Torsion angle analysis of glycolipid order at membrane surfaces

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ABSTRACT A method is presented for determining the average glycosidic torsion angles and motion about those angles for a glycolipid headgroup at a model membrane surface. Dipolar and quadrupolar coupling constants were previously collected on the headgroup of β-dodecyl glucoside embedded in phospholipid/detergent bilayers which orient in a magnetic field (Sanders, C. R., and J. H. Prestegard. 1991. *J. Am. Chem. Soc.* 113:1987–1996). These observables are expressed as averages of second order spherical harmonics, and Wigner rotation matrices are used here to transform the spherical harmonics from the laboratory frame to a set of frames which allow motional averaging to be described as the result of simple bond rotations. Euler angles corresponding to rotations about glycosidic torsion angles ϕ and ψ are chosen to best reproduce experimental coupling constants, using models which have varying degrees of motional averaging. These models include a rigid headgroup, axially symmetric headgroup motion, and independent motion about each torsion angle in a square well potential.

The square well model proves to be significantly better than the rigid model in reproducing experimental observations and it offers a more physically meaningful description of motion than the axially symmetric model. The structures obtained, assuming a square well potential, are compared to potential energy maps for the glycolipid torsional angles to illustrate the need for inclusion of the membrane interface in energetic modeling of glycolipid conformations.

INTRODUCTION

Carbohydrates occurring as parts of either glycolipids or glycoproteins at the surfaces of membranes frequently serve as receptors for molecules which enter or modify target cells (1–3). The conformational properties of these moieties at the membrane surface has therefore been the subject of a number of studies (4–12).

The recent development of phospholipid/detergent bilayers, which orient in a magnetic field, has provided a new medium for studying the structure and dynamics of membrane-associated molecules using NMR spectroscopy (13). Using this medium, dipolar couplings, quadrupolar couplings, and chemical shift anisotropies can be easily measured and used to probe the average orientation and motion of saccharide headgroups (14, 15).

Analysis of these NMR data in terms of an order matrix describing their structure and dynamics has been described (14). The order matrix approach is attractive because it is a completely general approach (16). It describes the average motion of a molecule about its principal axes regardless of the source of that motion. For molecules whose degrees of motional freedom are restricted to rotations about certain bonds, however, the order matrix solution cannot easily be interpreted as average torsion angles for bonds and motional averaging about those bonds. In the case of glycolipids, a solution describing the orientation and motion of the head group as rotations about torsion angles would be attractive.

We present here a new method for using the coupling constants and other data from oriented media to determine the average torsion angles and the motion about those angles for a molecule whose motional degrees of freedom are limited to bond rotations. The method is used to reinterpret the dipolar coupling data for β-dodecyl glucoside in the phospholipid/detergent bilayers. We superimpose the structures we obtain onto potential energy maps for the glycolipid torsional angles generated by molecular modeling with a term in the target energy function representing the interaction of the glycolipid with the membrane interface.

THEORY

For a molecule undergoing motional averaging, the dipolar coupling constant in the laboratory frame is given by

$$D_{i,j} = \frac{-\gamma_i \gamma_j h}{2\pi^2 r^3} \left\langle \frac{3\cos^2\theta - 1}{2} \right\rangle \qquad (1)$$

where θ is the angle between the internuclear vector and the applied magnetic field (17). The $\gamma_{i,j}$ are the magnetogyric ratios for the interacting spin 1/2 nuclei, h is Planck's constant, and r is the internuclear distance. In isotropic solution the average of the angular term, $\langle (3\cos^2\theta - 1)/2 \rangle$, equals zero, so dipolar coupling is not observed. In oriented samples, however, dipolar coupling is not averaged to zero, but is reduced by an amount dependent upon the motional averaging the sample is undergoing.

The motional averaging of a glycolipid headgroup at the surface of a phospholipid/detergent bilayer is the result of several different types of motions. The bilayer itself orients with the bilayer normal perpendicular to the magnetic field, and wobbles about this average orientation. This orientational averaging is dependent on micelle size, micelle concentration, and other factors. It can be represented by the axially symmetric order parameter S_{micelle}.

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The glycolipid is also rotating and oscillating within the bilayer, with the long axis of the acyl chain along the bilayer normal and the headgroup in solution. Again, the motional deviation of the acyl chain from alignment with the bilayer normal can be described at each position of the chain by the axially symmetric segmental order parameter, S_{mol}. The product of these two terms at any position in the chain can be determined experimentally by measuring the quadrupolar coupling constant for the molecule of interest deuterated near the attachment site of the acyl chain. The measured value of $S_{\mathrm{mol}}S_{\mathrm{micelle}}$ for β-dodecyl glucoside deuterated at the C1′ position and dissolved in DMPC/CHAPSO (3:1 mole ratio) bilayers which are 70% water by weight is −0.20 (14).

The monosaccharides of a polysaccharide headgroup may be treated as rigid rings, but the monosaccharide units themselves are free to rotate about the bonds connecting one saccharide to another and one saccharide to a lipid chain of the bilayer. Transformation of Eq. 1 from the laboratory frame to a frame whose z axis is parallel to the long axis of the acyl chain results in the following:

$$D_{i,j} = \frac{-\gamma_i\gamma_j h}{2\pi^2 r^3} S_{\mathrm{micelle}} S_{\mathrm{mol}} \left\langle \frac{3\cos^2\theta' - 1}{2} \right\rangle. \tag{2}$$

Here θ' is the angle between the internuclear vector and the long axis of the acyl chain. The averaging denoted by the brackets arises only from bond rotations. The motions about the various bonds may not be axially symmetric, so completely describing the orientation and dynamics of the headgroup requires specification of five independent elements of an order matrix for each saccharide unit of the headgroup.

In our original treatment, elements of an order matrix were introduced by transforming the spherical harmonic, $\langle(3\cos^2\theta - 1)/2\rangle$, in Eq. 1 from the lab frame with z axis along the magnetic field to a frame fixed in the saccharide ring (18).

$$\left\langle \frac{3\cos^2\theta - 1}{2} \right\rangle = A_{zz}S_{zz} + \frac{1}{3}(A_{xx} - A_{yy})(S_{xx} - S_{yy}) + \frac{4}{3}(A_{xy}S_{xy}) + \frac{4}{3}(A_{xz}S_{xz}) + \frac{4}{3}(A_{yz}S_{yz})$$

where

$$A_{p,q} = \frac{(3\cos\alpha_p\cos\alpha_q - \delta)}{2}$$

and

$$S_{p,q} = \left\langle \frac{(3\cos\theta_p\cos\theta_q - \delta)}{2} \right\rangle.$$

The $\cos\alpha_p$ are the direction cosines of the internuclear vector in the molecular frame and the order matrix elements $S_{p,q}$ are order parameters describing the orientational order of the molecular axes, p, q, with respect to the z axis. The direction cosines are fixed by molecular geometry for a rigid monosaccharide headgroup and five experimental measurements are required to measure the five independent $S_{p,q}$. Diagonalization of the order matrix is equivalent to transforming to a coordinate system in which the principal order axis is assumed to be along the bilayer normal. Orientation of the saccharide ring in this system can be derived and $S_{\mathrm{micelle}}S_{\mathrm{mol}}$ can be separated from the principal order tensor.

In the method we present here, motional averaging is interpreted as fluctuations about average torsion angles rather than about axes in a principal order tensor system. As illustrated in Fig. 1 for a simple alkyl glycoside, the motion of the lipid chain through position C1′ is assumed to be axially symmetric. The motion of the headgroup is then a sum of the motion of the acyl chain at carbon atom C1′ and motion about the torsion angles C1-O-C1′-C2′ and 05-C1-O-C1′ (see Fig. 1).

The $\langle(3\cos^2\theta' - 1)/2\rangle$ term of Eq. 2 is the spherical harmonic $Y(2, 0)$ and may be transformed through the series of coordinate systems illustrated in Fig. 1 using the second order Wigner rotation matrices (19). The initial coordinate system is the molecular frame, defined with its $+z$ axis along the C1-05 bond and x axis in the 05-C1-0 plane. The first transformation is made by rotating z into z' about the molecular y axis and then changing the phi torsion angle by rotating about z'. The rotations about y and z' are the Euler rotations (19) beta and gamma, respectively. The second transformation is made by rotating z' through β_2 into z'' and then rotating z'' through the torsion angle psi. The final transformation is from z'' to z''', the axis aligned with the long axis of the acyl chain. These transformations may be written sequentially as

$$\begin{aligned}\langle Y_0^2(\theta')\rangle &= \left\langle \sum_{m_4=-2}^{2}\sum_{m_3=-2}^{2}\sum_{m_2=-2}^{2}\sum_{m_1=-2}^{2}\sum_{m_0=-2}^{2} [D_{m_4,0}(0, \beta_3, 0) \right. \\ &\quad \times D_{m_3,m_4}(0, 0, \psi)D_{m_2,m_3}(0, \beta_2, 0)D_{m_1,m_2}(0, 0, \phi) \\ &\quad \left. \times D_{m_0,m_1}(0, \beta_1, 0)Y_{m_0}^2(\theta, \gamma)] \right\rangle. \end{aligned} \tag{3}$$

θ, γ, and θ' are the spherical polar coordinates of the internuclear vector in the original molecular frame and the frame of the acyl chain, respectively.

The bond angles β_1, β_2, and β_3 should not vary substantially, due to the motion of the headgroup. The motional averaging will therefore occur due to motion about the torsion angles ϕ and ψ and Eq. 3 may be written

$$\begin{aligned}\langle Y_0^2(\theta')\rangle &= \sum_{m_4=-2}^{2}\sum_{m_3=-2}^{2}\sum_{m_2=-2}^{2}\sum_{m_1=-2}^{2}\sum_{m_0=-2}^{2} [D_{m_4,0}(0, \beta_3, 0) \\ &\quad \times \langle D_{m_3,m_4}(0, 0, \psi)\rangle D_{m_2,m_3}(0, \beta_2, 0)\langle D_{m_1,m_2}(0, 0, \phi)\rangle \\ &\quad \times D_{m_0,m_1}(0, \beta_1, 0)Y_{m_0}^2(\theta, \gamma)] \end{aligned} \tag{4}$$

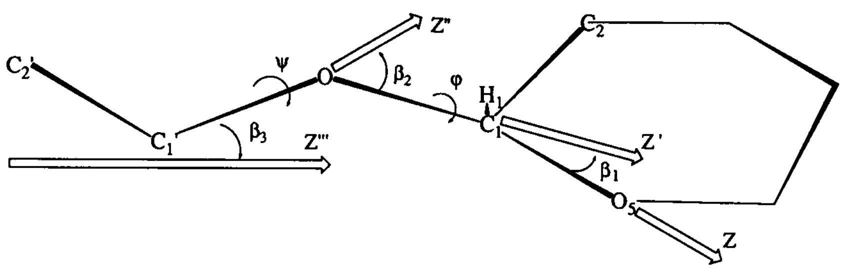

FIGURE 1 The three transformations relating the molecular frame with the z axis along the C1-O5 bond to the frame with the z axis along the acyl chain. The linkage angles β_1, β_2, and β_3 and the rotation angles ϕ and ψ are defined as positive in the direction of the arrows. ϕ and ψ torsion angles are defined according to IUPAC convention (see reference 23) as 05-C1-O-C1′ and C1-O-C1′-C2′, respectively.

Eq. 4 may be used to find the best fit of $\langle D(0, 0, \phi\rangle$ and $\langle D(0, 0, \psi)\rangle$ to experimental dipolar coupling constants. These Wigner elements are products of cosine and sine terms of ϕ, ψ, 2ϕ, and 2ψ. Since cosines and sines in these terms are related, the Wigner elements can be expressed in terms of the four independent variables $\langle\cos\phi\rangle$, $\langle\cos 2\phi\rangle$, $\langle\cos\psi\rangle$, and $\langle\cos 2\psi\rangle$. We note that this is one fewer variable than in the most general order matrix approach. The reduction in the number of variables results from limiting the motion of the molecule to rotations about torsion angles.

The program WIGNER5 was written in C++ to search for values of $\langle\cos\phi\rangle$, $\langle\cos 2\phi\rangle$, $\langle\cos\psi\rangle$, and $\langle\cos 2\psi\rangle$ that are consistent with the dipolar couplings determined from NMR experiments. Given the linkage angles β_1, β_2, and β_3, the program calculates the spherical polar coordinates for bond vectors in the original glycolipid frame. The program then increments the average Wigner rotation matrix elements for values of ϕ and ψ between 0 and 360°. It calculates values of the coupling constants in the laboratory frame and saves combinations of independent variables which reproduce the experimental data within a user-specified root-mean-squared deviation. The values of $\langle\cos\phi\rangle$, $\langle\cos 2\phi\rangle$, $\langle\cos\psi\rangle$, and $\langle\cos 2\psi\rangle$ which fit the data best must be interpreted in terms of particular models of motional averaging.

We reinterpreted the β-dodecyl glucoside dipolar coupling constants using WIGNER5 and assuming three different models of motional averaging. Initial structures were generated with the model-building program AMBER (20) and a set of force field parameters described previously (21). The data are from our recent publication (14) and include thirteen pieces of ^{13}C-^{13}C dipolar, ^{13}C-^{1}H dipolar, and ^{2}H quadrupolar data. The calculations were done on a Sun Sparc Station I. We note that WIGNER5 execution for a typical calculation incrementing angles by 10° required less than two hours, about one-half of the CPU time of a search over the elements of the order matrix, incrementing elements by 0.03.

RESULTS AND DISCUSSION

Rigid model

If the assumption is made that the phi and psi torsion angles are rigid, then $\langle\cos(\phi, \psi)\rangle = \cos(\phi, \psi)$ and $\langle\cos(2\phi, 2\psi)\rangle = \cos(2\phi, 2\psi)$. This allows the headgroup to undergo only the axially symmetric motions of its lipid chain and the bilayer fragment as a whole. It also reduces the number of variables in the search from four to two.

We ran a search over the Wigner elements, incrementing rigid phi and psi angles by 10° and saving solutions with rms deviation less than 15%. This corresponds to an error of approximately six times the linewidth for a determination of a ^{13}C-^{13}C coupling constant. All data were scaled by their maximum expected splitting before calculating an rms value. The solutions are shown in Fig. 2

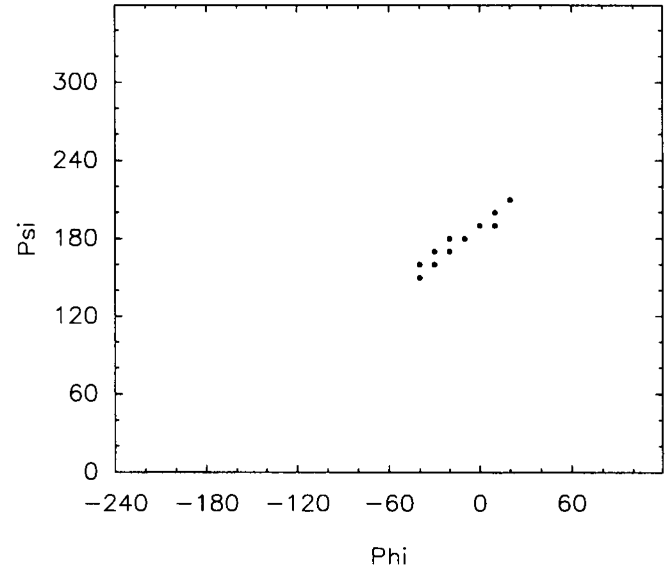

FIGURE 2 ϕ and ψ angles for β-dodecyl glucoside which fit the experimental data in Table 1 with rms deviation less than 15%, assuming a rigid headgroup and motional averaging of the acyl chain described by $S_{\text{micelle}}S_{\text{mol}}$. ϕ and ψ are defined in Fig. 1.

TABLE 1 Experimental and calculated dipolar/quadrupolar coupling constants for β-dodecyl glucoside

Nuclear vector	Experimental coupling constant[*]	Predicted coupling constants from WIGNER5			
		Rigid[‡]	Axially symmetric[§]		Square well[‖]
C1-C2	220 (10)	129	244	249	233
C3-C4	−225 (20)	−302	−200	−202	−184
C4-C5	265 (25)	190	290	295	289
C5-C6	−219 (10)	309	−203	−205	−185
C1-H1	−1,420 (250)	−2,189	−1,727	−1,752	−1,615
C2-H2	−1,520 (250)	−2,031	−1,860	−1,885	−1,943
C4-H4	−1,410 (250)	−2,512	−1,614	−1,640	−1,498
C5-H5	−1,430 (250)	−1,868	−1,858	−1,883	−1,866
C1-C3	|100| (15)	131	86	87	91
C1-C4	|42| (15)	7	39	39	44
C4-C6	0 (15)	11	−9	−9	−14
C3-D	9950 (1000)	6,515	10,990	11,134	10,588
C4-D	8650 (1000)	13,728	8,823	8,962	8,187

[*] Estimated errors in experimental data are in parentheses.

[‡] The rigid model assumes that the ϕ and ψ torsion angles are rigid, so that headgroup motion only results from the motions of its lipid chain and the bilayer fragment as a whole.

[§] The axially symmetric model describes the motion about the ϕ and ψ torsion angles as axially symmetric, adding one additional order parameter to those describing motion of the lipid chain and bilayer fragment. Two solutions for the axially symmetric model are shown, corresponding to the two structures described in the text which fit the data the best.

[‖] The square well potential uses independent parameters to describe motion about each torsion angle. See text for further description.

and Table 1. The structure which fits the data best is ϕ = 0 and ψ = 190, and the rms deviation is 12%. Table 1 shows that this structure does not fit the experimental data very well. The structure is also different from the best average structure determined from the order matrix, which had ϕ and ψ values of −68° and 180°, respectively (14).

Axially symmetric motion about ϕ and ψ

Under the assumption that motion about the ϕ and ψ torsion angles produces simply an additional axially symmetric motion of the headgroup, another order parameter, S_{axial}, is added to the rigid model. This order parameter allows additional motional averaging of the headgroup without attempting to attribute it to motion about either torsion angle.

We ran a search over the Wigner elements, incrementing average ϕ and ψ angles by 10° and incrementing S_{axial} by 0.005. Solutions with rms deviation less then 6% were saved, corresponding to an error of 2 linewidths. These solutions are still outside of experimental error, which we believe to be less than one linewidth. Fig. 2 shows that the average ϕ and ψ angles which fit the data best are clustered near $\phi = -120$, $\psi = 140$ and $\phi = -50$, $\psi = 220°$. The structures $\phi = -120$, $\psi = 140$ and $\phi = -50$, $\psi = 220$ degrees fit the data with rms deviation 3.7% and have S_{axial} values of 0.490 and 0.495, respectively.

The mean value of S_{axial} for the solutions with rms deviation less than 6% is 0.48, with a standard deviation of 0.05. The significant departure from a perfectly-ordered value of 1 suggests that the additional motional averaging of the head group by glycosidic bond rotation is substantial, so that the model of rigid torsion angles is probably not a good one.

Table 1 shows that the axially symmetric model fits the experimental data reasonably well. The solutions in Fig. 3 are also fairly close to the best order matrix solution of $\phi = -68$, $\psi = 180°$, suggesting that the model is useful in predicting the average orientation of the headgroup. Since the axially symmetric model describes the motion of the headgroup with a single order parameter, however, it has the disadvantage of not providing a description of the motion about the two glycosidic torsion angles.

Square well potential

To better describe the amplitude of the fluctuations about the ϕ and ψ torsion angles, a motional model must be assumed with independent parameters for each torsion angle. The correct motional model for β-dodecyl glucoside is not known, but the simplest approach is to use a square well potential. All angles within the well are assumed to occur with equal probability and those outside of it are not allowed. Describing the rotations about ϕ and ψ as square well potentials with center at x and width 2δ, we find

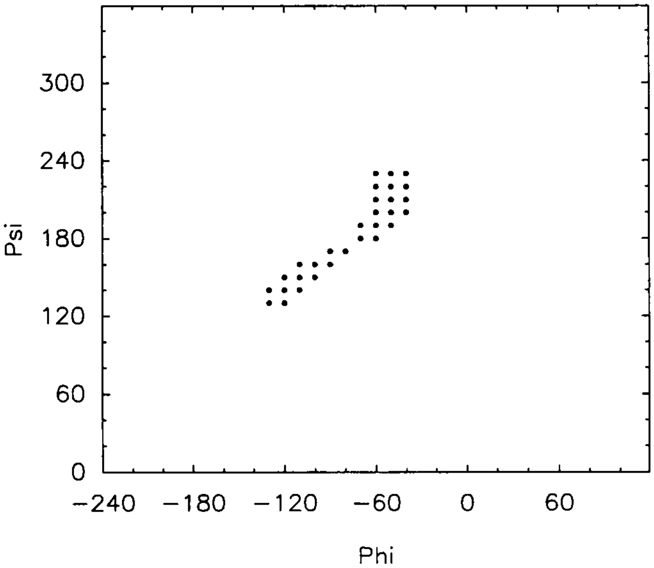

FIGURE 3 Average ϕ and ψ angles for β-dodecyl glucoside which fit the experimental data in Table 1 with rms deviation less than 6%, assuming axially symmetric motion of the headgroup described by S_{axial}. ϕ and ψ are defined in Fig. 1.

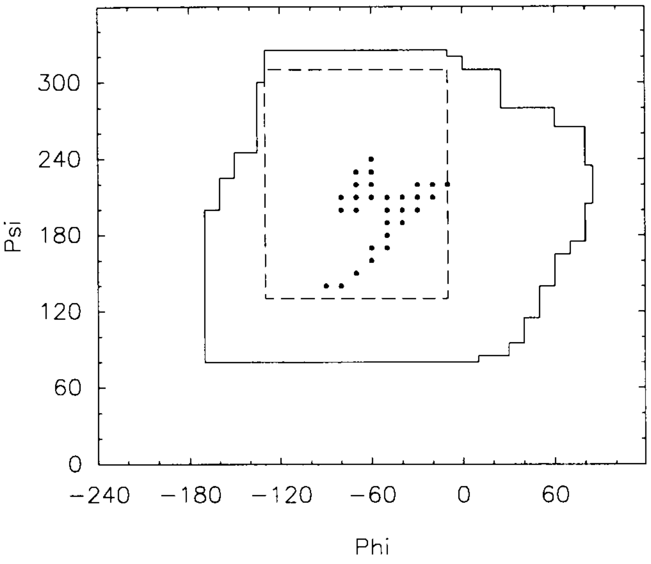

FIGURE 4 Average ϕ and ψ angles for β-dodecyl glucoside which fit the experimental data in Table 1 with rms deviation less than 5%, assuming motion of the headgroup in a square well potential, are plotted as filled circles. The square well potential is centered at ϕ and ψ and widths between 0 and 360° were sampled at 30° increments. The solid line encompasses all conformations which are in the range of motion for any of the solutions with rms deviation less than 5%. The dashed line encompasses all conformations which are within the range of motion for the solution with lowest rms deviation. ϕ and ψ are defined in Fig. 1.

$$\langle \cos \phi \rangle = \frac{\int_{x-\delta}^{x+\delta} \cos \phi}{2\delta} = \frac{\cos (x) \sin \delta}{\delta}$$

$$\langle \cos (2\phi) \rangle = \frac{\int_{x-\delta}^{x+\delta} \cos (2\phi)}{2\delta} = \frac{\sin (2\delta) \cos (2x)}{2\delta}.$$

These values were substituted into the Wigner rotation elements of WIGNER5.

We ran a search over the Wigner elements, independently incrementing the center of the potential wells for ϕ and ψ by 10° and the widths of the potential wells, 2δ, from 0 to 360° in 30° increments, and saving those solutions with rms deviation of less than 5%. The structure that fits the data best has the center of the square well potential for the ϕ rotation at −70°, the center of the square well potential of the ψ rotation at 220°, and an rms deviation of 3.7%. The widths of the square wells for the ϕ and ψ rotations are 120 and 180°, respectively. Table 1 compares predicted and experimental coupling constants for this average structure.

Fig. 4 depicts the distribution of allowed solutions. The dots show the centers of the square well potentials for the solutions with rms deviation less than 5%. The solutions cluster primarily near $\phi = -70$ and $\psi = 220$, with additional solutions near the best average structure, assuming axial symmetry. The solutions with rms deviation less than 10% cluster in the same region as the solutions in Fig. 4, but the distribution is about twice as broad (data not shown). The structures with centers of the ϕ and ψ potential wells near −70 and 220, respectively, are fairly close to the best average structures from the order matrix.

The area within the dashed box in Fig. 4 is the range of motion for the solution, assuming a square well potential with the lowest rms deviation from the experimental data. The amplitudes of the motions about the ϕ and ψ torsion angles are substantial, but this is consistent with the relatively small value of S_{axial} found when allowing only axially-symmetric motion and with the overall size of elements in the order matrix analysis. The amplitudes must also be interpreted cautiously since they are model dependent and a square well potential is certainly an oversimplification of the potential energy map for the headgroup at a bilayer interface.

Structures within the solid box in Fig. 4 are those within the square well potential for any of the calculated solutions with rms deviation from the experimental data of less than 5%. As expected, the solid line encompasses a larger region than the dotted box. Large regions of conformational space are still excluded, however, suggesting that certain conformations of the headgroup are preferred.

The best average structures for β-dodecyl glucoside, assuming a square well potential or axially-symmetric motion, have nearly identical rms deviations. The square well potential, however, has a clear advantage over the axially symmetric model, since it provides a better physical picture of the motion about the glycosidic torsion angles. The general order matrix makes no assumptions about the type of motion about the glycosidic torsion angles and since it fits the data somewhat better than either of our models, we conclude that neither model is ideal.

A better understanding of the conformational preferences of the β-dodecyl glucoside headgroup at a membrane interface may be obtained by combining our experimental data with information obtained from molecular modeling. Molecular mechanics and dynamics programs such as AMBER have been used to model the solution structures of oligosaccharides (21–25). Fig. 5 shows the calculated structures of Fig. 4 as filled dots and the range of motion of the best structure as dotted lines superimposed upon an AMBER-generated potential energy map of β-dodecylglucoside in solution. The local minimum near the calculated structures is quite broad, which is consistent with our finding of significant motional averaging of the headgroup and most of the calculated structures fall within a low-energy region of the potential energy map. However, the map's global minimum (marked with x) and most of the points within 1 kcal/mole of the minimum (marked with open circles) actually fall outside of the range of motion of the best structure.

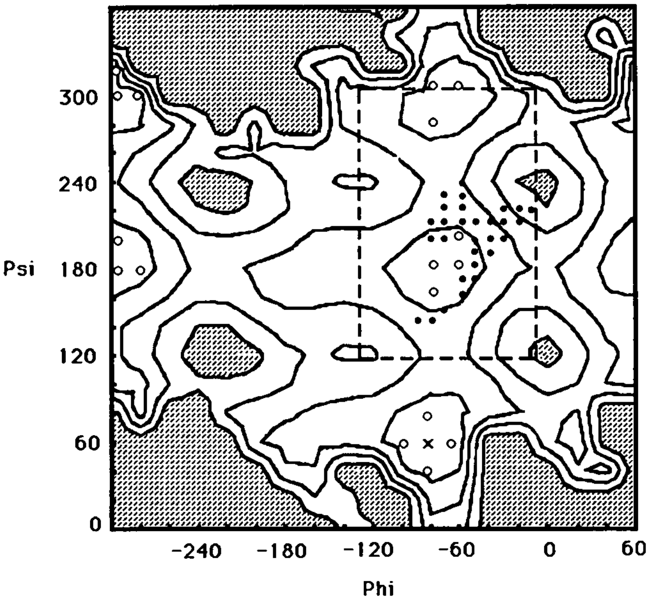

FIGURE 5 The solutions plotted in Fig. 4 are superimposed on a potential energy map. The x is the minimum energy structure in the map and the open circles are structures with energies within 1 kcal/mole of the global minimum. Contours are 1.5 kcal/mole apart. The energy of the cross-hatched area is at least 10 kcal/mole higher than the global minimum. The starting structures of the map were generated by a tree search of ϕ and ψ torsion angles at 20° intervals using the MULTIC option of Macromodel V3.1 (per reference 24). Each structure was minimized using Macromodel's version of the AMBER force field with the glycosidic torsions ϕ and ψ constrained to the starting values. The minimized structures were used as pdb overlays in the EDIT module of AMBER 4.0 and further minimized to convergence. In the AMBER minimizations, ϕ and ψ were constrained, a force field with improved parameterization for oligosaccharides was utilized (25) and the convergence criterion for the norm of the energy gradient was 0.01 kcal mole^{-1} A^{-1}.

The most obvious deficiency in the energetic modeling is the absence of any term describing the interaction of the headgroup with a membrane interface. The presence of the membrane would be expected to change the potential energy map by stabilizing certain conformations and destabilizing others. For example, structures with hydrophilic groups protruding into the membrane and hydrophobic groups protruding into the aqueous phase would be expected to be destabilized. Our laboratory has recently described an energy term to represent the interaction of a glycolipid with its surroundings in the vicinity of a lipid bilayer–water interface, which may be added to the target energy function in AMBER (22). The term includes energy of cavity creation and energy from dipole-induced dipole interactions in a medium of variable dielectric constant.

Fig. 6 shows the same calculated structures in Fig. 5 superimposed upon a potential energy map whose target function includes the membrane-interaction energy term. The inclusion of the membrane potential does not radically change the conformational energy profile. It does, however, stabilize the region near $\phi = -80$, $\psi = 180$ relative to the local minima at $\phi = -80$, $\psi = 60$ and $\phi = -300$, $\psi = 300$. This moves the global minimum to $\phi = -80$, $\psi = 180$, which is very near the best calculated structures, assuming motion in a square well potential. Most of the structures with energies within 1 kcal/mole of the global minimum are also within the range of motion of the best calculated structure.

The position of the calculated structures near the global minimum of the potential energy map when the membrane potential is included suggests that the membrane term improves the accuracy of modeling simple glycolipids at a membrane interface. In the future, information from modeling may be useful in solving the structure and dynamics of membrane-associated molecules, especially when insufficient experimental data exist to uniquely determine a solution.

CONCLUSIONS

A method has been presented which allows the orientational averaging for a glycolipid headgroup in solution at a phospholipid bilayer interface to be interpreted as rotations about torsion angles between the headgroup and the bilayer. The method is an improvement over the previous order matrix approach in that the number of variables is reduced from five to four by allowing only motion about the torsion angles. This speeds computation. The new method also facilitates comparison with predictions from molecular modeling. The average orientation and range of motion is found to be most consistent with predictions from molecular modeling studies when a

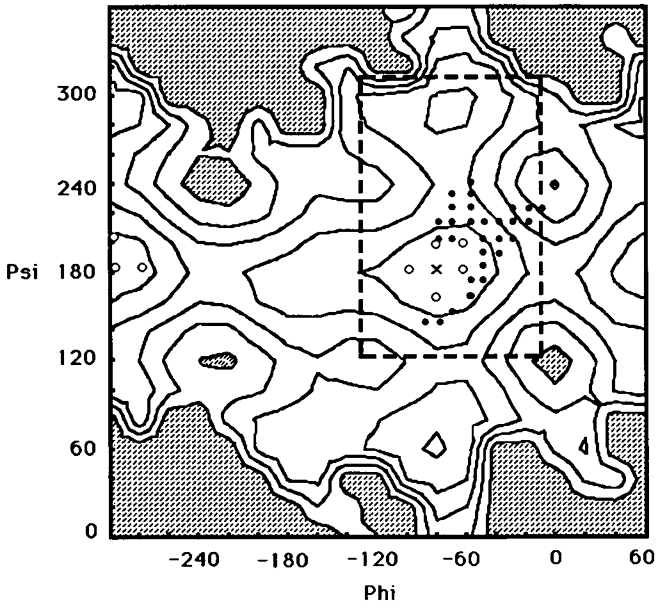

FIGURE 6 The solutions plotted in Fig. 4 superimposed on a potential energy map described in Fig. 5, except that a membrane interaction energy described in the text is included in the AMBER target energy function. Contours are 1.8 kcal/mole apart.

membrane potential is included in the target energy function.

The new method of data analysis may be easily extended to studying the structure and dynamics of polysaccharide headgroups by including rotations about torsion angles between the additional monosaccharide units. In general, dipolar coupling data for each ring can be independently acquired and, when combined with information from molecular modeling, adequate data should exist for a complete structural and motional analysis.

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