Physical Studies of Cell Surface and Cell Membrane Structure.

Deuterium Nuclear Magnetic Resonance Studies of N-Palmitoylglucosylceramide (Cerebroside) Head Group Structure

Robert Skarjune and Eric Oldfield

Abstract: Deuterium Fourier-transform nuclear magnetic resonance spectra of N-palmitoyl,2,3,4,6,6-2H5]glucosylceramide, N-palmitoyl[1-2H]glucosylceramide, N-palmitoyl-[5,6,6-2H3]glucosylceramide, and N-palmitoyl[6,6-2H2]-glucosylceramide have been obtained at 55.3 MHz (corresponding to a magnetic field strength of 8.5 T) for lipids as multilamellar dispersions in excess water at 90 °C, above the gel to liquid-crystal phase transition temperature (Tc = 82 °C). Spectra were also obtained for these same lipids dispersed with 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine, and cholesterol, all in excess water at 90 °C. The results are analyzed in terms of a model in which the lipid undergoes fast axial diffusion, together with a “wobbling” of the polar head group, by mathematical methods similar to those used previously for the choline and ethanolamine head groups in phosphatidylcholines and phosphatidylethanolamines [Skarjune, R., & Oldfield, E. (1979) Biochemistry 18, 5903-5909]. However, contrary to the results obtained in the previous study, which indicated many possible conformations for the choline and ethanolamine head groups, results with labeled cerebrosides yield at most a few orientations for the glucose head group in each of the systems studied. Furthermore, where multiple solutions do occur, they fall within a narrow orientational subspace so that all solutions exhibit the same general features. We also show that the order parameter describing the head group wobble is fully determined for each system, and it indicates a rather mobile structure for the cerebroside head group, in a variety of environments. In each system studied, the polar head group projects essentially straight up from the bilayer surface into the aqueous region, thereby permitting maximum hydration of the four glucose hydroxyl groups by bulk water molecules.

Knowledge of the structure and dynamics of cell surface glycosphingolipids may be of considerable importance for understanding functional differences between normal and transformed cell membranes (Hakomori, 1973, 1975; Tooze, 1973; Clarkson & Baserga, 1974; Lee & Smith, 1974). To date, many studies have shown that these lipids undergo dramatic changes in composition upon malignant transformation (Hakomori & Murakami, 1968; Hildebrand et al., 1971, 1972; Seifert & Uhlenbruck, 1965; Kostic & Buchheit, 1970; Karlsson et al., 1974). These changes involve primarily a simplification of the (polar) carbohydrate residues (Tooze, 1973; Hakomori, 1970, 1971; Robbins & Macpherson, 1971; Sakiyama et al., 1972; Critchley & Macpherson, 1973; Ki-jimoto & Hakomori, 1972; Nigam et al., 1973). This suggests that important biological properties of the glycosphingolipids may be associated with the polar head group region of these molecules. Clearly then, it should be of considerable interest to examine the head groups of various glycosphingolipids by nuclear magnetic resonance (NMR) spectroscopic and other physical methods in order to obtain an understanding of the static and dynamic structures of these species, an approach that might eventually provide valuable insights concerning cellular recognition processes at the molecular level.

In this paper we present the results of deuterium (2H) NMR experiments with aqueous multilamellar dispersions of N-palmitoylglucosylceramide (PGLC) specifically labeled with deuterium in the carbohydrate ring. In contrast to work previously done with 2H-labeled phosphatidylcholine and phosphatidylethanolamine, which yielded poorly defined results (Seelig et al., 1977; Seelig & Gally, 1976; Skarjune & Oldfield, 1979a), we show in this publication that all solutions for the glucose head group region of PGLC in a variety of environments show the same general orientational features. We use a very simple model of molecular motion in which the lipid molecules undergo fast axial diffusion, with the sugar moiety

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ABBREVIATIONS: PGLC, N-palmitoylglucosylceramide; [2,3,4,6,6-2H5]PGLC, N-palmitoyl[2,3,4,6,6-2H5]glucosylceramide; [1-2H]PGLC, N-palmitoyl[1-2H]glucosylceramide; [5,6,6-2H3]PGLC, N-palmitoyl-[5,6,6-2H3]glucosylceramide; [6,6-2H2]PGLC, N-palmitoyl- [6,6-2H2]-glucosylceramide; NMR, nuclear magnetic resonance; DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; DPPPE, 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine; SGADC, N-(2-hydroxystearyl)galactosyldiacylglycerol; PGAC, N-palmitoylgalactosylceramide.
and field-desorption mass spectrometry. It is estimated that an analogue (Skarjune monitored by thin-layer chromatography, purity of the final compounds, and their intermediates, was according to Mackie reacylation step (Skarjune above.

Dow-Corning antiform A was added to the reaction mixture, 8-gluconolactone. The procedure was essentially that of Lowenstein (1963) except for modifications as follows: (1) Dow-Corning antiform A was added to the reaction mixture, (2) the solution was deionized with Amberlite MB-3 whenever the conductivity significantly exceeded that of distilled water. The sugar was crystallized by adding either ethanol or glacial acetic acid to the thick syrup.

[1,2,3,4,5,6,6-2H]-d-Glucose was prepared according to Koch & Stuart (1977, 1978a,b) by catalytic exchange with deuterized Raney nickel. Decolorizing charcoal was added to the glucoside hydrolysis reaction during the last 30 min, and deionization of the sugar solutions was carried out with Amberlite MB-3 whenever the conductivity significantly exceeded that of distilled water. The sugar was crystallized by adding either ethanol or glacial acetic acid to the thick syrup.

[NMR Spectroscopy. 2H Fourier-transform NMR spectra were taken at 55.3 MHz with a "home-built" Fourier-transform NMR spectrometer that consists of an 8.5-T, 3.5 in. bore high-resolution superconducting solenoid (Oxford Instruments, Osney Mead, Oxford, U.K.), together with a variety of digital and radio-frequency electronics. We used a Nicolet 1180 computer, 293B pulse programmer, and Model NLC-2090 dual-channel 50-nsec transient recorder (Nicolet Instrument Corp., Madison, WI) for experiment control and rapid data acquisition, together with a Diablo Model 40 disc system for data storage (Diablo Systems, Inc., Haywood, CA). In order to generate radio-frequency pulses of power sufficient to cover the entire 2H spectral width (≈3-μs 90° pulse widths), we used an Amplifier Research (Amplifier Research, Souderton, PA) Model 200L amplifier to drive a retuned Henry Radio (Los Angeles, CA) Model 2006 transmitter to an ~1000-1500-W output power level. Deuterium NMR spectra were recorded on this instrument by using an 800-μL sample volume and a quadrupole-echo (Davis et al., 1976) pulse sequence. The 90° pulse width varied between 2.0 and 4.5 μs. The 90° pulse widths and phase quadrature between the two radio-frequency pulses were established by viewing quadrature free-induction decay signals of S-[methyl-2H]-methionine. The same settings were used for data acquisition on the 2H-labeled cerebroside dispersions. In essentially all cases, no phase corrections were necessary after Fourier transformation. Typically, data were collected with sampling rates of 5 μs/poin. The instrument zero frequency was established with a 1% D2O reference. The sample temperature was regulated by means of a heated air flow, the temperatures reported being measured with a calibrated Dicor trendicator (San Diego, CA) with a copper-constantan thermocouple. Gas-flow temperatures were measured next to the sample, and separate experiments indicate that this temperature is accurate to ±2 °C over the entire sample volume. Between 4000 and 100 000 scans were accumulated by using typically an 85-ms recycle time. Samples contained between 20 and 200 mg of lipid dispersed in excess deuterium depleted water (Aldrich Chemical Co., Milwaukee, WI) above the gel to liquid-crystal phase transition temperature (Tc = 82 °C). Spectra were recorded at 90 °C. Calculations were performed on a Control Data Corp. Cyber-175 computer, interfaced to a Tektronix 4006-1 graphics terminal and Model 4662 interactive digital plotter (Tektronix, Beaverton, OR) in our laboratory.

Computational Aspects. The mathematical analysis of the cerebroside head group conformation is somewhat similar to that for the choline and ethanolamine head groups in phosphatidyicholine and phosphatidylethanolamine (Seelig et al., 1977; Seelig & Gally, 1976; Skarjune & Oldfield, 1979a) but with the advantage that the cerebroside head group almost certainly contains a rigid carbohydrate ring. Because the carbohydrate ring exists as a well-defined structure (Pascher & Sundell, 1977; Abrahamsson et al., 1977), the results of NMR experiments with deuterium labels at the several different sites should therefore strongly reflect the orientation of the sugar residue. The major limitation in the previous work undergoing an additional "wobbling" motion. Our results suggest that the 2H NMR method should permit determination of polar head group organization in more complex glycolipids, both in model systems and in biological membranes themselves.
with the choline and ethanolamine head groups was that there were too few NMR parameters to describe a structure in which there are clearly numerous likely possibilities for rotational isomerism (Skarjune & Oldfield, 1979a).

In order to interpret our $^1$H NMR data, we have therefore developed the following simple model for head group motion. First, as with other liquid-crystalline lipids, the cerebroside molecules rotate rapidly about their long molecular axes (Skarjune & Oldfield, 1979a,b). This motion is not perfectly collinear with the bilayer normal, and a symmetric wobble of the long axis, or the polar head group, about the bilayer normal may be introduced to account for such disorder. Analogous calculations with phospholipids used a two-site "enantiomeric" exchange motion to describe the motions of the choline and ethanolamine head groups (Seeig et al., 1977; Seeig & Gally, 1976; Skarjune & Oldfield, 1979a). This model was suggested by examination of X-ray crystallographic structures of related compounds, which showed two enantiomeric molecules in the unit cell (Abrahamsson & Pascher, 1966). In the case of cerebroside X-ray crystallographic studies, two independent molecules in the unit cell are also seen, but the differences reflect packing effects in the nonpolar region of the molecule; i.e., the head group regions are very similar (Pascher & Sundell, 1977). We use only a single head group in our model. Thus, the motional model we have chosen is one in which there is a fixed head group conformation and the presence of fast rotation about the bilayer normal, together with a head group wobble of undetermined amplitude about the rotation axis. Previous work (Skarjune & Oldfield, 1979b) has suggested that the hydroxymethyl group of the carbohydrate is effectively rigid relative to the ring; however, recent results indicate the possibility that a two-site exchange occurs for this group both above and below the phase transition temperature (R. G. Griffin, private communication). As a result, no assumption is made about the hydroxymethyl group motions, and the results from deuterium labels at this position are not used in the calculations. The model considers only head group motions, and the remainder of the molecule may undergo multisite reorientations such as the gauche–trans isomerizations thought to occur in the fatty acid side chain (Huang et al., 1980).

In earlier work with the choline head group, the all-trans conformation was chosen as the reference for which all torsion angles were 0° (Skarjune & Oldfield, 1979a). The choline head group is essentially a rigid system, so there is no ambiguity in choosing an all-trans conformation. However, the rigid nonplanar sugar ring in the cerebroside head group requires a different definition of the reference conformation. Analogous to the treatment of choline, in which the glyceryl C1C2C3 was included in the reference, C1 and C2 of sphingosine will be part of the PGLC reference. Proceeding toward the sugar moiety, all torsion angles are made trans (i.e., 0°). The carbohydrate ring is oriented so that C2 and C3 of the glucose residue lie in the plane defined by the previous trans torsion angles. Furthermore, the hydroxymethyl group is oriented so the C2C3C4O5 torsion angle is trans. This arbitrary reference conformation is illustrated in Figure 2.

Also included in Figure 2 is the labeling scheme used for the atoms and torsion angles and the coordinate system of the bilayer normal. C2 of sphingosine (C32) is the origin of the coordinate system. While free rotations about the bilayer normal make this coordinate system axially symmetric, the x and y axes are differentiated to define various rotations and to aid in visualizing the bilayer plane. Thus, all head groups may be freely rotated about the z axis.

![Figure 2: Torsion angle reference conformation and coordinate system. (A) Reference conformation showing labeling for torsion angles and carbon atoms. C2C3C15C6O1C5, and C2 lie in the yz plane. The x axis is the bilayer normal. (B) Rotation about z axis showing the definition of θ1z and labeling scheme used for the oxygen atoms.](image)
determining coordinates for the sphingosine region of the reference conformation while the psychosine was used to calculate the glucose coordinates.

In the previous choline head group calculations, the conformations were tested by rotating about all adjustable torsion angles within a small window about the crystallographic values (Seelig et al., 1977; Seelig & Gally, 1976; Skarjune & Oldfield, 1979 a). Despite the fact that a very small fraction of the total conformational space was examined, a large number of conformations consistent with the NMR results were obtained. Anticipating the advantage offered by the several ring deuterons for determining the orientation of the carbohydrate ring, we thought it possible to examine the entire conformational space without finding unreasonably large numbers of computational solutions. However, with three torsion angles freely variable over 360° and one variable over 180°, the total number of conformations for 1° increments is 8.4 x 10^6. To reduce this number significantly without sacrificing a complete search of ring orientations, we therefore devised an alternative approach. This is accomplished by defining another coordinate system based only upon the orientation of the carbohydrate ring: Figure 3 is a schematic representation. In the new coordinate system, C2 is at the origin, and the z axis is defined by the C2H2 vector. The C2C1 vector is taken to lie on the positive y side of the xy plane, and the z axis is chosen to complete a right-handed Cartesian coordinate system. If A4 is defined as in the previous coordinate system, this new reference orientation is unambiguous. Only three angles, θ, A4, and A6, are needed to completely specify the orientation of the carbohydrate ring (hydroxyl torsion angles neglected as before). θ is a positive rotation about the y axis, and A4 is a rotation about the C2H2 bond axis. As in the torsion angle coordinate system, the z axis is defined to lie along the bilayer normal, and the x and y axes are presented for mathematical and visual convenience. Arbitrary θ, A4, and A6 rotations will generate any possible orientation of the sugar ring, and the resulting orientations of the various CD vectors can be used to calculate the expected quadrupole splittings since

\[
\Delta r_Q = \frac{3}{4} \left( \frac{e^2 q Q}{\hbar} \right) \left( \frac{3}{2} \cos^2 \theta - \frac{1}{2} \right) S_{BN}
\]

where \( e^2 q Q / \hbar \) is the quadrupolar coupling constant, θ is the angle between the CD vector and the bilayer normal, and \( S_{BN} \) is the order parameter describing the magnitude of the rotational wobble. The quadrupolar coupling constant appropriate for labeled carbohydrates was measured from a 2H NMR spectrum of anhydrous, polycrystalline [2,3,4,6,6-2H]-D-glucose and was estimated to be 164 kHz.

That this approach is more efficient than the torsion angle search is immediately apparent from the requirement of only three angles to describe the ring orientation. Neglecting \( A_6 \) as before, the total number of conformations at 1° increments is only 64,800, a much more tractable number.

Our computer program performs the calculations as follows: For each possible positive value of \( S_{BN} \) (positive and negative splittings are indistinguishable), the program mathematically rotates the carbohydrate ring about all possible orientations and determines which of these could yield the experimental data. Recalling that the C1D2 vector is aligned along the bilayer normal in the reference structure, it is clear that there is a simple relationship between the values of \( \theta \), \( S_{BN} \), and the splitting expected for this label. Furthermore, the choice of the C2D2 vector as the \( A_4 \) rotation axis makes the splitting of deuteron 2 (D2) independent of the value of \( A_4 \). The calculations are based upon the experimental spectra obtained from N-palmitoyl[2,3,4,6-2Hs]glucosylceramide ([2,3,4,6,6-2H]PGLC). Solving eq 1 for \( \theta \) indicates that there are a maximum of four values of \( \theta \) for a given splitting and \( S_{BN} \). With the experimental spectrum potentially consisting of five different splittings, a maximum of 20 values of \( \theta \) are possible for each value of \( S_{BN} \). Each of the [2,3,4,6,6-2H]PGLC experimental splittings is assigned to D2 for each value of \( S_{BN} \). The possible values of \( \theta \) are then calculated, and subsequent rotation of \( A_4 \) yields all possible orientations of the carbohydrate ring. The expected splitting for all nonhydroxyl deuterons is calculated by means of eq 1 for each of these. By using 1° increments, this procedure reduces the number of orientations to be tested by a factor of about 18, yet it does not exclude any orientational space where solutions may be found. Each time a splitting is assigned to D2, the remaining experimental splittings are assembled into all possible combinations of two to test for consistency with the calculated splittings for deuterons 3 and 4 (D3 and D4). In this way, every possible orientation that will yield three of the five experimental splittings for D2, D3, and D4 is considered to be a tentative solution. Additionally, each solution found by this procedure must give calculated values for deuterons 1 and 5 (D1 and D5), which are consistent with the values obtained from experiments with dispersions of N-palmitoyl[1-2H]-glucosyleramido ([1-2H]PGLC), N-palmitoyl[5,6,6-2H]-glucosyleramido ([5,6,6-2H]PGLC), and N-palmitoyl[6,6,6-2H]-glucosyleramido ([6,6,6-2H]PGLC). Note that while "exact" values of \( \theta \) can be calculated, \( A_4 \) must be incremented. Experience has shown that 1° increments provide the best combination of resolution and reasonable computer execution time. However, it can be shown from eq 1 that a splitting can change as much as 3338 Hz for only a 1° change in CD vector orientation. Moreover, the crystallographic structures used to generate the reference coordinates report calculated fractional coordinates for the protons, so a certain amount of flexibility must be allowed in the CD vector reference orientations due to uncertainty in the X-ray data. The preliminary calculations allow a ±3338S_{BN} Hz spread about each of the experimental splittings in which the calculated splittings must fall to be considered consistent. This assures that valid solutions are not "jumped over" during the incrementing and allows an uncertainty in the crystal structures on the order of a few degrees. Once the general region of the orientational space in which solutions may be found is determined, the spread is increased from a low value in increments of 100 Hz to ±S_{BN} Hz where X is the minimum value that produces solutions. When these calculations are performed over the entire orientational space for the carbohydrate ring (\( \theta = 0 \rightarrow 180°; A_4 \),


Results and Discussion

We show in Figure 4 typical experimental $^1$H NMR spectra of dispersions of [2,3,4,6,6-$^2$H$_3$]PGLC, [1-$^2$H]PGLC, and [5,6,6-$^3$H$_3$]PGLC, at 90 °C. Splittingfs are determined by comparison with spectral simulations (Figure 4) and are listed in detail in Table I. The spectra of Figure 4 (and other unpublished results) show the presence of a small but relatively broad central component. Simulations including this resonance indicate that the worst case relative intensity (i.e., assuming no contribution from residual HO$_2$H) is about 5% of the total intensity, for each spectrum. When the cerebroside was very carefully repurified by column chromatography, no difference in the spectra was apparent. These facts suggest that the central intensity may be due to a small (≤5%) amount of cerebroside that is not incorporated into the normal bilayer structure but has perhaps been incorporated into some micellar or other disordered structure, which permits averaging of the quadrupole splittings to the small line widths (<1 kHz) observed. The excellent agreement between the rest of the $^1$H spectra and their simulations, obtained with $\eta = 0$ values, strongly suggests, however, that most (≥95%) of the $^2$H line shape is characterized by a zero asymmetry parameter.

Table I: Experimental Quadrupolar Splittings (kHz) for Labeled N-Palmitoylglucoycylceramide

<table>
<thead>
<tr>
<th>PGLC label</th>
<th>PGLC$^b$</th>
<th>PGLC$^c$</th>
<th>PGLC$^d$</th>
<th>PGLC$^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>[2,3,4,6,6-$^2$H$_3$]</td>
<td>5.2</td>
<td>5.8</td>
<td>2.4</td>
<td>4.9</td>
</tr>
<tr>
<td>1-$^2$H</td>
<td>16.0</td>
<td>12.0</td>
<td>11.75</td>
<td>13.5</td>
</tr>
<tr>
<td>[1-$^2$H]</td>
<td>18.5</td>
<td>16.0</td>
<td>12.0</td>
<td>14.0</td>
</tr>
<tr>
<td>[5,6,6-$^3$H$_3$]</td>
<td>19.2</td>
<td>17.0</td>
<td>12.5</td>
<td>14.0</td>
</tr>
<tr>
<td>5-$^3$H</td>
<td>5.2</td>
<td>5.8</td>
<td>2.4</td>
<td>4.9</td>
</tr>
<tr>
<td>[6,6-$^3$H$_3$]</td>
<td>18.5</td>
<td>12.0</td>
<td>12.0</td>
<td>13.5</td>
</tr>
</tbody>
</table>

$^a$ Quadrupole splittings were determined by means of spectral simulations (Figure 4 and additional unpublished data). All spectra were recorded at 90 °C by means of the quadrupole-echo method. All samples were dispersed in excess water; the estimated error is ±0.5 kHz. $^b$ Pure N-palmitoylglucoycylceramide (PGAC). $^c$ Equimolar mixture of PGLC with cholesterol (CHOL). We did not determine if excess cholesterol is present outside the bilayer. $^d$ 17 wt % PGLC in 1,2-dipalmitoyl-sn-glycero-3-phosphocholine. $^e$ 17 wt % PGLC in 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine.

0 → 360°, sets of angles that are possible solutions to the head group orientation are obtained. Furthermore, the entire positive range of $S_{BN}$ is incremented during the calculations, and its value for each solution is noted.

Table II: Calculated Head Group Orientations for Pure PGLC

<table>
<thead>
<tr>
<th>$\theta$</th>
<th>$A_1$</th>
<th>$S$</th>
<th>Spread</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>98</td>
<td>352.0</td>
<td>0.32</td>
</tr>
<tr>
<td>II</td>
<td>103</td>
<td>17.0 - 18.0</td>
<td>0.31</td>
</tr>
<tr>
<td>III</td>
<td>82</td>
<td>172.0</td>
<td>0.32</td>
</tr>
<tr>
<td>IV</td>
<td>77</td>
<td>197-198</td>
<td>0.31</td>
</tr>
</tbody>
</table>

$^a$ All angles in degrees. Spread in hertz.

Table II lists the results of the computer search described above. In this search, all possible combinations of assignments were considered, as well as values of the order parameter ($S_{BN}$) ranging from 0.0 to 1.0, in increments of 0.01. These are analogous to the "quasi-conformations" seen for choline and ethanolamine in a related paper (Skarjune & Oldfield, 1979b); however, in the present case, the advantages offered by the larger number of NMR parameters and the rigidity of the carbohydrate ring have combined to yield only four solutions, each with a spread of no more than 2° in the $A_1$ dimension and discrete values in the $\theta$ dimension.

These results actually represent two sets of symmetry-related solutions. Equation 1 is symmetric about $\theta = 90°$, so a CD vector oriented at $\theta^C$ with respect to the bilayer normal will give the same splitting as another vector oriented at 180° - $\theta^C$. This symmetry is generated in the glucose head group when an additional rotation about $A_1$ of 180° is added. Thus, in Table II the members of the two pairs, I–III and II–IV, are complementary with respect to $\theta$ and supplementary with respect to $A_1$. In the reference coordinate system used in the calculations, this relationship will always hold, and the best refinement possible with no further assumptions is a single pair of solutions.

However, it seems reasonable to suggest that all head group orientations may not be possible for the cerebroside molecule. For example, conformations that would result in a penetration of the polar glycolipid head group into the nonpolar bilayer matrix would not be expected. A 180° rotation of $A_1$ (Figure 2) would be an example of such an unlikely conformation. Specifically, we shall consider any conformation that generates a negative $z$ coordinate, in the coordinate system of Figure 2, for one or more atoms directly bound the carbohydrate ring, to be energetically unlikely and will therefore be deleted. For each set of angles ($\theta_{12}, A_{12}, A_1, A_2$) in the coordinate system of Figure 2, it is possible to determine the corresponding value of ($\theta, A_1$) in the coordinate system of Figure 3. Figure 5 shows the results of a computer mapping between the two coordinate systems. In these calculations, $\theta_{12}, A_{12}, A_1, A_2$ were incremented in 15° steps over their entire ranges, and ($\theta, A_1$) values were calculated for each conformation. If a particular set of torsion angles led to a likely conformation according to the criterion above, the appropriate ($\theta, A_1$) point was plotted; points were not plotted for unlikely conformations. Figure 5 shows the results for the total search, for PGLC. It is clear that there is a sizable ($\theta, A_1$) subspace that is unpopulated.
according to the above criterion. More importantly, the results of Figure 5 show that two of the four solutions listed in Table II fall within the unpopulated subspace. By neglecting these, the number of glucose orientations is limited to solutions I and II in Table II, and the calculated parameters are listed in Table III. Good agreement exists between the experimental and calculated splittings that are consistent with the $\pm 1300 S_{\text{BN}}$ Hz spread allowed.

A “picture” of one of the PGLC head group orientations, showing two views of the glucose ring relative to the bilayer normal, is given in Figure 6A, and it is apparent that the head group is essentially fully extended. This orientation suggests to us that interaction with the water layer is the major determinant of head group orientation. The other solution looks very similar, the primary difference being a clockwise rotation of the ring of 25° in the first view in Figure 6A. What is perhaps surprising is the $S_{\text{BN}}$ of 0.32, which is significantly smaller than the 0.66 value determined for pure DPPC bilayers (Gally et al., 1975; Seelig & Takagi, 1977) to check for consistency. Although the CD vector orientations are somewhat different in the two calculated structures, values of $S_{\text{BN}}$ and $\theta$ are essentially identical.

Lipid Mixtures. Intact biological membranes contain a wide variety of lipids and sterols, and cerebrosides are generally dispersed at rather low concentration, except in the case of the myelin membrane. It is therefore of some interest to examine the effects of changing the lipid composition around the cerebroside upon the head group orientation. We have therefore carried out some preliminary work with [2,3,4,6,6-3H]PGLC dispersed with various phospholipids and cholesterol. At a concentration of 17 wt % PGLC dispersed in each of dipalmitoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylethanolamine (DPPC), and dipalmitoylphosphatidylcholine (DPPC) just above the phase transition temperature of the phospholipid component, liquid-crystalline spectra were obtained, despite the fact that the phase transition temperature of pure DPPC is some 20-60 °C higher than the gel to liquid-crystal phase transition temperatures of the lipids investigated. This concentration of cerebroside is slightly higher than the ~13 wt % galactocerebroside found in myelin (McIlwain & Bachelard, 1971), suggesting that cerebrosides in biological tissues will also be in a liquid-crystalline state. Furthermore, the observed patterns (data not shown) of the quadrupole splittings when compared with those seen in pure PGLC dispersions suggest that the head group orientations are different. To test this idea, we have carried out calculations for PGLC dispersed in DPPC, DPPE, and cholesterol, all in excess water at 90 °C. The phospholipid systems contained

### Table III: Characteristics of the PGLC Conformational Solutions Calculated

<table>
<thead>
<tr>
<th>system $^b$</th>
<th>$\theta$ (deg)</th>
<th>$A_2$ (deg)</th>
<th>$S_{\text{BN}}$</th>
<th>$D_1$ (kHz)</th>
<th>$D_2$ (kHz)</th>
<th>$D_3$ (kHz)</th>
<th>$D_4$ (kHz)</th>
<th>$D_5$ (kHz)</th>
<th>spread (kHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGLC</td>
<td>98</td>
<td>352</td>
<td>0.32</td>
<td>19.6</td>
<td>18.5</td>
<td>18.2</td>
<td>18.7</td>
<td>18.9</td>
<td>1.3</td>
</tr>
<tr>
<td>PGLC:CHOL</td>
<td>103</td>
<td>17-18</td>
<td>0.31</td>
<td>19.1</td>
<td>16.0</td>
<td>18.9</td>
<td>18.2</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>PGLC:DPPC</td>
<td>99</td>
<td>356-357</td>
<td>0.28</td>
<td>17.2</td>
<td>16.1</td>
<td>16.4</td>
<td>16.4</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>PGLC:DPPE</td>
<td>96</td>
<td>16-17</td>
<td>0.21</td>
<td>12.0</td>
<td>12.2</td>
<td>12.8</td>
<td>12.2</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>7-6</td>
<td>0.21</td>
<td>12.8</td>
<td>11.8</td>
<td>12.8</td>
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<td>2.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>98</td>
<td>40</td>
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<td>14.0</td>
<td>14.3</td>
<td>13.9</td>
<td>1.3</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ All angles in degrees. The quadrupole splittings and spread are in kilohertz. $^b$ Samples were all dispersed in excess water and spectra recorded at 90 °C. Systems containing two lipid components were mixed by dissolution in chloroform-MeOH, followed by lyophilization and a 24-h period of evacuation. Equilibration with H$_2$O was carried out at 100 °C.

17 wt % PGLC while we used a 1:1 mole ratio with cholesterol. The splittings obtained from the experimental spectra are listed in Table I, and the characteristics of the solutions are listed in Table III. Typical head group orientations are shown schematically in Figure 6. Note that all of the orientations are essentially fully extended with respect to $\theta$, the major differences between the various systems being in the degree of extension with respect to $A_\perp$. In the case of the DPPC and DPPE mixtures, the $A_\perp$ extension is essentially complete while the cholesterol mixture is somewhat less extended. In the phospholipid dispersions, there are roughly five phospholipid molecules per cerebroside, so it is possible that the more numerous choline and ethanolamine head groups “crowd” the glucose head groups into a more extended orientation.

Note also that the values of $S_{\text{BN}}$ are lower for PGLC dispersed with each of the other lipids, especially the phospholipids (Table II). Considering the qualitative similarity of the orientations of Figure 6, the primary effect of dispersing cerebroside with other lipids is therefore to increase the disorder in the sugar–lipid head group region, due presumably to the increased overall “fluidity” of the lipid bilayer in the region of the sugar head group.
Our calculations for cerebroside show that the carbohydrate head group is essentially fully extended away from the bilayer surface for each of the systems we have examined. The observation of multiple solutions is of limited significance since all orientations have the same general appearance (Figure 6). Thus, these results, based on a search of the entire orientational space of the head group, are far more revealing than similar searches performed for the choline and ethanolamine head groups, which examined a more limited conformational space (Seelig et al., 1977; Seelig & Gally, 1976; Skarjune & Oldfield, 1979a).

Conclusions

The results discussed in this paper represent the first attempt at detailing the structure of a glycolipid in the sugar head group region, in the liquid-crystalline phase. With use of four selectively deuterated glycolipids, and a simple motional model involving only fast axial lipid diffusion together with a wobbling of the sugar residue, the orientation of the sugar residue may be confined to a narrow orientational subspace and the order parameter describing the head group fluctuations determined.

The general features derived from the calculations indicate that the carbohydrate head group is extended away from the bilayer to allow maximum interaction with the bulk water layer. Furthermore, the head group order parameter is quite small, suggesting large amplitude fluctuations of the sugar moiety.

Our results also indicate that ~17 wt % glycolipid may be readily dispersed into liquid-crystalline phosphatidylcholine and phosphatidylethanolamine bilayers and that these lipids have a small but measurable effect on the cerebroside head group structure. Similar experiments and calculations could yield the orientation of cerebroside head groups in a variety of environments, giving information on, for example, protein–lipid interactions, both in model systems and in biological membranes themselves. A similar approach should also be applicable to determining the structures of individual monosaccharide units in more complex glycosphingolipids.

References


