Influence of the membrane surface on glycolipid conformation and dynamics

An interpretation of NMR results using conformational energy calculations

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ABSTRACT Glycolipids constitute an important class of biomolecules that are involved in biomolecular recognition. The importance of carbohydrate head group conformation in such processes is well recognized. Glycolipids typically occur as minor components of the complex heterogeneous matrix of a biological membrane. As a result, the membrane surface may not only influence head group conformation but also serves as a spatial frame in which the glycolipid is oriented and recognized.

In this study, conformational energy calculations have been used to assess the conformational space available to the glucose head group of 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (β-DTGL) in a liquid-crystalline membrane matrix. ^{2}H NMR quadrupolar splittings are calculated and compared with those observed experimentally. This study demonstrates the importance of including surface interactions when considering the conformational space accessible to cell surface carbohydrates. The empirical approach taken here provides considerable insight at the molecular level, and offers the possibility of exploring even more complex systems.

INTRODUCTION

Over the past decade the significance of the biophysical roles of cell surface carbohydrates has been progressively better appreciated and understood. This is particularly true in the fields of blood transfusion (Rydberg et al., 1988), membrane structure (Curatolo, 1987), and tumor immunology (Hakamori, 1986). The elucidation of both the three-dimensional structure and the dynamical properties of the carbohydrate head groups has been of primary concern, and principal difficulty. Three-dimensional structural analysis probes the distribution of conformations of these molecules, whereas dynamical studies involve the investigation of the types, amplitudes, and rates of motion, including conformational equilibria, present in a given system.

Nuclear magnetic resonance (NMR) has been most useful in both the structural and dynamical analysis of glycolipids. In particular, deuterium (^{2}H) NMR has been used to address these very complex but biologically relevant problems in partially ordered environments, such as model membrane systems (Seelig, 1977; Seelig and Seelig, 1980; Smith, 1989; and Davis, 1991). ^{2}H labeling effectively isolates the molecular segment of interest because the intramolecular ^{2}H quadrupolar interaction dominates the NMR spectrum (Abragam, 1961). As a result, the ^{2}H NMR spectrum can be interpreted in terms of an order parameter for the labeled molecular segment *S*. *S* is a measure of the averaged amplitude of motion that the segment undergoes within the time frame of the experiment, allowing the investigation of ordered systems in large superstructures such as are found in biological membranes.

In our recent studies of carbohydrate structure in partially ordered systems, a consistent trend has been observed: the head group *S* is smaller than that of the entire lipid molecule portion (typically, for the head group S = 0.35–0.53 and for lipid S = 0.65; Carrier et al., 1989; Renou et al., 1989; Jarrell et al., 1987*a*, 1987*b*). This indicates that there is some motional averaging about the carbohydrate-glycerol anchor. Although *S* may quantitate the degree of motion, it does not lead directly to a reasonable physical picture of the molecular motion(s) giving rise to the order parameter. Recognizing the potential nonrigid nature of the glycosidic bond, Prestegard and co-workers (Scarsdale et al., 1986, 1988) have described an equilibrium between two possible head group conformations of glycolipids in dilute solution. For the calculations they used NOE results in an NMR pseudoenergy approach (Scarsdale et al., 1986) with the molecular mechanics program AMBER (Assisted Molecular Building with Energy Refinement; Weiner et al., 1986). As the next step in defining the molecular details of the recognition processes involving carbohydrates at membrane surfaces, it is very important to appreciate the types, amplitudes, and rates of motion that the head groups may undergo in highly organized structures such as lamellae.

We have devoted much attention to interpreting the dynamics of the glycolipid 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (β-DTGL; Fig. 1, *left*) in a partially ordered environment (Winsborrow et al., 1991; Auger et al., 1990; Jarrell et al., 1986, 1987*a*, *b*). β-DTGL is a reasonable analogue of many glycosphingolipids where the first sugar unit within the oligosaccharide head group is often glucose. Before addressing the potentially complex motions of the β-DTGL head group in the biologically relevant liquid-crystalline phase, a hierarchy of motions of the glycerol backbone (^{2}H labeled at the glycerol *sn* C3 position) were elucidated. Investigations were carried out first in the gel (Auger et al., 1990) and then in the liquid-crystalline states (Winsborrow et al.,

Abbreviations used in this paper: β-DTGL, 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; ^{2}H-NMR, deuterium nuclear magnetic resonance.

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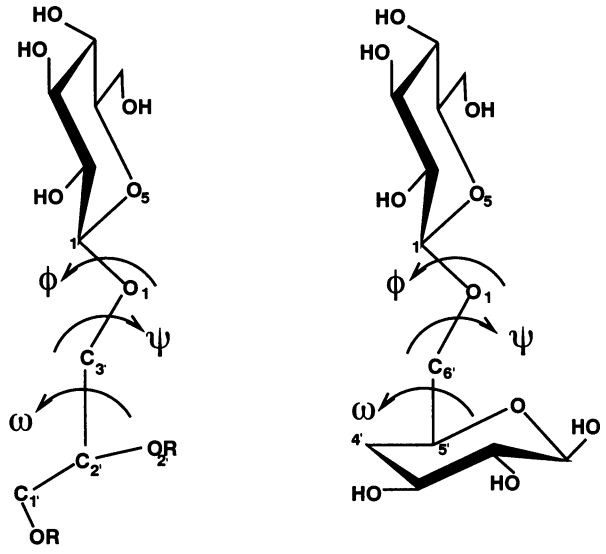

FIGURE 1 β-DTGL molecule and analogue structures. Torsional angles about the glycosidic bond are defined by: ϕ = O5, C1, O1, Cx'; ψ = C1, O1, Cx', C(x − 1)'; ω = O1, Cx', C(x − 1)', O(x − 1)'. Here, x represents position number 3' of glycerol and 6' of 4-deoxy-glucose aglyconic units. Hence, (x − 1) represents position number 2' of glycerol and 5' of 4-deoxy-glucose aglyconic units. (*Left*) Structure used in experiment, β-DTGL ($R = -C_{14}H_{29}$), and analogue structures C and D ($R = -COC_{13}H_{27}$). (*Right*) Structures A and B, 4-deoxy-gentiobiose (note that the 4-deoxy-glucose C6', C5', and C4' positions correspond to the glycerol C3', C2', and C1' atoms, respectively).

1991). By the analysis of orientation-dependent longitudinal relaxation times (T_{1Z} and T_{1Q}) the following three motions were defined in the liquid-crystalline state: an internal large angle three-site jump about the glycerol C2–C3 bond ($\tau_c = 6.7 \cdot 10^{-10}$ s; fast limit, $\omega_0\tau_c \ll 1$) with site populations 0.46, 0.34, 0.20; axial rotation about the molecular long axis ($\tau_c = 8.3 \cdot 10^{-9}$ s; near the T_1 minimum, $\omega_0\tau_c \approx 0.65$), and molecular fluctuations about the order director which gives rise to the glycerol (whole molecule) segmental order parameter, $S = 0.65$. In order to incorporate this model into an overall description of the molecular dynamics the above treatment was also extended to the glucose head group. Although it had been reported previously that there is additional segmental motion about the glycosidic bond ($S = 0.45$, Jarrell et al., 1987*b*), for simplicity the model had considered no motion about the glucose–glycerol linkage. The result was a reproduction of the basic relaxation features of the glucose head group, but it was apparent that motion about the glycosidic linkage was taking place and must be included to give a more quantitative motional description. The glycolipid head group is at the membrane surface, hence, it is implicated in a number of cellular events. Therefore, it is stressed again that the ultimate goal has been to elucidate the glycolipid head group conformation, surface orientation, and dynamics. Although experimental results point to some degree of motion about the glycosidic bond, it is not obvious what such motion(s) entails in terms of conformational averaging.

Insight into the conformational equilibrium that may exist about the glycosidic bond can be gained by means of conformational energy calculations, which define the allowed conformational space for the carbohydrate moiety. Such calculations use a potential energy function and determine the energy of particular conformations through pairwise interactions based on the molecular coordinates. The conformational energy of the structure is minimized by altering the torsional angles about the glycosidic bond (ϕ, ψ, ω, see below; Tvaroska and Perez, 1986). Application of these calculations to the β-DTGL system can provide insight into the nature of the conformational energy surface near the minimum energy structure(s) that may be sampled during conformational exchange.

In this study, conformational energy calculations have been used to assess the conformational space available to the glucose head group of β-DTGL in a liquid-crystalline membrane matrix. ^{2}H NMR quadrupolar splittings are calculated and compared with those observed experimentally. This study demonstrates the importance of including surface interactions when considering cell surface carbohydrate conformations.

COMPUTATIONAL METHODS

The structures

In these calculations molecular coordinates, which are not available from experiment for 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (β-DTGL), are necessary for the evaluation of the conformational energy. Therefore, two types of β-DTGL analogues were constructed in the initial phase of the study. The first, 4-deoxy-gentiobiose, used atom positions taken from the crystal structure of methyl β-D glucopyranoside hemihydrate (Jeffrey and Tagaki, 1977) to construct both the glucose head group and the aglyconic unit (Fig. 1, *right*, structure A). The glucose residue that was intended to serve as an analogue to the β-DTGL glycerol fragment was reduced at the C4' position, generating 4-deoxy glucose. Glucose was linked β 1 → 6 to the 4-deoxy-glucose, generating 4-deoxy-gentiobiose. The aglyconic C6', C5', and C4' positions now correspond to the glycerol C3', C2', and C1' β-DTGL positions, respectively. This is a reasonable representation because the atomic species in the interfacial region that are near enough to the head group to affect its energetics are of the correct type.

A second analogue, 1,2-dilauroyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (Fig. 1, *left*, structure C), was created to represent more closely a lipid molecule and to test the dependence of the results on the choice of models. This molecule was constructed with the same glucose crystal structure but the aglycon is the diacylglycerol portion of the phosphatidyl ethanolamine crystal structure (Elder et al., 1977). This construct is very similar to β-DTGL, except that the lipid chains are ester- and not ether-linked to the glycerol C1 and C2 positions.

Finally, because of potential concerns in using x-ray crystallographic positions of the above hydrogen atoms, in both cases the glucose structure was also used after the methyl β-D glucopyranoside hemihydrate was relaxed by applying molecular mechanics minimization MM2(87) (Allinger 1977; Lii et al., 1989). Fig. 2 is an overlay of the two glucose structures used; the main differences are in the positions of the hydroxymethyl groups and the ring hydroxyl oxygen atoms. Orientation of the

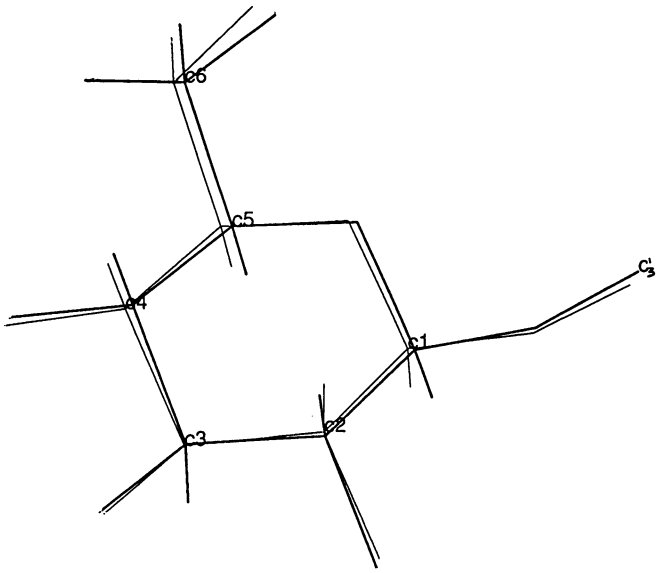

FIGURE 2 Overlay of glucose structures. Light lines are structures using crystal atom positions. Dark lines are structures using crystal atom positions relaxed by MM2.

hydroxyl hydrogen atoms is not important because they are not considered in the conformational energy calculations. Hence, structure B is 4-deoxy-gentiobiose and structure D is 1,2-dilauroyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol, where the crystal glycose molecule is relaxed by MM2 for both structures. In effect, four β-DTGL analogues were generated (structures A–D). In all cases the bridging C–O–C angle was 114° and the glucose hydroxymethyl torsional angle was gauche-plus (g^+, defined by O5, C5, C6, O6).

Calculation of conformational energy

As a first step to understanding the conformational space that the glucose head group of β-DTGL might occupy, PFOS (potential functions of oligosaccharides) conformational energy calculations (Tvaroška and Pérez, 1986) were used to evaluate the conformational potential energy over ϕ, ψ, ω space. These angles are shown in Fig. 1: ϕ = O5, C1, O1, Cx', ψ = C1, O1, Cx', C(x − 1)′, and ω = O1, Cx', C(x − 1)′, O(x − 1)′. Here, x represents position number 3 of glycerol containing aglyconic units (*left side* of Fig. 1) or position number 6 of 4-deoxy-glucose aglyconic units (*right side* of Fig. 1). Hence, (x − 1) represents position numbers 2′ and 5′ of glycerol and 4-deoxy-glucose aglyconic units, respectively. All calculations were carried out on a Micro VAX 3500 computer (Digital Equipment Corp., Marlboro, MA).

These calculations evaluate the van der Waals interactions between nonbonded atoms (using a Leonard-Jones 6–12 potential), torsional contributions about the glycosidic bond, and an exo-anomeric term (Tvaroska and Perez, 1986):

$$\text{PFOS} = E_{nb} + E_t + E_{exo}. \quad (1)$$

The torsional contribution had a threefold periodicity and a barrier V_3 = 1.0 kcal/mol about ϕ, and V_3 = 0.5 kcal/mol about ψ.

The angle ω describes the isomerization about the glycerol C2–C3 bond. In a previous NMR lineshape and relaxation study on β-DTGL in the liquid-crystalline state (Winsborrow et al., 1991) this motion was defined to be a large angle jump with site populations 0.46, 0.34, 0.20: most likely these sites can be characterized by isomerization between the conformers gauche-plus (g^+, 60°), t (trans, 180°), and gauche-minus (g^-, 300°). The angles ϕ and ψ are the angles about the glycosidic bond that were assumed to be fixed in the same study, for the sake of developing a whole body motional model in the liquid-crystalline state. In these calculations ω has the values corresponding to the conformations g^+, t, and g^- as proposed above, and both ϕ and ψ are varied over 360° (with 5° increments).

Surface potentials

A surface term (E_{surf}) was used which included both a surface barrier (V_s), and an intermolecular energy (E_{im}) between an annulus of six static β-DTGL analogue molecules and the central β-DTGL molecule. E_{im} calculated pairwise nonbonded interactions using a Leonard-Jones 6–12 potential. The annular molecules were translated around the central molecule (Fig. 3) and then held fixed for the duration of the calculation with the glycerol C2′–C3′ (or 4-deoxy-glucose C5′–C6′) bond perpendicular to the membrane surface and the glycerol C2′ (or 4-deoxy-glucose C5′) atom at the origin ($\phi \approx t$, $\psi = t$, $\omega = t$). In addition, a surface barrier (V_s) of 30 kcal/mol, similar to that proposed by Nyholm et al. (1989), was applied at the glycerol C2′ (or 4-deoxy-glucose C5′) position, which disallowed any conformation where the hydrophilic head group would lie in the hydrophobic lipid region below this position. The potential energy of the system (E_{sys}) is now described by:

$$E_{sys} = \text{PFOS} + E_{surf} \quad (2)$$

$$E_{surf} = E_{im} + V_s. \quad (3)$$

Another feature of this treatment is the variation in the radius of the surface annulus (Fig. 3). Monolayer and calorimetric studies have estimated the cross-sectional area occupied by β-DTGL in the liquid-crystalline phase to be 102 ± 4 Å^2/molecule (radius ≈ 5.7 Å; Hinz et al., 1985). Therefore, we placed the annulus of surface molecules within a range of radii, which bracketed twice the cross-sectional radius (7.5–15.0 Å), around β-DTGL in order to test the sensitivity of the allowed conformational space to surface constraints.

Calculation of quadrupolar splittings

For a deuterated molecular fragment in a partially ordered environment the ^{2}H NMR spectrum is dominated by the interaction between

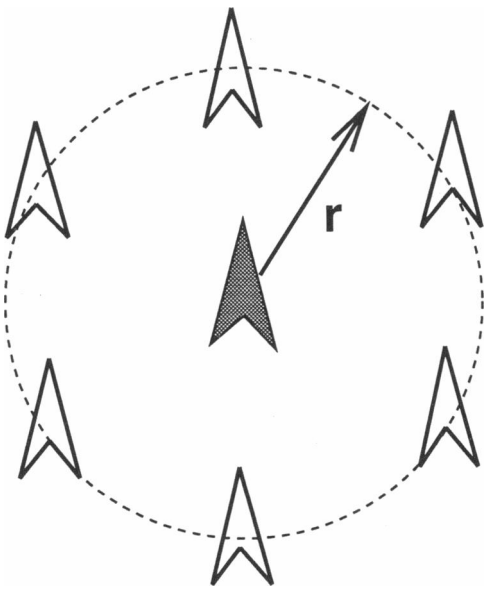

FIGURE 3 Schematic diagram, as viewed from above, of the central β-DTGL analogue molecule (*shaded arrow head*) surrounded by six translated surface analogue molecules that are static ($\phi \approx t$, $\psi = t$, and $\omega = t$). r and the dashed circle indicate that the annulus size is variable.

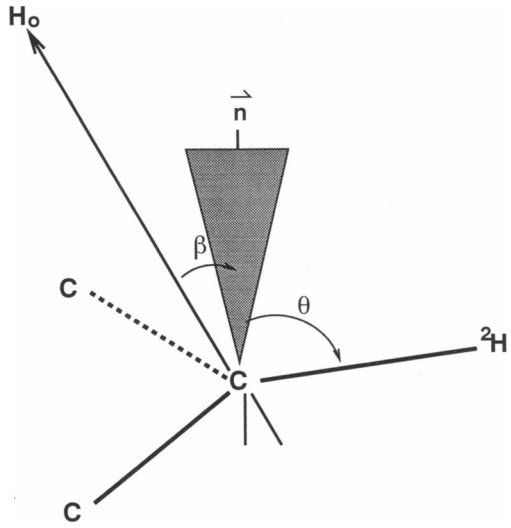

FIGURE 4 Terms used in Eqs. 1 and 2 to assess the quadrupolar splitting. θ defines the angle that the C–^{2}H bond makes with the order director ($\vec{n}$). β defines the angle that $\vec{n}$ makes with the applied magnetic field (H_0). The shaded region represents the molecular motional averaging about n which is quantified by S.

the deuterium nuclear quadrupole moment (eQ) and the electric field gradient (eq) at the nuclear site. This results in a quadrupolar splitting of the ^{2}H resonance. The description of the quadrupolar splitting ($\Delta\nu_{Qi}$) for C–^{2}H$_i$ bonds in experimental systems executing axially symmetric motions is (Seelig, 1977):

$$\Delta\nu_{Qi} = (3/2h)e^2qQ \cdot S \cdot (3\cos^2\theta_i - 1)/2 \cdot (3\cos^2\beta - 1)/2, \quad (4)$$

where e^2qQ is the quadrupolar coupling constant (164 kHz for carbohydrates; Jarrell et al., 1986), θ is the angle between the C–^{2}H bond and the molecular frame, and β is the angle between the order director ($\vec{n}$, which is coincident with the molecular long axis) and the applied magnetic field (H_0, Fig. 4). S is the segmental order parameter (0.45 for the glucose head group; Jarrell et al., 1986), which is a measure of the motional averaging of the molecular fragment with respect to the order director. This is depicted by the shaded region about $\vec{n}$, in Fig. 4. Therefore, each deuterated position (i) on the glucopyranose ring of β-DTGL has a quadrupolar splitting ($\Delta\nu_{Qi}$), which is dependent upon the corresponding θ_i for each C–^{2}H$_i$ bond.

For the calculation of the quadrupolar splitting, it is necessary to know the direction cosine ($\cos\theta$) made by a C–^{2}H vector with the axis about which the order is symmetric ($\vec{n}$, coincident with the molecular long axis, and also, parallel with the β-DTGL glycerol C2–C3 bond; Jarrell et al., 1987*a*). For every ϕ, ψ, ω combination a new set of molecular coordinates are generated; hence, the direction cosine and quadrupolar splitting can be evaluated for each glucose C–^{2}H$_i$ bond at all conformations (with $\beta = 90°$):

$$\Delta\nu_{Qi(\phi,\psi,\omega)} = (3/8h)e^2qQ \cdot S_{gl} \cdot (3\cos^2\theta_i - 1)_{(\phi,\psi,\omega)}, \quad (5)$$

where e^2qQ and θ are the same as in Eq. 4, and S_{gl} is the glycerol segmental order parameter (0.65 for this molecule in the liquid-crystalline state), which, for this model, is used as the molecular order parameter (Winsborrow et al., 1991).

Both S (Eq. 4) and S_{gl} (Eq. 5) have been experimentally determined. However, S for the sugar ring reflects the motional averaging of the glycerol C3 position and the glucose head group about the glycosidic bond, and S_{gl} reflects only the former motion. Therefore, in order to appropriately compare the experimentally determined quadrupolar splitting, $\Delta\nu_{Qi}$ (Eq. 4), with the calculated one, $\Delta\nu_{Qi(\phi,\psi,\omega)}$ (Eq. 5), the calculated quadrupolar splitting has to include the motional averaging of the head group about the glycosidic bond. This is carried out by averaging $\Delta\nu_{Qi(\phi,\psi,\omega)}$ over ϕ, ψ, ω space, as weighted by their conformational populations. The populations of the individual conformers were calculated from their relative energies assuming a Boltzmann distribution:

$$\text{population}(\phi, \psi, \omega) \propto \exp(-E_{sys}(\phi, \psi, \omega)/kT), \quad (6)$$

where $E_{sys}(\phi, \psi, \omega)$ is the conformational energy calculated for a particular ϕ, ψ, ω conformation, and both k and T have their usual meanings. The averaged calculated quadrupolar splitting, therefore, reflects the predicted degree of conformational averaging about the glycosidic linkage superimposed upon the whole body motions defined by the glycerol backbone. The resultant quadrupolar splitting for each deuteron may be compared with that observed experimentally to assess the closeness in fit between the predicted and actual internal motional amplitudes.

RESULTS AND DISCUSSION

Isolated molecule

For brevity, only one set of results of the conformational energy calculations will be presented in detail (from a choice of three ω angles: g^+, t, g^-; and four β-DTGL analogues, structures A–D); any differences in results between molecular models will be discussed in the text. The left panel in Fig. 5 is the ϕ, ψ contour map of structure A ($\omega = g^+$). The global minimum is at $-95°/55°$, for ϕ/ψ, respectively. Similar results were produced with the four analogues that were constructed. Moreover, the results for the gentiobiose based analogues (structures A and B) are similar to those of Neuman et al. (1990) for methyl gentiobioside.

All sets of data indicated that the preferred ϕ conformation was around $-80° \pm 15°$ and a broad range of ψ conformations were energetically allowed. It is more readily apparent that there is a "trough" of local minima along $\phi = -90°$ for structure A, $\omega = g^+$, (and all other analogues along $\phi = -80° \pm 15°$) when the population map is considered (Fig. 5, right). The populations are evaluated at 55°C (to match the experimental conditions) from the potential energies determined for each conformation, assuming a Boltzmann distribution.

The ψ range was 40–300° for the molecules with the 4-deoxy glucose aglyconic unit (structures A and B) and 100–270° for the molecules with the diacylglycerol aglyconic unit (structures C and D). The more restricted conformational space for the latter molecule is not unexpected because this lipid is more bulky at the glycerol backbone due to the carbonyl oxygen atoms that are not present in either structure A, B, or β-DTGL. In fact, these carbonyl oxygen atoms do experience interactions with the ring hydroxyl oxygen atoms when the van der Waals interactions are examined for the individual pairs of atoms.

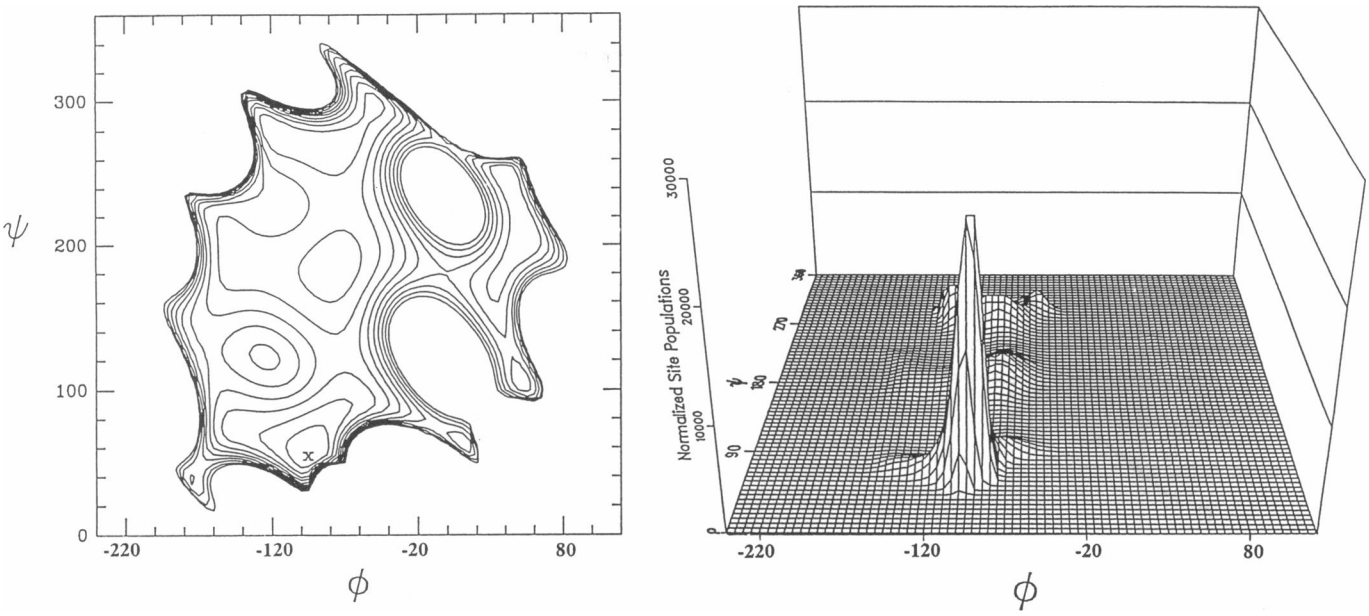

FIGURE 5 (*Left*) Conformational energy as a function of the angles ϕ and ψ for the β-DTGL analogue in vacuo. The analogue molecule is structure A ($\omega = t$). There are 10 contour levels, each contour is 1 kcal; *X* represents the global minimum. (*Right*) ϕ, ψ population map of the same molecule, where the conformational energies have been given a Boltzmann weighting. Note that the most populated sites are ϕ ($-110°$ to $-90°$) and ψ (40° to 300°).

The relative populations for the three global minima, $\omega = g^+, t, g^-$, of structure A are 0.52, 0.32, and 0.16, respectively. Although this compares well with 0.46, 0.34, 0.20, determined independently in NMR line shape and relaxation studies on β-DTGL in the gel state (Auger et al., 1990), the agreement must be fortuitous because the conformational energy calculations have been made only for an isolated molecule. So far there has been no consideration of any membrane surface environment and any conclusions concerning the validity of the conformational description at this point would be premature.

Membrane embedded molecule

Under conditions in which their biological roles are expressed, these lipids clearly do not exist in vacuo; in a membrane matrix the allowed conformational space is very likely quite different. It is certain that some conformations are not possible, for example, the hydrophilic head group should not be found in the hydrophobic lipid region ($-120° < \phi < 100°$ when $\psi < 140°$) which is energetically unfavorable. In analogy with the approach of Nyholm et al. (1989), a hydrophilic/hydrophobic barrier was implemented. A 30 kcal/mol penalty was imposed whenever any carbohydrate atoms were positioned below the surface (which begins at glycerol C2′ and 4-deoxy-glucose C5′). A number of additional conformations were disallowed (result not shown) and the conformational space became more restricted relative to the isolated molecule. However, simply disregarding these conformations in order to simulate a membrane molecule at the membrane surface does not recognize interactions with neighboring molecules which may influence the conformation(s) allowed.

Through a series of steps where softer surface interactions were applied, a more complicated surface matrix was created by spatially translating six static β-DTGL analogue molecules around the central β-DTGL molecule (Fig. 3). Monolayer and calorimetric studies have estimated the cross-sectional area occupied by β-DTGL in the liquid-crystalline phase to be 102 ± 4 Å^2/molecule (radius $\approx$ 5.7 Å; Hinz et al., 1985). Therefore, the surface molecules were initially positioned 11 Å ($2 \cdot r$) away from the central β-DTGL molecule. The conformationally averaged $\Delta\nu_Q$ values were determined by calculating quadrupolar splittings for the glucose deuterons ($^2H_1$, $^2H_2$, $^2H_3$, and $^2H_4$) for each ϕ, ψ, ω conformation and weighting by their probability, based on the conformational population. In the course of the conformational energy calculations the hydroxymethyl group was maintained at only one conformation (g^+). However, it has been established that the exocyclic hydroxymethyl group of glucose in β-DTGL exists in two rotameric forms which are in slow exchange on the ^{2}H NMR timescale (Jarrell et al., 1987*a*). Therefore, the quadrupolar splittings due to the hydroxymethyl deuterons have not been calculated.

The calculated splittings for structure A (Table 1) agree well with those observed experimentally (Jarrell et al., 1987*a*). In order to test the sensitivity of the conformational space to the surface environment these calculations were carried out for all analogue structures (A–D). A variety of surface conditions were used (Table 1): the

TABLE 1 **Conformationally averaged quadrupolar splittings for different surface constraints**

		Quadrupolar splittings (kHz) of glucose deuterons	
β-DTGL Experimental result*		^{2}H1	22.9 kHz
		^{2}H2	22.8
		^{2}H3	24.0
		^{2}H4	26.0

		Conformationally averaged quadrupolar splittings (kHz) of glucose deuterons							
		Radii of surface molecules (Å)							
β-DTGL analogue	^{2}H position	7.5	9.5	10.0	10.5	11.0	11.5	13.0	15.0
	C1	−60.5	**25.1**‡	**20.1**	**20.2**	**20.6**	18.5	−23.4	−10.7
A	C2	−58.0	**23.9**	**18.7**	**17.7**	**18.1**	12.3	−27.9	−14.0
	C3	−55.8	**26.6**	**21.2**	**20.8**	**20.5**	8.2	−31.4	−15.6
	C4	−55.1	**33.5**	**27.6**	**30.8**	**29.3**	15.0	−34.0	−15.2
	C1	−63.3	**22.6**	**28.0**	**23.2**	7.4	5.0	−0.8	−2.8
B	C2	−60.0	**19.5**	**20.7**	**26.7**	5.4	6.5	−5.3	−1.4
	C3	−56.6	**16.4**	**22.7**	**22.6**	9.8	0.7	−10.6	−4.3
	C4	−55.9	**22.4**	**29.4**	**21.4**	8.2	3.4	−0.9	1.8
	C1		**26.2**	**15.2**	9.7	18.5	19.1		
C	C2		**24.7**	**12.8**	7.3	14.4	13.2		
	C3		**27.5**	**16.4**	11.5	13.7	11.5		
	C4		**35.3**	**27.9**	25.4	19.4	11.5		
	C1		**24.4**	**27.1**	**24.9**	0.4	−1.2		
D	C2		**27.2**	**25.1**	**18.6**	−13.6	−11.9		
	C3		**25.5**	**25.3**	**19.5**	−6.9	−9.0		
	C4		**22.9**	**27.1**	**25.9**	3.9	2.4		
	C1		**29.6**		5.2				
E§	C2		**28.5**		1.4				
	C3		**26.6**		3.6				
	C4		**21.3**		15.9				
	C1		36.6		**22.6**				
F‖	C2		31.1		**18.1**				
	C3		31.5		**14.3**				
	C4		37.4		**22.6**				

* Jarrell et al. 1987*a*. ‡Bold indicates calculated quadrupolar splittings that correspond closely with experimental results. §Structure A but surface molecules are rotated by 180°. ‖Structure B but surface molecules are rotated by 180°.

surface annulus was positioned at a range of radii (7.5–15.0 Å) and the static surface molecules were also rotated by 180° about their molecular long axes (structures E and F). As the surface radius was enlarged from 7.5 to 15 Å the conformational area of the contour maps for structure A ($\omega = g^+$, Fig. 6) progressively grew from a small spot to approach resemblance with the contour of the isolated molecule (Fig. 5, *left*). As mentioned above, for brevity, only one set of results is presented because the contour plots for the other analogue structures at all three ω positions (g^+, t, g^-) are similar.

The sensitivity of the conformational space to local environment is demonstrated dramatically when the ϕ/ψ population map for membrane-embedded structure A ($r = 9.5$ Å, $\omega = g^+$; Fig. 7) is compared with that of the isolated molecule (Fig. 5, *right*). The conformational space is very restricted when membrane surface interactions are included in the calculations. The head group conformational space of the membrane-embedded β-DTGL analogue molecule is centered around a conformational well −150°/145°, ϕ/ψ, with a distribution of populated sites (Fig. 7): $-170° \leq \phi \leq -130°$ and $120° \leq \psi \leq 155°$. In contrast, the conformational space of the isolated molecule (Fig. 5, *right*) is centered about −95°/55°, ϕ/ψ, with a much broader distribution of populated sites, particularly in the ψ direction: $-140° \leq \phi \leq -40°$ and $40° \leq \psi \leq 300°$. When ω is set to either g^- or t the conformational space is equally restricted. For $\omega = g^-$ the populated sites are centered about $\phi = -80 \pm 20°$ and $\psi = 170 \pm 15°$. In the case of $\omega = t$, $\phi = -70 \pm 20°$ and $\psi = 235 \pm 15°$. The results for the three different ω values clearly demonstrate that the confor-

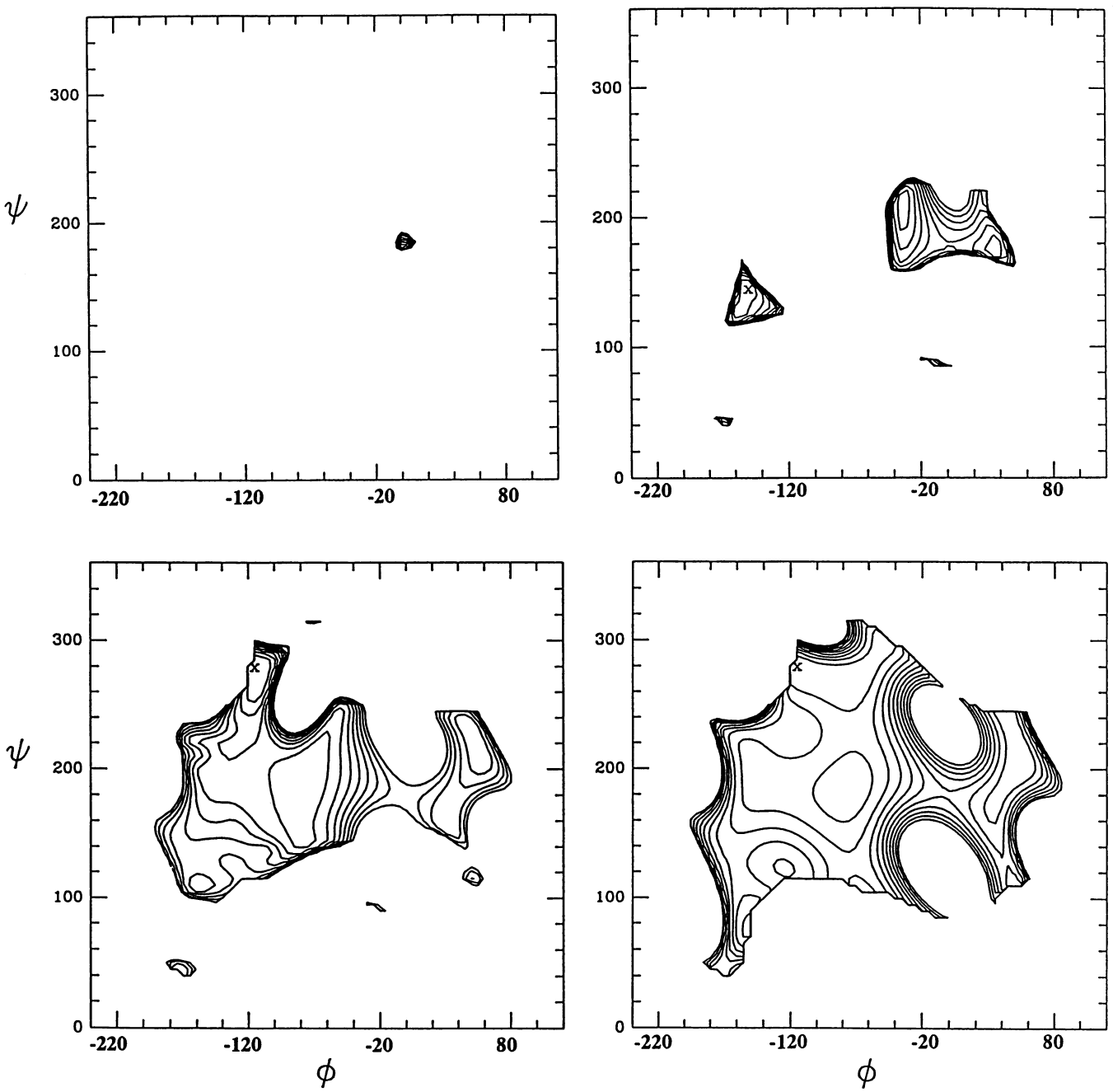

FIGURE 6 Conformational energy as a function of the angles ϕ and ψ for the β-DTGL analogue surrounded by an annulus of six surface analogue molecules at a distance of: (*top left*) 7.0 Å, (*top right*) 9.5 Å, (*bottom left*) 11.5 Å, (*bottom right*) 15.0 Å. The analogue molecule is structure A ($\omega = t$). There are 10 contours, each contour is 1 kcal; *X* represents the global minimum.

mational space is restricted relative to the isolated molecule.

In Table 1 the calculated quadrupolar splittings that agree most closely with those determined experimentally can be seen in bold print. The results suggest that the conformational space of all of the central β-DTGL analogue molecules is tolerant to the presence of surface molecules within a range of distances (9.5–11.0 Å). Upon closer inspection it is apparent that the quadrupolar splittings change more quickly with increasing radius for structure B than for structure A (MM2 and crystal glucose atom positions in 4-deoxy-gentiobiose, respectively). Such rapid changes in the calculated quadrupolar splittings, as displayed with structure B, are usually indicative of a dominant repulsive term (due to the r^{-12} dependence in the Leonard-Jones nonbonding potential). Consideration of the individual interactions reveals that the source of the repulsion is between the glucose hydroxyl oxygen atoms of the central molecule and those of the surface molecules. This implies that the conforma-

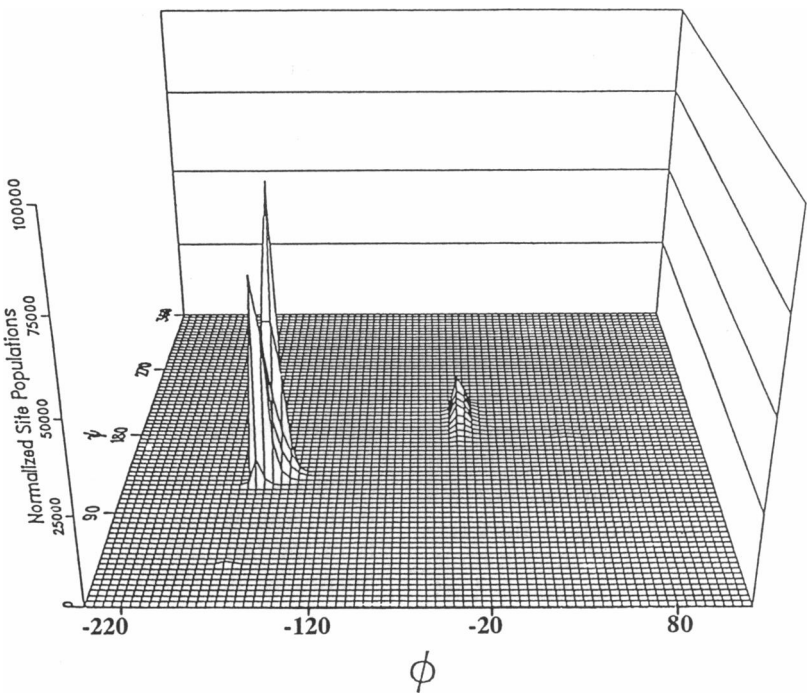

FIGURE 7 ϕ/ψ population map of β-DTGL analogue (structure A, $\omega = t$) surrounded by an annulus of six surface analogue molecules at a distance of 9.5 Å. Note that the most populated sites are ϕ ($-170°$ to $-130°$) and ψ (120° to 155°).

tional space is very sensitive to its environment, particularly to the positioning of the hydroxyl oxygen atoms. Although the change in potential energy around the lowest energy conformations may be small (as can be seen in Fig. 6) this has a dramatic effect on the weighting of the quadrupolar splittings. The angles that the C–^{2}H bond vectors make with the order director in these low energy conformational regions are close to an angle (55.4°, the "magic angle") that causes the quadrupolar splittings to change drastically with small changes in conformation.

The effect of the environment on the conformational space is again noted when the six static surface molecules are rotated by 180° about their molecular long axes (structures E and F, Table 1), effectively changing the apparent nature of the lipid annulus. Not only do the quadrupolar splittings change but the relative sensitivity to the neighboring hydroxyl oxygen atoms for structures A and B are reversed: the quadrupolar splittings for structure A deviate from the experiment at a smaller radius (9.5 Å, structure E) than structure B (10.5 Å, structure F). The energy differences around the energy minima are small, again demonstrating the sensitivity of the quadrupolar splitting to conformation in this region where the angle between the C–^{2}H bond and the order director is near the magic angle.

The results of the calculations on the second type of β-DTGL analogue with the diacylglycerol aglyconic unit (structures C and D) demonstrate a trend similar to that observed for the above systems. Note that the results for structures A and C, with the crystal glucose structure, are very different, whereas those for structures B and D (glucose structure relaxed by MM2) are similar. It is apparent that the conformational space of structure C is more affected by intramolecular repulsions between the glucose and aglyconic atoms than structure D. In particular, the glucose O3 hydroxyl oxygen atom and the glycerol C2 carbonyl oxygen atoms contribute strong repulsions to the energetics of the system. In general, with a molecular annulus spaced 9.5 to 11.0 Å around any of the four β-DTGL analogue molecules, the conformational space of the head group is centered around a conformational well $-150°/145°$, ϕ/ψ, when $\omega = g^+$ (the lowest energy ω conformation) with a distribution of populated sites: $-170° \leq \phi \leq -130°$ and $120° \leq \psi \leq 155°$. When $\omega = g^-$ the populated sites are centered about $\phi = -80° \pm 20°$ and $\psi = 170° \pm 15°$, and for $\omega = t$, $\phi = -70° \pm 20°$ and $\psi = 235° \pm 15°$. It should be reemphasized that although the details (specific conformations and populations) are influenced by the nature of the β-DTGL analogue molecule and annulus, the conclusions are not.

One final result (not shown) is the effect of positioning the membrane surface with respect to the molecular long axis of the central molecule. The deeper into the membrane the molecule was placed (within 2 Å), the more restricted the conformational space became. This approach may be useful in studies concerning the effect of varying lipid chain lengths on heterogeneous membrane systems, lectin binding, and drug–lipid interactions.

COMPARISON WITH PREVIOUS WORK

The concept of modeling surface-bound glycolipids has been considered in the HSEA (hard sphere exoanomeric)

analysis of the conformations of blood group A-active glycosphingolipids (Nyholm et al., 1989). A membrane barrier to head group atoms was implemented which disregarded any conformations with any part of the head group below the membrane barrier. This approach was a necessary first step in the description of a membrane surface, but we have now demonstrated the importance of considering the energetics of membrane interactions (intermolecular effects) more specifically.

Another study has included membrane surface energetics in their calculations. Rudolph et al. (1990) used AMBER to identify minimum energy conformations of saccharide/DMPC systems. They modeled saccharide-lipid interactions by calculating intra- and intermolecular energies between lipid molecules and a saccharide molecule. This approach has similar features to ours, but there are three significant differences. First, we have represented the membrane by a hexagon of surface molecules, and whose radius of separation was modified systematically, whereas only one rectangular matrix was used per saccharide in the earlier study. In addition, we propose a complex equilibrium of conformations, whereas Rudolph et al. focused their attention on a single minimum energy conformation. Finally, we test our results by comparison with experimental observations on a relevant glycolipid system.

In considering the potential nonrigid nature of the glycosidic bond, Prestegard and co-workers (Scarsdale et al., 1986 and 1988) have used an NMR pseudoenergy approach with AMBER to describe an equilibrium between two possible head group conformations of glycolipids in dilute liquid solution. NMR distance constraints were used as a pseudoenergy term that directed the conformational energy minimization calculations. This approach was also used to identify one favored conformation of membrane surface glycolipid analogues and trehalose in oriented systems (Ram and Prestegard, 1988; Ram et al., 1989; Sanders and Prestegard, 1991). As in our previous studies a number of simplifying assumptions had to be made in order to approach the more complicated partially ordered systems. However, as a means for testing our assumptions and to prevent any bias in the calculations, we chose to compare the calculated results with those observed in NMR experiments, instead of using the NMR results as part of the minimization procedure.

Molecular dynamics simulations of oligosaccharides in solution have been used to demonstrate that the head groups are not rigid but do exhibit a great deal of flexibility in the picosecond timescale (Edge et al., 1990; Yan and Bush, 1990). This type of calculation would be ideal for completing the dynamic description of the carbohydrate-aglyconic linkage in partially ordered environments. However, the timescale of the motions for the β-DTGL system has been identified, by ^{2}H NMR relaxation studies, to be in the nanosecond range (Winsborrow et al., 1991). Although a molecular dynamics approach is not practical for partially ordered environments, Pastor and co-workers (1988*a*, *b*) have demonstrated that a Brownian dynamics simulation can be used to describe quantitatively the motions of membrane bilayer lipid chains in the nanosecond timescale. However, the authors also pointed out that assumptions had been made that obviated conformational energy calculations of head groups. Therefore, at this point in time, neither molecular nor Brownian dynamics simulations are feasible for the ordered glycolipid system most relevant to the biological situation.

CONCLUSIONS

Previous ^{2}H NMR analysis has shown that the time scale of the head group motion for liquid-crystalline β-DTGL is in the fast limit motional regime ($\omega_0\tau_c \ll 1$) (Winsborrow et al., 1991) and that the glycosidic bond of a glycolipid may be flexible. In order to define what is meant by "flexible" a quantitative description of these motions is necessary. At this point the conformational space of the β-DTGL head group is best described as a complex equilibrium of conformations centered about a conformational well (the ϕ/ψ minimum is around $-150°/145°$), with a distribution of populated sites: $-170° \leq \phi \leq -130°$ and $120° \leq \psi \leq 155°$.

Admittedly, the present approach uses an extremely simplified description of the restraints on β-DTGL head group conformations because of the restrictive assumptions that have been made; no solvent was used, the surrounding membrane molecules were held fixed on a lattice, and the interactions have no temporal scale (as incorporated in simulations of molecular and Brownian dynamics). However, the predicted NMR parameters are in reasonable agreement with experiment, which indicates that additional insight into the nature and amplitude of the head group motion (not available directly from experiment) is derived from such a simple approach.

The use of an expandable surface annulus has shown that molecular energies are sensitive to spatial packing. Ideally, it would be beneficial to explore further the effect of modifying the surface annulus in an attempt to represent more realistically the actual membrane surface (i.e., vary the surface molecule conformations and include other membrane molecules). One feature that was noted but not pursued in this study was the effect of positioning the height of the membrane with respect to the molecule under consideration. The empirical approach taken here provides considerable insight at the molecular level, and offers the possibility of exploring even more complex systems.

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