison of the spectra of [$^2H_5$]DTGL at 52 °C and [$^2H_5$]DTGL in DMPC at 35 °C (data not shown) indicates that the head group orientations are different. To quantitate the changes, the head group orientation of DTGL in DMPC and the value of $S_{mol}$ were determined (Table I). The sugar ring is more extended with respect to the rotation axis and to rotation about the C2′–$^2H$ bond than it is in the pure DTGL system. This result is similar to that reported for GC in PC and in PE. Interestingly, the value of $S_{mol}$ is the same as that found for DTGL alone. This contrasts with GC, for which dispersal in a PC or PE matrix introduced a crowding of the glucose head group into a complete extension of the sugar with respect to $\beta$ and $\gamma$. Furthermore, GC in PC and in PE had a lower value of $S_{mol}$ (0.21–0.24) than that of GC alone (0.32). A detailed interpretation of the results requires more extensive study of the DTGL/DMPC lipid system. However, the present data do confirm that the physical properties of surface residues are sensitive to the presence of other surface constituents.

*Orientation of the Hydroxymethyl Group.* Inspection of Figures 3 and 4 reveal that the deuterons at C6′ give rise to a superposition of two powder spectra. The relative integrated intensity of the two resonances as estimated from the dePaked spectra is 2/1; the total intensity of the two powder patterns corresponds to that expected for two deuterons. These observations eliminate the possibility that inequivalence of $^2H6'$ and $^2H6''$ within a single rotamer is responsible for the two observed quadrupolar splittings. Since the longitudinal relaxation times ($T_1$) of the two powder spectra are the same, the results suggests that either two or more rotational isomers about the C5′–C6′ bond of the glucopyranose residue are present and of unequal populations or there are two orientations of the sugar ring that are in slow exchange on the $^2H$ NMR time scale ($10^{-5}$ s). The presence of two head group orientations with only one rotameric state of the hydroxymethyl group may be dismissed as unlikely since the differences in ring orientations that are required to give rise to the observed differences in quadrupolar splittings would also lead to correspondingly large differences for the ring deuterons. It is clear from Figure 4 that the deuterons on the pyranose ring give rise to only one splitting. The presence of two or more rotameric states of the hydroxymethyl group is more likely for several reasons. First, for simple glycosides high-resolution $^1H$ NMR studies have shown that the hydroxymethyl function may exist in two rotameric states, which are in fast exchange on the time scale of 10 $s^{-1}$ (DeBruyn & Anteunis, 1976). Second, studies on a galactocerebroside have shown that the C6′ deuterons give rise to two powder patterns of equal intensity, which indicated that the two C–$^2H$ bond vectors made different angles with respect to the director of motional averaging (Skarjune & Oldfield, 1979) but that only one head group orientation was present. Finally, glucocerebroside has been suggested to have two rotamers about the C5′–C6′ bond that are in fast exchange (Skarjune & Oldfield, 1982). In this study, the orientation of the pyranose ring with respect to the rotation axis and the value of $S_{mol}$ are known, so that the dependence of $\Delta\nu_Q$ for the C6′ deuterons may be calculated

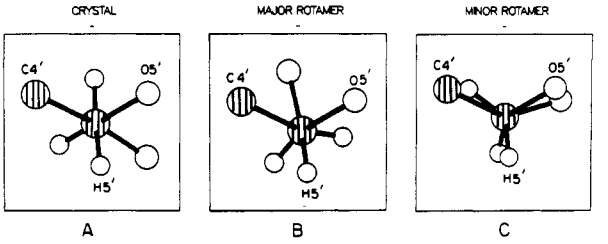


FIGURE 6: Orientation of the hydroxymethyl group in DTGL in the lamellar structure as viewed along the C5′–C6′ bond. Atomic positions as calculated from (A) X-ray crystallographic data (Jeffrey & Takagi, 1977) and (B and C) $^2H$ NMR data (Table II). The major rotamer gives rise to $\Delta\nu_Q$ values of 35.6 and 2 kHz; the minor rotamer gives rise to $\Delta\nu_Q$ values of ca. 2 kHz for both deuterons.

Table II: Calculated Quadrupolar Splittings of $^2H6'$ and $^2H6''$ as a Function of Rotation about the C5′–C6′ Bond

| angle (deg)[a] | quadrupolar splitting (kHz)[b] | deuteron |
|---|---|---|
| 0 | 21.4 | 6′ |
| 0 | 47.9 | 6″ |
| 219 ± 2 | 35.9 ± 2.1 | 6′ |
| 223 ± 1 | 2.3 ± 1.0 | 6″ |
| 305 ± 3 | 2.2 ± 0.6 | 6′ |
| 307 ± 1 | 2.4 ± 1.0 | 6″ |

[a] Angle is the angle of rotation about the C5′–C6′ bond: the rotation is clockwise as viewed from C5′ to C6′. The error estimates reflect the range of angles that gave the required quadrupolar splitting and correspondence between rotation angle for the pairs of deuterons. [b] Error estimates are those corresponding to the uncertainty in the rotation angle.

as a function of rotation about the C5′–C6′ bond. Starting with the coordinates of the hydroxymethyl group defined by the X-ray crystallographic data for methyl $\beta$-D-glucopyranoside, new deuteron positions were calculated by a rotation about the C5′–C6′ bond and the corresponding $\Delta\nu_Q$ values calculated as described under Materials and Methods. Analyses of the dePaked spectra of [6′,6″-$^2H_2$]DTGL indicates that the spectra can be explained by the superposition of two spectra; one having $\Delta\nu_Q$ of 35.6 and 2.0 kHz and the other having both $\Delta\nu_Q$ values of approximately 2.0 kHz. Examination of the calculated splittings for each deuteron as a function of rotation about the C5′–C6′ bond gave the following results. The quadrupolar splittings calculated for the hydroxymethyl group orientation (Figure 6A), as given by X-ray diffraction data for methyl $\beta$-D-glucopyranoside, differ considerably from the observed values (Table II, 0°). The C6′–O6′ bond in DGTGL cannot be gauche to the C5′–O5′ and C5–H5 bonds. In addition, there was no rotamer that gave rise to both deuterons having a splitting of 35.6 kHz. There was only one rotamer that gave rise to $\Delta\nu_Q$ values of 35.6 and 2.0 kHz and one that had splittings of ~2.0 for both deuterons. The calculated and experimental data are summarized in Table II. Table II also displays the angle through which the hydroxymethyl group was rotated about the C5′–C6′ bond to give the required splitting for each of the C6′ deuterons. Since the accuracy of the calculations is dependent on the accuracy of the X-ray data and of the experimental $\Delta\nu_Q$ values, it is not surprising that there is some decrepancy in the rotation angle as calculated for each deuteron. The results presented in Table II also indicated that the splittings associated with each of the two prochiral deuterons can be assigned unambiguously. The two rotamers are represented in Figure 6. The major rotamer (ca. 66%) that gives rise to two splittings of 35.6 and 2.0 kHz is labeled B in Figure 6 while the minor rotamer is labeled C. High-resolution NMR studies on methyl $\beta$-D-glucopyranoside have concluded that there are two rotamers that

are in fast exchange, in a ratio of approximately 2/1, and that the major rotamer is very similar to B and the minor one similar to that given by the crystal data (Figure 6A).

## Conclusions

These results demonstrate that the head group orientation and dynamics of glycolipids are sensitive to other surface components, to lipid aggregate structure (hexagonal and lamellar), and to the type of lipophilic group that anchors the carbohydrate to the hydrophobic matrix. The ability to detect rotational isomers about the C5′–C6′ bond by $^2$H NMR is of particular interest since in more complex surface residues it may be possible to observe conformational isomers about the various glycosidic linkages. Such information, in conjunction with studies of the corresponding oligosaccharide sequences without the hydrophobic anchors, may provide valuable insight into cell surface phenomena (e.g., ligand receptor interactions).

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**Registry No.** DMPC, 18194-24-6; DTGL, 85648-57-3; *sn*-DTGL, 81281-23-4.

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