Articles

Determination of Conformational Properties of Glycolipid Head Groups by ^2H NMR of Oriented Multibilayers†

Harold C. Jarrell,* P. Å. Jovall,‡ J. B. Giziewicz, L. A. Turner, and Ian C. P. Smith
Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R6
Received September 29, 1986; Revised Manuscript Received December 4, 1986

ABSTRACT: The conformations and orientations of the glucose and glycerol moieties of a monoglucosyl lipid in hydrated bilayers have been determined in detail by deuterium nuclear magnetic resonance (^2H NMR). Multibilayer membranes of 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)glycerol (DTGL), of dimyristoylphosphatidylcholine (DMPC), and of a mixture of DTGL and DMPC were oriented between glass plates. The glucolipid was selectively labeled with deuterium on the pyranose ring and at C3 of glycerol, whereas DMPC was labeled at the C4 position of the *sn*-2 chain. Quadrupolar splittings were measured as a function of the angle between the bilayer normal and the magnetic field direction. The results establish that the director of motional averaging, the direction about which motion and order are axially symmetric, is the bilayer normal for all the head group, the glycerol backbone, and the hydrophobic core. Segmental order parameters were determined to be 0.45, 0.65, and 0.40, respectively, for the various regions of DTGL in the membranes. The latter results indicate that there is some motion on the time scale of 10^5 s^{-1} about the C1′(glucose)–O–C3(glycerol) glycosidic bond but that its amplitude is very restricted. Comparison of ^1H-decoupled and ^1H-coupled ^2H NMR spectra of the C3-labeled glycolipid gave estimates of the ^2H–^2H dipolar coupling between the deuterons at this position. The orientation of the glycerol C3 hydroxymethylene subunit was calculated relative to the bilayer normal, and the C2–C3 bond was found to be tilted away from the bilayer normal by 3 ± 1°. An estimate of the ^1H–^2H dipolar coupling between the hydrogen at C2 and the deuterons at C3 of glycerol of DTGL was obtained. The results allowed calculation of the dihedral angle about the C2–C3 bond and the determination of the average conformation of this part of the glycerol backbone. The conformation about the glycosidic bond of the glycolipid is such that the sugar ring is fully extended away from the bilayer surface. This conformation differs considerably from that associated with simple glycosides.

Although glyceroglycolipids occur in plants (Gigg, 1980; Quinn et al., 1978), bacteria (Gigg, 1980; Boggs, 1980), and mycoplasma (De Kruijff et al., 1972), their role is not well understood. Recent studies have attempted to elucidate the effect of the carbohydrate head group on the physical properties of biomembranes and model membranes (Wieslander et al., 1978; Lakdar-Ghazal & Tocanne, 1981; Endo et al., 1983; Iwamoto et al., 1982; Hinz et al., 1985) and to interpret the results in terms of effects specific to the membrane surface. Glycolipids having one or two sugar residues have been reported to resemble the corresponding phosphatidylethanolamines with respect to hydration capacity (Wieslander et al., 1978; Hinz et al., 1985), phase transition temperature (Wieslander et al., 1978; Endo et al., 1983), and the ability to form nonlamellar mesophases (Wieslander et al., 1978; Endo et al., 1983; Hinz et al., 1985; Shipley et al., 1973). The observed physical properties of glyceroglycolipid membranes have been interpreted in terms of strong direct intermolecular hydrogen bonds between the hydroxyl groups of the sugar moieties (Hinz et al., 1985), head-group flexibility (Iwamoto et al., 1982), and molecular shape (Iwamoto et al., 1982; Wieslander et al., 1980). Detailed X-ray diffraction studies on such compounds in hydrated lamellar structures are not possible due to the disorder and motion in such systems. To date, only one direct measurement of glyceroglycolipid head-group orientation, flexibility, and motion has been reported (Jarrell et al., 1986). An analogous study on a glycosphingolipid was the first such deuterium nuclear magnetic resonance (^2H NMR)[1] study to be reported (Skarjune & Oldfield, 1982).

In order to assess the contribution of the glycolipid head group to the membrane surface properties, several parameters must be examined: the orientation of the carbohydrate head group relative to the membrane surface, which may affect hydration properties (Wieslander et al., 1978; Endo et al., 1983; Hinz et al., 1985); the conformation within the head group; and the motional properties of the surface residues. Deuterium (^2H) NMR, which is particularly useful for the elucidation of orientational and motional properties of molecules in an anisotropic environment such as obtains in a membrane (Seelig, 1977; Davis, 1983; Smith, 1984), affords an excellent tool with which to probe membrane surfaces and to observe surface components directly.

An earlier study from this laboratory established that in multilamellar aqueous dispersions of the glycolipid 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)glycerol, the head group

† Issued as NRCC Publication No. 27447.
‡ Present address: Department of Biochemistry and Biophysics, Chalmers University of Technology, 5-41296 Göteborg, Sweden.

[1] Abbreviations: DTGL, 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)glycerol; PE, phosphatidylethanolamine; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; TLC, thin-layer chromatography; ^2H NMR, deuterium nuclear magnetic resonance.

0006-2960/87/0426-1805$01.50/0

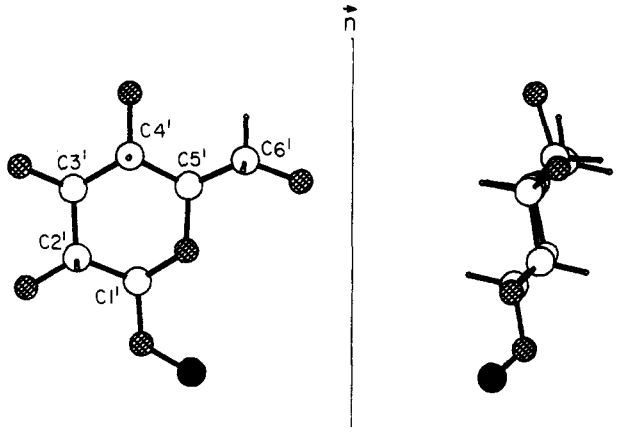

FIGURE 1: Orientation of the glucose moiety of DTGL (Jarrell et al., 1986) in the lamellar phase at 52 °C relative to the director of motional averaging, $\vec{n}$. The right figure corresponds to the left figure rotated about $\vec{n}$ by 90°. The carbon atom (dark circle) attached to O1′ is shown to indicate the point of attachment to the bilayer surface. (○) Carbon; (◎) oxygen; (∘) hydrogen.

was undergoing axially symmetric motion in the liquid-crystalline phase (Jarrell et al., 1986). The sugar ring was described as rotating rapidly about a segmental axis which was fluctuating or wobbling about a preferred direction. The time-averaged amplitude of the wobbling, given by the order parameter S_{mol}, was found to be 0.45 (Jarrell et al., 1986). The orientation of the rigid glucopyranose ring relative to the segmental rotation axis is that shown in Figure 1 and is similar to that reported for a glycosphingolipid (Skarjune & Oldfield, 1982) as discussed in detail elsewhere (Jarrell et al., 1986). In order to relate this ring orientation with respect to the bilayer plane, the director for the anisotropic motion or wobble of the rotation axis must be related to the bilayer plane or specifically to the bilayer normal. The present study establishes that the director of motional averaging for the glycerol backbone and for the carbohydrate ring of DTGL is the bilayer normal. In addition, the orientation of the C2–C3 bond of the glycerol backbone relative to the bilayer normal has been measured. An estimate of the conformation about the C2–C3 bond of glycerol and about the glycosidic linkage is deduced from the ^{2}H NMR results. A detailed molecular view of the membrane surface may thus be obtained.

Materials and Methods

Dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC) were obtained from Sigma, St. Louis, MO. DMPC, ^{2}H-labeled at C4 of the *sn*-2 chain, was prepared as described previously (Perly et al., 1984). 1,2-Di-*O*-tetradecyl-3-*O*-(β-D-[1-$^2H_1$]glucopyranosyl)-*rac*-glycerol was prepared as described elsewhere (Jarrell et al., 1986).

1,2-Di-*O*-tetradecyl-*sn*-[3-$^2H_2$]glycerol was prepared by treating 1,2-di-*O*-tetradecyl-*sn*-glycerol (0.99 g) (Ogawa & Beppu, 1982) in 60 mL of acetone with Jones' reagent diluted in acetone (1:10) until the color persisted for 10 min. Excess reagent was destroyed with 2-propanol. Celite and sodium sulfate were added, and the mixture was stirred overnight. The filtered reaction mixture was concentrated to dryness and the residue dissolved in diethyl ether. The ether solution was washed with water and concentrated. After evaporation with benzene, the crude product in diethyl ether was added to a suspension of lithium aluminum deuteride (Aldrich Chemical Co.) (160 mg, 4 mmol) in ether (50 mL). The mixture was refluxed for 1 h. Celite (1 g), sodium sulfate (1 g), and water were added to decompose excess reagent. After 15 min, the mixture was filtered and the filtrate concentrated. Chromatographic purification on silica gel with acetone–hexane (4:100 v/v) afforded, after recrystallization from methanol, pure product (680 mg, 70% yield) having a melting point of 40 °C [lit. mp 42–43 °C (Ogawa & Beppu, 1982)]. The product had the same mobility on TLC [ethyl acetate–hexane–chloroform (1:3:2 v/v)] as authentic material and gave a molecular ion at $M + 2$.

1,2-Di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-*sn*-[3-$^2H_2$]-glycerol was prepared from 1,2-di-*O*-tetradecyl-*sn*-[3-$^2H_2$]-glycerol in 82% yield by established procedures (Jarrell et al., 1986; Ogawa & Beppu, 1982); mp 116–117 °C [lit. mp 118–119 °C (Ogawa & Beppu, 1982), 116–118 °C (Jarrell et al., 1986)].

For the preparation of oriented samples, the required amount of each lipid was dissolved in chloroform–methanol (2:1 v/v). The lipid sample was then treated by one of the two following procedures:

(A) The lipid mixture was dried under a flow of nitrogen followed by 3 h under high vacuum. It was hydrated with an equal weight of ^{2}H-depleted water (Aldrich Chemical Co.), cyclically heated to 55 °C with vortexing, and freeze–thawed until homogeneous. The lipid mixture was smeared between 22 × 7 × 0.15 mm glass plates, maintaining the sample at ca. 55 °C. The NMR samples consisted of 32–36 plates in a 10-mm (o.d.) sample tube. They were allowed to dehydrate in the NMR tube at 55 °C and subsequently rehydrated by adding a few drops of ^{2}H-depleted water and sealing the tube.

(B) The lipid mixture was dropped on 22 × 7 × 0.15 mm glass plates and allowed to dry. The plates were stacked in a 10-mm (o.d.) NMR tube and dried under vacuum for 3 h. The sample was hydrated overnight at 55 °C under a humid atmosphere of ^{2}H-depleted water. A drop of ^{2}H-depleted water was added and the tube sealed. NMR samples typically consisted of 30–60 mg of lipid.

^{2}H NMR spectra were obtained at 30.7 MHz with data treatment as described previously (Jarrell et al., 1986). Spectra were acquired with a 90° pulse width of 4–5 μs (10-mm solenoid coil) and a recycle time of 100–150 ms ($>5T_1$). Sample rotation was achieved by rotating the sample manually outside the magnet bore. The orientation having the plane of the glass plates orthogonal to the magnetic field direction is defined as the 0° orientation. The estimated accuracy of the angular settings is ±2°, as determined by multiple settings of a particular orientation.

Results and Discussion

Hydrophobic Region. For a C–^{2}H bond executing axially symmetric motion, the observed quadrupolar splitting is given by (Seelig, 1977)

$$\Delta\nu_Q(\theta) = \frac{3}{2}\frac{e^2qQ}{h}S_{CD}\frac{3\cos^2\theta - 1}{2} \qquad (1)$$

where e^2qQ/h is the quadrupolar coupling constant, S_{CD} is the C–^{2}H bond order parameter, and θ is the angle between the symmetry axis for the motion and the magnetic field direction. The bilayer normal is generally assumed to be the director of motional averaging for lipid molecules in a membrane. This has been confirmed for the *N*-methyl group of choline in egg phosphatidylcholine (Stockton et al., 1974), for the phospholipid head group (Seelig & Gally, 1976; Hemminga & Cullis, 1982), and for the hydrophobic core of dipalmitoylphosphatidylcholine (Seelig & Seelig, 1974). In order to determine whether this is also true for dimyristoylphosphatidylcholine (DMPC) and hence for the 1,2-di-*O*-

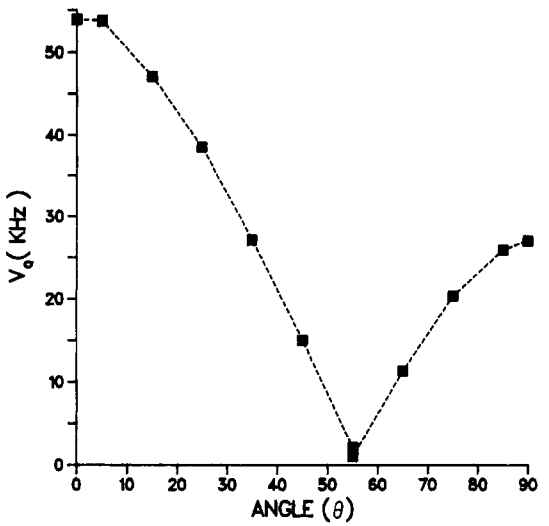

FIGURE 2: Dependence of the quadrupolar splitting ($\Delta\nu_Q$) on the angle θ between the normal to the glass plates and the magnetic field direction for oriented multibilayers of DMPC ^{2}H labeled at C4 of the *sn*-2 chain at 44 ± 1 °C.

tetradecyl-3-*O*-(β-D-glucopyranosyl)glycerol (DTGL)–DMPC system, DMPC, selectively ^{2}H labeled at the C4 position of the *sn*-2 acyl chain, was oriented as multibilayers between glass plates and the ^{2}H NMR spectrum measured as a function of the angle between the plane of the glass plates and the magnetic field direction. The 0° orientation corresponds to the plane of the glass plates orthogonal to the magnetic field direction. The dependence of the quadrupolar splitting on the angle θ shown in Figure 2 reveals a 3 $\cos^2\theta - 1$ dependence with the splitting collapsing at 54.7°. This establishes that the director of motional averaging in the hydrophobic region of the DMPC membrane, and by inferrence in the DTGL–DMPC system, is the bilayer normal.

Glycerol Backbone and Glycolipid Head-Group Region. ^{2}H NMR spectra of multilamellar dispersions of a mixture of DTGL, labeled at C3 of glycerol, exhibit two quadrupolar splittings (Figure 3A), indicating that the two C–^{2}H bonds at this position make different angles with respect to the axis of motional averaging. The spectra also demonstrate that the motion is axially symmetric. Similar results have been reported for the C3 position of glycerol in phospholipids (Wohlgemuth et al., 1980; Browning & Seelig, 1980; Gally et al., 1981). Oriented sample spectra of a mixture of DTGL (same labeled position) and DMPC (1:1 mole ratio) are shown as a function of the angle between the normal to the glass plates and the magnetic field direction (figure 3B). The dependence of the two quadrupolar splittings on the angle θ is shown in Figure 4. For both positions, a 3 $\cos^2\theta - 1$ dependence is evident; note the collapse of the splittings when θ is ca. 54.7°. This shows that the director of motional averaging for the glycerol C3 position is coincident with that of the hydrophobic core and is the bilayer normal.

Oriented multibilayers of a mixture of DTGL, labeled at C1′ of the glucopyranose residue, and DMPC (1:1 mole ratio) were prepared, and the dependence of $\Delta\nu_Q$ on θ was measured (Figure 4). The equivalence of the bilayer normal with the director of motional averaging of the rigid pyranose ring is demonstrated by the 3 $\cos^2\theta - 1$ dependence of $\Delta\nu_Q(\theta)$. The results demonstrate that for the entire glycolipid molecule the axially symmetric motions associated with the various molecular segments are related to the same director, the bilayer normal. If the glucose residue were only rotating about the bilayer normal with no angular fluctuations away from this direction (i.e., S_{mol} is 1.0), the average orientation of the sugar

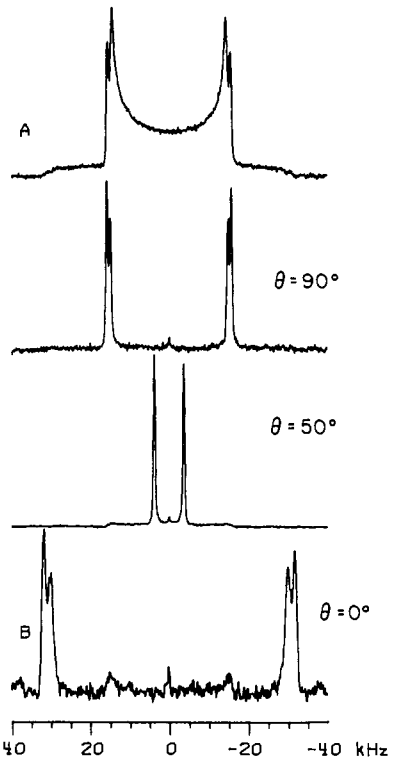

FIGURE 3: ^{2}H NMR spectra obtained at 30.7 MHz of (A) an aqueous multilamellar dispersion of DTGL ^{2}H labeled at C3 of glycerol. Sample temperature is 52 °C. (B) Oriented multibilayers of a mixture of DTGL–DMPC (1:1 mole ratio) at 45 °C as a function of the angle θ between the normal to the glass plates and the magnetic field direction. DTGL is ^{2}H labeled at C3 of glycerol.

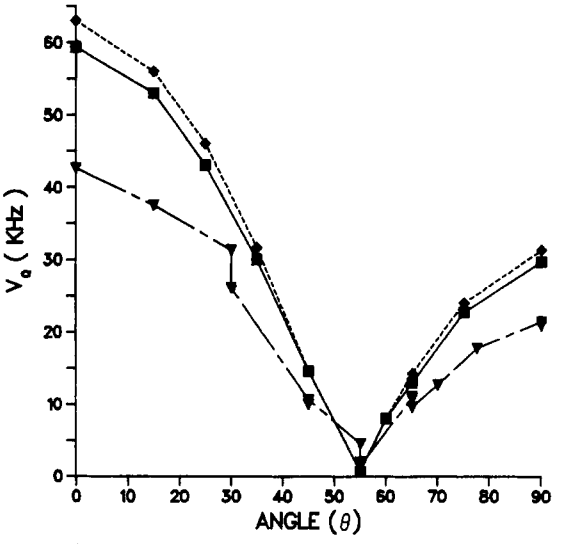

FIGURE 4: Dependence of the quadrupolar splitting on the angle θ between the normal to the glass plates and the magnetic field direction for oriented multibilayers of DTGL–DMPC (1:1 mole ratio) at 45 °C: (♦ and ■) DTGL labeled at C3 of the glycerol unit; (▼) DTGL labeled at C1′ of the glucose moiety.

ring relative to the bilayer surface is as shown in Figure 1. On the time scale of the ^{2}H NMR measurement ($<10^5$ s^{-1}), the head group is oriented away from the bilayer surface into the aqueous phase.

Glycerol Backbone Orientation. As described previously, ^{2}H NMR spectra of DTGL labeled at C3 of glycerol exhibit a superposition of two axially symmetric powder spectra. The spectra indicate that the two C–^{2}H bonds at C3 make different angles with respect to the motional axis. If the motion is described as rapid rotation about a segmental axis which is

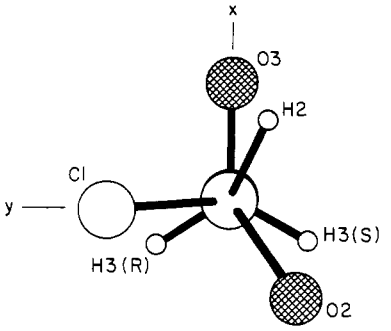

FIGURE 5: Molecule-fixed axis system attached to the glycerol backbone. View is along the C2–C3 bond from C2 toward C3. O3 lies in the X–Z plane. The conformation shown is that calculated from the estimated H2–^{2}H(S) and H2–^{2}H3(R) dipolar couplings.

fluctuating (wobbling) about the bilayer normal, eq 1 may be rewritten as (Taylor et al., 1981)

$$\Delta\nu_Q = AS_{mol}\frac{3\cos^2\beta - 1}{2} \qquad (2)$$

where $\Delta\nu_Q$ is the quadrupolar splitting for the 90° orientation of the director with respect to the magnetic field direction. The constant A is $3e^2qQ/4h$, S_{mol} is the molecular order parameter describing the anisotropic motion of the molecular rotation axis relative to the bilayer normal (the wobbling), and β is the angle between the C–^{2}H bond and the molecular rotation axis. If the value of S_{mol} is known for the glycerol C3 hydroxymethylene group, the orientation of this segment of glycerol can be calculated. For the corresponding group of phospholipids, an S_{mol} of 0.65 ± 0.01 has been reported for several head groups (Wohlgemuth et al., 1980; Browning & Seelig, 1980; Gally et al., 1981; Strenk et al., 1985). However, because DTGL differs considerably from the phospholipids with respect to the head group, the assumption of similar C3 segmental order parameters or conversely similar orientation of the glycerol C2–C3 bond does not seem justified. In order to describe the orientation and ordering of this segment, at least one additional order parameter is required.

Inspection of Figure 3B reveals that for the C3 positions in DTGL the spectrum consists of two quadrupolar splittings which have different widths for the individual pairs of resonances. One possible source of the additional line broadening is ^{1}H–^{2}H dipolar coupling between the protons on C2, and/or C1, and the deuterons at C3. If the C2–C3 segment of glycerol is considered (Figure 5), the dipolar couplings H2–^{2}H(S) and H2–^{2}H(R) (S and R refer to the *pro-S* and *pro-R* deuterons of C3) are dependent on the conformation about the C2–C3 bond and may be significantly different. Similarly, the dipolar couplings between the protons at C1 and the deuterons at C3 will be dependent on the glycerol backbone conformation.

Oriented sample spectra obtained with ^{1}H decoupling (Figure 6B) reveal that the two quadrupolar doublets have essentially the same line width, supporting the speculation that there is a differential ^{1}H–^{2}H dipolar coupling. A sample of DTGL–DPPC (4:1 mole ratio) was used to facilitate the measurements as a result of the higher DTGL content relative to the DTGL–DMPC system. The ^{1}H-decoupled spectra were analyzed as described in the Appendix to give the orientation and segmental order parameter of the C3 segment. This shows that the C2–C3 bond of glycerol is tilted away from the bilayer normal by 3 ± 1° (Figure 7) and that the segment has an S_{mol} value of 0.65. Interestingly, the latter value is very similar to that obtained for phospholipids (Wohlgemuth et al., 1980; Browning & Seelig, 1980; Gally et al., 1981; Strenk et al., 1985). In the case of phospholipids, the C2–C3 bond of

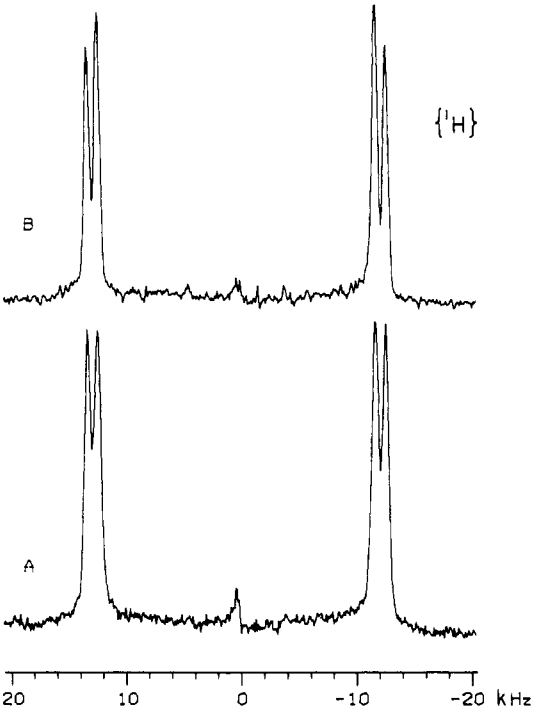

FIGURE 6: ^{2}H NMR (30.7 MHz) spectra of oriented multibilayers (θ = 78°) of a mixture of DTGL–DPPC (1:1 mole ratio) at 52 °C; DTGL is ^{2}H labeled at C3 of glycerol: (A) proton coupled; (B) proton decoupled.

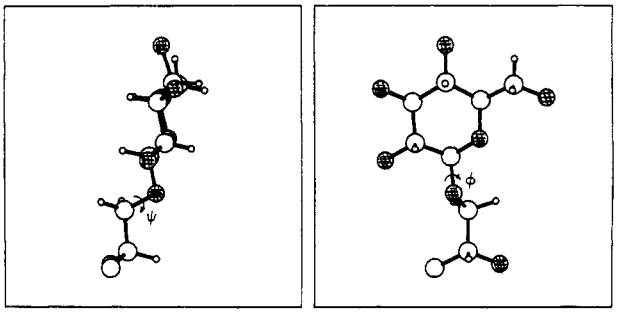

FIGURE 7: Head-group orientation of DTGL relative to the bilayer normal as calculated from ^{2}H NMR data. The conformation corresponds to that of B in Table I. The angles ψ and ϕ as defined in Table I are shown. The calculated conformation about the C2–C3 bond of glycerol (as shown in Figure 5) is included for completeness.

glycerol was assumed to be parallel to the bilayer normal (Wohlgemuth et al., 1980; Browning & Seelig, 1980; Gally et al., 1981), an assumption which was based upon X-ray crystallographic data for a single crystal of dilauroyl-PE (Elder et al., 1977). A recent study of multilamellar dispersions of DMPC concluded that the glycerol backbone region has an S_{mol} of 0.6 ± 0.1 and that the C2–C3 bond is tilted with respect to the bilayer normal (Strenk et al., 1985). The present results suggest that the glycerol backbone conformation and ordering are not very sensitive to the nature of the head group. An S_{mol} value of 0.45 for the rigid pyranose ring of DTGL was determined previously (Jarrell et al., 1986). The decrease in S_{mol} on going from the C2–C3 segment of glycerol to the carbohydrate head group reflects motion about the glycosidic bond (C1′–O3–C3) which is fast (>10^5 s^{-1}) on the scale of the ^{2}H quadrupolar splittings. The angular amplitude of the head-group motion relative to that of the C2–C3 bond of glycerol is ca. 10° greater and may still be described as very restricted.

The orientations of the glucopyranose ring and of the C3 hydroxymethylene group of glycerol have been established relative to the bilayer surface. In addition, because the two

Table I: Calculated Conformation of the Glucose–Glycerol Linkage in DTGL[a]

glycerol orientation[b]	ϕ[c] (deg)	ψ (deg)
A	159	168
B	13	173
C	148	168
D	22	156

[a] Orientation of the glucopyranose relative to the bilayer normal is that reported previously (Jarrell et al., 1986) and corresponds to β = 103° and γ = 235°. [b] Orientations A and B are those of the C2–C3 bond calculated as described in the Appendix from the glucopyranoside model, while orientations C and D derived from mannopyranoside results. See the Appendix for definitions of A–D. [c] ϕ is the angle defined by the H1′–C1′–O3–C3 segment. [c] ψ is the angle defined by the C1′–O3–C3–C2 segment.

molecular segments share a common atom, namely, the O3 atom, the average conformation about the glycosidic bond may be determined by connecting the glucopyranose and glycerol C3 subunits according to their orientations relative to the bilayer normal (Figure 7); this is only strictly valid if the order parameters of the two segments are the same which, in the present case, is when S_{mol} is 1.0 for both segments. There is some ambiguity in this procedure since a given orientation of the glycopyranose ring can be connected to either of two orientations calculated for the C3 subunit of glycerol (see Appendix); as a result, two possible conformations about the C1′–O3–C3 glycosidic linkage are obtained (Table I). In addition, the atomic positions for all the atoms of the C3 hydroxymethylene group of glycerol have not been determined by X-ray or neutron diffraction methods for DTGL or a phospholipid. The results are therefore limited by the choice of the model used for the atom positions in the C3 unit. For convenience, a pictorial representation of only one of the two possible conformations is shown in Figure 7. For the following discussion, the definitions of the angles ϕ and ψ are shown in Figure 7 and defined in Table I. The angle ψ, which reflects the conformation about the O3–C3 bond, is not very sensitive to which orientation of the glycerol is chosen or to the model used to obtain atomic positions (Table I). The C1′–O3 bond is almost antiperiplanar (trans) to the C3–C2 bond, and as a result, the glucose ring is extended away from the bilayer plane (Figure 7). The conformation about the C1′–O3 bond, as given by the angle ϕ, is less well-defined (Table I); this may be because the angle is determined by using a hydrogen atom position which is less accurately measured by X-ray or neutron diffraction than are oxygen or carbon atom positions. At present, there is no strong justification for selecting one conformation (ϕ) over the other. However, for simple β-glycopyranosides, ϕ values of ca. 50° seem to be preferred in single crystals (Jeffrey et al., 1977), suggesting that a ϕ angle in the region of 13–22° may be reasonable. In either case, the extension of the carbohydrate ring relative to the bilayer normal is essentially the same. Therefore, Figure 7 gives a reasonable representation of the average conformation of the β-glucose–C3 glycerol segment of DTGL.

If the motion of the head group of DTGL is described as a wobble about the bilayer normal of amplitude given by S_{mol}, and if the pivot of the wobble is in the plane of the bilayer and is at C2 of glycerol, an estimate of the surface area per molecule of the head group may be obtained on the basis of the conformation given in Figure 7. In the liquid-crystalline phase, an area of 90 Å^2 is calculated, surprisingly close to a value of 102 ± 4 Å^2 per molecule determined by surface pressure studies of DTGL in the liquid-expanded state (Hinz et al., 1985). For the gel state, in which the head group is assumed to be undergoing axial rotation with essentially no angular fluctations away from the bilayer normal (S_{mol} = 1.0), the surface area per molecule for the head group is estimated to be 50 Å^2, a result which is much larger than that of the condensed state, 40 Å^2, and less than that of the intermediate state, 92 Å^2, as determined by monolayer studies of DTGL (Hinz et al., 1985). ^{2}H NMR spectra of DTGL in the gel state suggest that the assumption of simple rotation about the bilayer normal in the gel state is not correct since the spectra are not indicative of axial symmetry (Jarrell et al., 1986). As a result, the surface area per molecule may be expected to be larger than 50 Å^2. A previous study of multilamellar dispersions of DTGL concluded that at 58 °C the lipid undergoes a lamellar to hexagonal mesophase transition (Jarrell et al., 1986). If the glycerol backbone conformation is assumed to be similar in both phases, an estimate of the head-group area may be made, again assuming that C2 is the pivot locus and an S_{mol} of 0.38 (Jarrell et al., 1986). A value of 55 Å^2 per molecule is obtained which is dramatically smaller than that obtained for the lamellar phase. Monolayer studies have shown that DTGL has a much larger surface area (102 ± 4 Å^2) than does dimyristoylphosphatidylethanolamine (82 Å^2) (Hinz et al., 1986). Glycolipids having only one sugar residue resemble the corresponding phosphatidylethanolamines (PE's) with respect to the gel to liquid-crystalline transition temperatures (Wieslander et al., 1978; Endo et al., 1983; Hinz et al., 1985) but exhibit a much lower temperature for the transition from bilayer to hexagonal mesophase (Wieslander et al., 1978; Jarrell et al., 1986). The shape of, or area required by, the lipid head group has been proposed to be intimately involved in the predisposition of a lipid to form nonlamellar structures (Wieslander et al., 1980). The dramatic reduction in the head-group area of DTGL, ca. 90 Å^2 (lamellar) to ca. 55 Å^2 (hexagonal), offers a reasonable explanation of the ease of formation of nonlamellar structures by monoglycosylglycerides. No correspondingly large change in the surface area per lipid molecule has been reported for the lamellar to hexagonal mesophase transition of PE.

Conformation about the Glycerol C2–C3 Bond. In the above discussion, the conformation of the glycerol backbone dealt specifically with the orientation of the C3 hydroxymethylene group with respect to the bilayer normal and the conformation about the glycosidic linkage. Comparison of ^{1}H dipolar-coupled and -decoupled spectra (Figure 6) gives an estimate of the ^{1}H–^{2}H dipolar coupling between H2 and ^{2}H3(S) as 125 Hz. The assignment of this coupling to the H2–^{2}H3(S) rather than to dipolar coupling between the protons on C1 and ^{2}H3(S) is not unambiguous but is reasonable (vide infra). The dipolar coupling is given by (Emsley & Lindon, 1975)

$$D_{HD} = \frac{-\gamma_D \gamma_H h}{8\pi} S_{mol} \left\langle \frac{3\cos^2\alpha - 1}{r_{HD}^3} \right\rangle \frac{3\cos^2\theta - 1}{2} \quad (3)$$

where all symbols have their usual meaning (Emsley & Lindon, 1975) and α is the angle between the ^{1}H–^{2}H internuclear vector and the motional axis and r_{HD} is the internuclear distance; both α and r_{HD} depend on the conformation about the C2–C3 bond (Figure 5). The inequivalent broadening of the ^{2}H(S) and ^{2}H(R) resonances indicates that motion about the C2–C3 and the C1–C2 bonds is not free or of large amplitude since either of these situations is expected to lead to similar ^{1}H dipolar broadening for both C3 deuterons. The glycerol backbone is thus essentially rigid on the ^{2}H NMR time scale; that is, it has a fixed conformation and moves as a whole about the bilayer normal. A recent ^{2}H NMR study of DMPC as a multilamellar aqueous dispersion concluded that the glycerol

Table II: Orientation of Rotation Axis for the C3 Segment of Glycerol in DTGL and Calculated Order Parameters

orientation	angles[a] (deg) β'	γ'	$S_{CD}(S)$[b]	$S_{CD}(R)$[b]	S_{DD}[b]	S_{mol}
A[c]	184	156	0.23 (0.23)	0.25 (0.25)	0.32 (0.33)	0.65
B[c]	176	336	0.23 (0.23)	0.25 (0.25)	0.32 (0.33)	0.65
C[d]	182	146	0.23 (0.23)	0.25 (0.25)	0.33 (0.33)	0.66
D[d]	178	326	0.23 (0.23)	0.25 (0.25)	0.33 (0.33)	0.66

[a] Angles refer to the orientation of the rotation axis in the axis system shown in Figure 5. [b] Values in parentheses are the experimental parameters. [c] Atom positions and direction cosines of C–^{2}H and ^{2}H–^{2}H vectors derived by using the C5–C6 segment of methyl α-D-glucopyranoside (Jeffrey et al., 1977). Note that in the axis system having the rotation axis as the z direction, A and B are related by a 180° rotation about this z axis. [d] Atom positions and direction cosines of C–^{2}H and ^{2}H–^{2}H vectors derived by using those of methyl α-D-mannopyranoside (Jeffrey et al., 1977). Note that C and D are related in the same way as A and B.

backbone has a fixed conformation and moves about the director as a rigid unit (Strenk et al., 1985). The present results are consistent with the concept of a rigid glycerol backbone. In order to elucidate the conformation about the glycerol C2–C3 bond, the dipolar coupling was calculated according to eq 3 as a function of the dihedral angle given by O2–C2–C3–O3. It must be emphasized that such calculations suffer from the need to use atomic coordinates derived from a model of the glycerol C2–C3 segment. In the present case, the atomic positions of the C5–C6 segment of methyl α-D-manno- and glucopyranoside (Jeffrey et al., 1977) were used. Calculations based upon these two models of the C2–C3 segment of glycerol give dihedral angles O2–C2–C3–O3 of −140 ± 4° and C1–C2–C3–O3 of 98 ± 4° (Figures 5 and 7). For 1,2-dilauroyl-DL-phosphatidylethanolamine (DLPE), values of 65° and 52°, respectively, were determined from X-ray diffraction data of single crystals (Elder et al., 1977). For DMPC, values of 58° and 169° were reported for the C1–C2–C3–O3 dihedral angle (Pearson & Pascher, 1979). One ^{2}H NMR study (Strenk et al., 1985) has concluded that the conformation of the glycerol backbone in a multilamellar dispersion of DMPC resembles the X-ray crystal structure DMPC-B (Pearson & Pascher, 1979). The X-ray crystallographic conformations of the glyceryl moieties in DMPC (Pearson & Pascher, 1979) and DLPE (Elder et al., 1977) were examined for the expected values of the ^{1}H–^{2}H dipolar couplings between the deuterons at C3 and the protons at C2 and C1 according to eq 3. None of the conformations reproduced the present experimental results. However, the X-ray crystallographic glycerol conformation DMPC-A (Pearson & Pascher, 1979) gives the closest fit of the expected couplings to the observed, with that between H2–^{2}H3(S) being the largest. At present, there are insufficient data to allow a more definitive determination of the glycerol backbone conformation. Additional labeling of the glycerol moiety is required to enable a more precise definition of the glycerol conformation. The present results indicate that DTGL adopts a conformation about the glycerol C2–C3 bond which differs considerably from those reported for phospholipids. They support the suggestion (Pearson & Pascher, 1979) that there is no generally preferred conformation about the glycerol C2–C3 bond for lipids.

Conclusions

The present study establishes that the C2–C3 bond of the glycerol backbone of the simple glyceroglycolipid DTGL has an orientation relative to the bilayer normal which is very similar to that reported for phospholipids (Wohlgemuth et al., 1980; Browning & Seelig, 1980; Gally et al., 1981). The orientational order parameter, S_{mol}, of DTGL in the liquid-crystalline phase is essentially the same as that reported for phospholipids (Wohlgemuth et al., 1980; Browning & Seelig, 1980; Gally et al., 1981; Strenk et al., 1985), suggesting that in general the glycerol backbone properties are common to all glycerolipids and are determined by the lipid lattice rather than by the head-group region. Oriented sample results confirm that the bilayer normal is the director of motional averaging for the entire lipid molecule. As a result, the orientation of the head group relative to the bilayer surface may be determined and is established to be essentially extended away from the surface. Estimates of the surface area per molecule, which are based upon the angular amplitude of the head-group motion and on the head-group orientation relative to the bilayer normal, provide a possible explanation of the ease in forming nonlamellar structures of the monoglycosyl glycerides relative to the corresponding phosphatidylethanolamines. In constrast to the *orientation* of the C2–C3 bond of the glyceryl fragment, the *conformation* about this bond appears to vary significantly from lipid to lipid.

Appendix

Oriented sample spectra obtained with proton decoupling (Figure 6B) were analyzed as follows. The difference in quadrupolar splittings of the two deuterons at C3 of the glyceryl moiety is 2 kHz while the ^{2}H–^{2}H dipolar coupling is <100 Hz. The ^{1}H-decoupled spectrum is thus expected to be two doublets each of which is composed of three lines of nearly equal intensity (Wemmer, 1978; Emsley & Lindon, 1975). In addition, the transverse relaxation time was determined by measuring the signal amplitude as a function of the spacing (t) between the $\pi/2$ pulse of the quadrupolar echo sequence (Davis, 1983). The decay of the echo was exponential in t and gave a T_2 of 1.2 ms, yielding an estimated line width of 250 Hz for the individual lines in Figure 6B. Each quadrupolar doublet was fitted with three lines of equal spacing and width of 250 Hz using the standard Nicolet software. A ^{2}H–^{2}H dipolar coupling of 75 Hz was estimated. The dipolar coupling is given by (Emsley & Lindon, 1975)

$$D_{DD} = \frac{-\gamma_D{}^2 h}{4\pi^2 r^3} S_{mol} \frac{3\cos^2\alpha - 1}{2} \frac{3\cos^2\theta - 1}{2} \quad \text{(A1)}$$

where α is the angle between the ^{2}H–^{2}H internuclear vector of length r and the segmental rotation axis, θ is the angle between the director and the magnetic field direction, and S_{mol} describes the anisotropic motion of the segmental rotation axis. All other symbols have their usual meaning. Two bond order parameters (S_{CD}) may be calculated from the two quadrupolar splittings for the C3 hydroxymethylene segment of glycerol, and the order parameter S_{DD} may be calculated from the ^{2}H–^{2}H dipolar coupling via eq A1. The order parameters S_i are related by

$$S_i = S_{mol} \frac{3\cos^2\alpha_i - 1}{2} \quad \text{(A2)}$$

and therefore differ only as a result of the different orientation (α_i) of the interaction vector with respect to the segmental rotation axis. A segment-fixed axis system was defined for the C3 segment of glycerol in which the z axis is along the C2–C3 bond with the origin at C3 and O3 lies in the X–Z

plane (Figure 5). The segmental rotation axis was located in the segment-fixed axis system by the procedure outlined previously (Jarrell et al., 1986; Taylor et al., 1981). In order to calculate direction cosines of the two C–^{2}H bonds, and of the ^{2}H–^{2}H vector, atomic positions were required. Since no atomic positions have been reported for the entire C3 unit of glycerol in a glycerolipid, those of the C5–C6 segment of methyl α-D-manno- and glucopyranoside (Jeffrey et al., 1977) were used. From the ratios of the order parameters, S_i, and the direction cosines of the three interaction vectors, the orientation of the segmental rotation axis was calculated according to established procedures (Taylor et al., 1981). The results are summarized in Table II. The segmental order parameter, S_{mol}, is not sensitive to the choice of the model system used to obtain atomic positions while the orientation of the rotation axis is slightly affected. Because eq A1 is symmetric about 90° and 180°, two orientations of the motional axis relative to the C3 segment are obtained (Table II) which differ only by a 180° rotation about the segmental motional axis. Interchanging the assignments of the two quadrupolar splittings did not affect the value of S_{mol} but gave a poorer fit of the calculated S_i values to the experimental S_i. In Table II, the angle β' represents the tilt of the C2-C3 bond away from the segmental rotational axis and therefore, in the absence of fluctuations of this axis, represents the tilting of the bond away from the bilayer normal.

References

Boggs, J. M. (1980) *Can. J. Biochem. 58*, 755–770.

Browning, J. L., & Seelig, J. (1980) *Biochemistry 19*, 1262–1270.

Davis, J. H. (1983) *Biochim. Biophys. Acta 737*, 117–171.

De Kruijff, B., Demel, R. A., & Van Deenen, L. L. M. (1972) *Biochim. Biophys. Acta 255*, 331–347.

Elder, M., Hitchcock, P., Mason, R., & Shipley, G. G. (1977) *Proc. R. Soc. London, A 354*, 157–177.

Emsley, J. W., & Lindon, J. C. (1975) *NMR Spectroscopy Using Liquid Crystal Solvents*, Pergamon Press, Elmsford, NY.

Endo, T., Inoue, K., Nojima, S., Sikiza, T., Ohki, K., & Nozawa, Y. (1983) *J. Biochem. (Tokyo) 93*, 1–6.

Gally, H.-U., Pluschke, G., Overath, P., & Seelig, J. (1981) *Biochemistry 20*, 1826–1831.

Gigg, R. (1980) *Chem. Phys. Lipids 26*, 287–404.

Hemminga, M. A., & Cullis, P. R. (1982) *J. Magn. Reson. 47*, 307–322.

Hinz, H.-J., Six, L., Ruess, K.-P., & Lieflander, M. (1985) *Biochemistry 24*, 806–813.

Iwamoto, K., Sunamoto, J., Inoue, K., Endo, T., & Nojima, S. (1982) *Biochim. Biophys. Acta 691*, 44–51.

Jarrell, H. C., Giziewicz, J. B., & Smith, I. C. P. (1986) *Biochemistry 25*, 3950–3957.

Jeffrey, G. A., McMullan, R. K., & Takagi, S. (1977) *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem. B33*, 728–737.

Lakhdar-Ghazal, F., & Tocanne, J. F. (1981) *Biochim. Biophys. Acta 644*, 284–294.

Ogawa, I., & Beppu, K. (1982) *Agric. Biol. Chem. 46*, 255–262.

Pearson, R. H., & Pascher, I. (1979) *Nature (London) 281*, 499–501.

Perly, B., Dufourc, E. J., & Jarrell, H. C. (1984) *J. Labelled Compd. Radiopharm. 21*, 1–4.

Quinn, P. J., & Williams, W. P. (1978) *Prog. Biophys. Mol. Biol. 34*, 109–173.

Seelig, J. (1977) *Q. Rev. Biophys. 10*, 353–418.

Seelig, J., & Seelig, A. (1974) *Biochem. Biophys. Res. Commun. 37*, 406–411.

Seelig, J., & Gally, H.-U. (1976) *Biochemistry 15*, 5199–5204.

Shipley, G. C., Green, J. P., & Nichols, B. W. (1973) *Biochim. Biophys. Acta 311*, 531–544.

Skarjune, R., & Oldfield, E. (1982) *Biochemistry 21*, 3154–3160.

Smith, I. C. P. (1984) *Biomembranes 12*, 133–168.

Stockton, G. W., Polnaszek, C. P., Leitch, L. C., Tulloch, A. P., & Smith, I. C. P. (1974) *Biochem. Biophys. Res. Commun. 60*, 844–850.

Strenk, L. M., Westerman, P. W., & Doane, J. W. (1985) *Biophys. J. 48*, 765–773.

Taylor, M., Akiyama, I., & Smith, I. C. P. (1981) *Chem. Phys. Lipids 33*, 153–177.

Wemmer, D. E. (1978) Ph.D. Thesis, University of California.

Wieslander, A., Ulmius, J., Lindblom, G., & Fontell, K. (1978) *Biochim. Biophys. Acta 512*, 241–253.

Wieslander, A., Christiansson, A., Rilfors, L., & Lindblom, G. (1980) *Biochemistry 19*, 3650–3655.

Wohlgemuth, R., Waespe-Šarčevič, N., & Seelig, J. (1980) *Biochemistry 19*, 3215–3321.