Computer modelling of glycolipids at membrane surfaces

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ABSTRACT Interactions of membrane anchored molecules such as glycolipids with a membrane surface are important in determining headgroup conformation. It is therefore essential to represent these membrane surface interactions in molecular modeling studies of glycolipids and other membrane bound molecules. We introduce here an energy term that represents the interaction of molecules with a membrane bilayer. This membrane interaction energy term has been added to the potential energy function of a molecular dynamics and mechanics program and has been parameterized using partition coefficients between an aqueous solution and a vesicular membrane for two model glycolipids.

INTRODUCTION

Conformational properties of molecules anchored at the surface of cellular membranes are clearly of importance to their role as receptors for a variety of physiologically and pathologically active agents. The oligosaccharide headgroups of glycolipids, in particular, are known to serve as receptors for viruses and bacterial toxins (1), as well as markers for cell differentiation and development (2). The combined use of molecular modeling and experimental observation has proven useful in defining solution structures for oligosaccharide headgroups of some glycolipids and membrane glycoproteins (3–7). However, it is conformation as it exists at a membrane surface which is truly of interest. Some experimental methods for the study of oligosaccharides at the surface of membranes are beginning to evolve (8–11). It is clear that these studies would be complemented by an adequate representation of the energetics of membrane surface interactions in programs used to model oligosaccharide conformations.

There have been some efforts directed at computational modeling of membrane surfaces and the interaction of these surfaces with carbohydrates. Some are based on simple exclusion of conformers which dip hydrophilic groups below a nominal interfacial plane (12). Others have attempted explicit inclusion of lipid molecules in a relatively rigid membrane-like array (13). In principle, the latter could be extended to include interfacial mobility using molecular dynamics trajectories of sufficient length, but even for pure lipid bilayers these calculations are very time consuming (14). A simple empirical procedure for representing a time average of membrane surface interactions without the need for long dynamics trajectories should have some utility, particularly when used to complement experimental studies of membrane bound glycolipid conformation.

Membrane bilayers are composed primarily of amphiphilic phospholipid molecules arranged with polar headgroups extended into the aqueous environment and hydrocarbon tails forming the hydrophobic interior. At a minimum, a representation of a membrane interface must include a transition from a hydrocarbon like interior to the polar aqueous exterior as one moves along the normal to the bilayer. A continuous change in effective dielectric constant on polarity of the environment is one way to treat this transition. This treatment fails to recognize the explicit hydrogen bond formation which is so often the focus of membrane surface interactions, however, experimental data suggest that a more general consideration of hydrophobicity and hydrophilicity of carbohydrate moieties may be equally valid (15).

Variations in polarity in both model membrane systems and cellular membranes have been studied by several authors (15, 16). A noteworthy study employs nitroxide spin labels anchored at various positions along lipid chains. The ESR spectra of nitroxide spin labeled compounds are sensitive to polarity changes and the isotropic ^{14}N coupling constant, ΔA, changes as a function of the position of the spin label on the lipid chain. These data provide a preliminary view of a possible smooth polarity variation through the membrane interface. These polarity variations can be expressed in terms of a dielectric constant which in turn modulates both intra- and inter-molecular electrostatic energies. Such energies are key parts of solvent interactions with hydrophilic groups. It is possible that interactions classified as hydrophobic could also be described as depending on similar dielectric constant variations.

Molecular mechanics and molecular dynamics programs such as AMBER (17) have been very useful in modelling conformational preferences of a variety of molecular systems. It would make sense to add an interfacial energy representation to such a program. These programs normally use a dielectric representation of solvation, but only to mediate charge-charge interactions among discrete sites. Unless solvent molecules have been explicitly included, no adequate representation of solute-solvent or solute-membrane interactions are employed. In this paper we will attempt to represent the energy of interaction of an amphiphilic molecule with a membrane surface using a theoretically justified functional form for the energy of interaction of the amphiphile with a dielectric medium which is continuous but

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varies in dielectric properties as one moves along the bilayer normal.

The interactions of molecular dipoles with continuous dielectric media has been studied by Debye, Onsager, and others (18, 19). Onsager developed a formulation for this problem in 1936, as an extension of the Debye theory for the dielectric properties of gases and liquids (19). If we introduce a rigid dipole of moment d into a cavity of radius a in a liquid of uniform dielectric constant ϵ, the electric field which acts on the dipole as a result of the electric displacements induced by its own presence is given by:

$$R = \frac{2(\epsilon - 1)}{2\epsilon + 1}\frac{d}{a^3}. \quad (1)$$

R is also called a reaction field. The energy of interaction of the dipole with the reaction field is given by:

$$E = -\frac{2(\epsilon - 1)}{2\epsilon + 1}\frac{d^2}{a^3}. \quad (2)$$

These energies of interaction are favorable, negative energy contributions which become more negative in higher dielectric solvents such as water.

An expression of this type is most easily integrated with the molecular energy representation of a program like AMBER, by making use of bond dipoles, $r_{ij}(q_i - q_j)/2 = d_{ij}$ and a cavity radius that is a sum of the van der Waals radius, a_{ij}. In practice we will fix a_{ij} at 1.6 Å, a value approximately correct for a C—H bond. Charges are assigned as previously described (20). The energy contribution is then represented by summing over bonds as if they independently interacted with the solvent

$$E = -\sum_{\text{bonds}} \frac{2(\epsilon - 1)}{2\epsilon + 1}\frac{d_{ij}^2}{a_{ij}^3}. \quad (3)$$

This is of course not rigorously correct, but serves adequately, given our objectives of introducing an empirical function of an appropriate mathematical form. Use of bond dipoles rather than one molecular dipole also allows ϵ to vary for different parts of the molecule.

There are other energy contributions not represented in the above expression. One class is loosely referred to as hydrophobic interactions. To represent the hydrophobic interaction we have included a term that is proportional to molecular surface, as described by Sinanoglu and others (21, 22). Terms of this form make positive contributions to the free energy and can be viewed as the energy expended in creating a cavity of appropriate size in a given solvent. These energies are normally expressed in terms of a solvent surface free energy. Rather than introducing a second solvent dependent parameter, however, we will rely on a general tendency for surface free energies to rise with increasing dielectric constant, and for convenience, assume the cavity energy to rise with the same dependence on dielectric constant as the reaction field energy. We will also assume that we can approximate the surface of the molecule by summing over spheres enclosing bonds.

We will weight this hydrophobic term with a constant C. In principle, this constant is related to the surface free energy of the cavity, but given the empirical nature of our approach, we will allow it to be an adjustable parameter. This parameter may also in some way, compensate for our neglect of other surface localized interactions such as specific hydrogen bonds involving water molecules. The complete energy term E_{solv} can therefore be represented as:

$$E_{\text{solv}} = \sum_{\text{bonds}} (2(\epsilon - 1)/(2\epsilon + 1))(\text{Ca}_{ij}^2 - d_{ij}^2/a_{ij}^3). \quad (4)$$

The choice of ϵ is critical to the modelling of a membrane bilayer using the above energy expression. The dielectric constant must vary slowly enough to make expression 3 valid and lie between the two extremes of the aqueous environment and the hydrocarbon interior. As mentioned earlier there is adequate evidence to suggest that the dielectric variation through membranes is gradual and seldom reaches either extreme. The variation in polarity observed in ESR studies by Griffith et al. (15), as one moves along the bilayer normal, provides the starting point for representing the dielectric constant. Griffith's ESR data have a linear dependence on the dielectric constant of the medium in the extremes of the hydrocarbon interior and the aqueous exterior. We have therefore used this data to set the limits of our dielectric function and its overall shape.

The following z dependent dielectric function, where z is a linear measure of displacement along the bilayer normal will mimic the variation shown in Griffith's data

$$\epsilon = A \arctan(nz) + B. \quad (5)$$

A and B are constants adjusted so that calculated dielectrics lie between 2 and 80 at the limits of the arctan function. These correspond to the accepted values of the dielectric constant in a pure hydrocarbon phase and an aqueous phase. A and B are calculated to be 0.4333 and 41.0, respectively. n is a parameter that affects how rapidly variation in ϵ occurs as one penetrates the bilayer. A value of 0.2 approximates the dependence depicted by Griffith et al. (15). Fig. 1 represents this function plotted with $n = 0.2$ along with Griffith's ESR data. The fit to data is well within the limits of experimental uncertainty, which for this case is quite high. We now have, however, an equation that is able to represent available experimental data on the polarity variation through a membrane.

The parameter C, introduced above, is best chosen on the basis of agreement of computed energies with experimental data. Relevant free energies of binding are easily obtained from membrane-water partition coefficients. In what follows, we present binding data on two simple glycolipids, octyl glucoside, and hexyl glucoside. These molecules are commonly used non-ionic detergents and

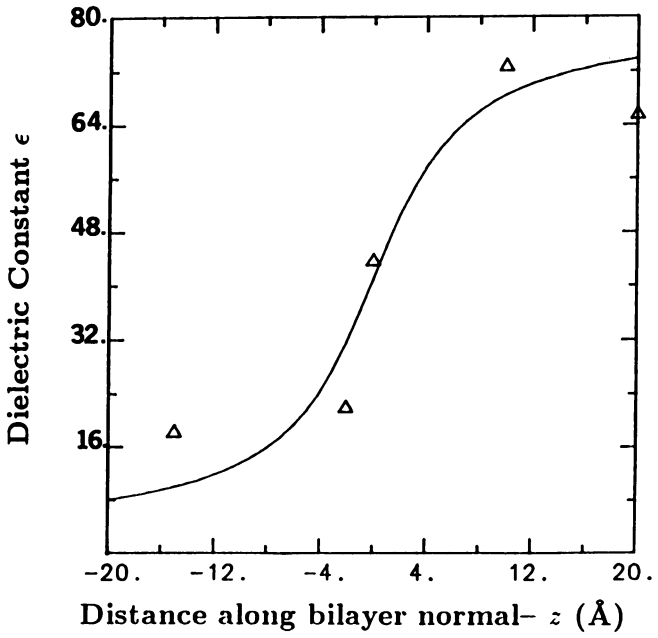

FIGURE 1 The dielectric variation through a membrane. (Δ) Experimental ESR data (8).

are homologous to longer chain alkyl glycosides that we are studying by NMR methods (10). They are small enough to bind to vesicles with constants in a range that makes their determination by a simple dialysis method possible. They are also sufficiently small to make possible optimization of C through iterative molecular mechanics and molecular dynamics calculations.

MATERIALS AND METHODS

Octyl glucoside (Og) was obtained from Sigma Chemical Co. (St. Louis, MO) and used without further purification. Hexyl glucoside (HG) was synthesized using the methods of Paulsen et al. (24). $SnCl_4$ was added to 1-hexanol and β-glucose penta acetate under nitrogen in anhydrous methylene chloride, at −15°C. The reaction mixture was maintained at this temperature for 4 h followed by 4 h at 0°C and 4 h at room temperature. Acetate groups were then cleaved by slow addition of catalytic amounts of sodium methoxide, after which the mixture was maintained at room temperature for 24 h. The product β-hexyl glucoside (65% yield), was purified by flash chromatography.

Phospholipid vesicles composed of diacylglycero-phosphatidylcholine from egg yolk (EYPC) obtained from Avanti Polar Lipids, Inc., were used to provide a membrane surface for glycolipid association. EYPC (0.2 g) was dissolved in 1.8 ml water and sonicated until the sample became translucent (for ~1 h at room temperature in a Branson model E Ultrasonic Generator). 1.42 ml of this solution was placed in the bottom chamber of a Technilab dialysis cell along with a magnetic stir bar. 1.5-ml solutions of OG or HG at various concentrations, were placed in the other chamber. The two chambers were separated by a dialysis membrane, from Spectropor Inc., with a molecular weight cutoff of 6,000-8,000. This allows passage of HG and OG monomers but no redistribution of phospholipid vesicles. The setup was equilibrated, with stirring, for 14 h at room temperature (25°C). 1 ml of glycoside solution in the upper chamber was then transferred to a 10 cm polarimeter cell to measure optical rotation in a Perkin Elmer model 241 polarimeter. Standard runs were used to determine dialysis cell constants and all subsequent, measurements were corrected for dilution based on these constants. Rotations were measured to ±0.001° and converted to concentrations assuming a specific rotation of $[\alpha]_D^0 = -32.0°$ for each compound.

We used the molecular mechanics program AMBER as a basis for our calculations. To the normal potential energy function of AMBER (25) which is made up of a series of empirical energy terms, representing various bonding and non-bonding contributions, we have added the solvation energy term described above. The forcefield for the carbohydrate moiety is that used in our earlier work on modeling structure in the presence of NMR derived experimental constraints (8, 20, 26). Calculations were performed on a Vaxstation 3200 using the double precision version of the AMBER energy minimization software. Both steepest descent and conjugate gradient minimization routines were used and the convergence criterion for the norm of the energy gradient was set to be 0.01 kcal mol^{-1} $Å^{-1}$.

In the initial stages of the calculation only the membrane dielectric term, bond and angle energy terms were included, with the dielectric term initially weighted one order of magnitude higher than the bonding and angular energy terms. Next all the energy terms were added and the weighting on the dielectric term was reduced to one. With this protocol, the average CPU time for convergence of both phases of calculations was 2–3 h. To ensure a real minimum in the molecular mechanics calculations, the minimizations were started from three different positions along the z axis. Depending on the starting position the first step resulted in 10–15 Å displacement along the bilayer normal. After final convergence the first carbon atom of the acyl chain on all three reached the same position on the bilayer normal within ±2 Å. The minimized structures were finally allowed to relax further during constant temperature molecular dynamics simulations. These simulations were typically run for 25 ps, with a 0.002-ps step size and required 2 CPU h. 25 ps are sufficient to effect a displacement of the molecule by up to 20 Å when far removed from the equilibrium point. We feel satisfied therefore that the dynamics run following the molecular mechanics is sufficient to allow for any further movement and to overcome any local minima traps. The final molecular dynamics step produced less than 1 Å displacement of the molecule, when starting from the molecular mechanics results, suggesting a reasonably efficient convergence of the molecular mechanics step.

For comparison of the membrane anchored molecule with one in an aqueous environment, additional calculations were carried out with the final conformations found for the membrane phase but with ϵ of Eq. 4 now set to 80. The differences in these two calculated energies, $(E_{mem} - E_{aq})$, will be compared to the energies, calculated from experimental partition coefficients, K_{exp}.

RESULTS

Partition coefficients for octyl glucoside and hexyl glucoside binding to phospholipid vesicles are presented in Table 1. In all cases these are ratios of concentrations in

TABLE 1 **Partition coefficients**

Concentration (g/ml)		
Initial	Final	Partition coefficient
Octyl glucoside		
0.0150	0.0016	79
0.025	0.0025	85
0.0315	0.0042	59
Hexyl glucoside		
0.0290	0.0104	9.9
0.0650	0.0257	7.1

the membrane phase to concentrations in the aqueous phase. Units are in volume fractions, calculated using a density of 1 g/ml for octyl glucoside, hexyl glucoside, EYPC, and water. As discussed below these units were chosen to minimize entropy of mixing contributions to ΔG^0. Note that the partition coefficients for the final concentrations below 0.0042 g/ml for octyl glucoside and below 0.0257 g/ml for hexyl glucoside appear to be independent of concentration. This is to be expected for a system with minimal interactions between glycolipids at the membrane surface. This is reasonable in that even at higher initial concentrations, the glycolipid is surface dilute. If all glycolipid was bound, only one in five membrane molecules would be a glycolipid. At 0.0042 g/ml, the highest final solution concentration, the partition coefficient for octyl glucoside seems to have decreased slightly. This is easily rationalized. The critical micelle concentration for octyl glucoside is $1.3–2.5 \times 10^{-2}$ M (0.0038–0.007 g/ml) (27). Above this point the chemical potential of octyl glucoside in solution increases little with increasing concentration and we would expect binding to the membrane to plateau. A fixed membrane concentration with increasing amounts of OG in solution would result in a reduced apparent binding constant.

DISCUSSION

The partition coefficients given for octyl glucoside compare favorably to those reported by Ueno (28). Ours are ~30% lower but well within expectation, given the number of assumptions involved in conversion of units. Conversion of the above partition coefficients to free energies of membrane binding is straightforward. The free energy of the glycolipid in water is given by:

$$G_w = G_w^0 + RT \ln X_w, \quad (6)$$

where G_w^0 is the free energy in the standard state and we let X_w be the volume fraction of glycolipid in water. The standard state corresponds to pure glycolipid in a hypothetical state in which all neighbor interactions are actually with water. An exactly equivalent expression applies for the free energy of the glycolipid in the phospholipid vesicles or the membrane phase, with the standard state again a pure glycolipid phase, but now with all the neighbor interactions with the molecules of the membrane interface

$$G_v = G_v^0 + RT \ln X_v. \quad (7)$$

At equilibrium the chemical potential of the glycolipid in the bilayer is equal to that in water: $G_v = G_w$. The unitary free energy of transfer is thus given by combining Eqs. 6 and 7

$$G_v^0 - G_w^0 = RT \ln (X_w/X_m). \quad (8)$$

The ratio X_w/X_v is the inverse of the partition coefficient between bilayers and water expressed in volume fraction units. In the limit where molecules are of similar size, either mole fraction or volume fraction units can be used for the glycolipid concentration in the above expressions to produce hypothetical standard states that are pure glycolipid phases in both cases and minimize contributions from entropy of mixing to ΔG^0 (29). For molecules of unequal size arguments can be made for using Flory-Huggins corrected volume fractions (29). Such corrections have, however, not been applied here.

TABLE 2 **Optimization of hydrophobic parameter c**

	$E_{mem} - E_{aq}$ kcal/mol	
C	OG	HG
0.76	−0.83	2.54
0.83	−1.13	0.25
1.01	−2.44	−1.48
1.26	−6.52	−3.34

The changes in free energies for octyl glucoside and hexyl glucoside, on going from solution phase to the vesicle phase, ΔG_{solv}, are −2.6 kcal/mol and −1.4 kcal/mol, respectively. The change in free energy on adding two methylene groups to the alkyl chain (hexyl to octyl glucoside) is 1.2 kcal/mol, and is in good agreement with 1.3 kcal/mol for partitioning of *n*-alkyl alcohols into EYPC bilayers (23). The free energy obtained above is actually a combination of internal energy, an entropy-temperature term and a pressure-volume work term. Since volume changes are negligible in our system, the last term can be neglected. Some of the contributions to entropy, such as entropy of mixing, can also be assumed to be negligible, based on choice of standard states. Other contributions, arising from conformational degrees of freedom, or solute-solvent interactions for example, cannot be assumed to be negligible. It is the combination of internal energy and residual entropy terms that we wish to model with AMBER calculations. Normally, energies calculated in a molecular mechanics program are considered to be internal energies. However residual entropy-temperature terms are at least partially represented in the hydrophobic interaction term with the adjustable parameter C, now added as a part of the solvation energy.

Molecular mechanics runs were made for both molecules, as described above, followed by a 25-ps molecular dynamics run. These sets of calculations were performed with several different values of the adjustable parameter C, and the resultant energies are represented in Table 2. As expected, a higher value of the hydrophobic effect parameter, C, results in more favorable aqueous to membrane partition energies. Also, higher values of C result in deeper penetration of the molecules into the membrane bilayer. These results are entirely consistent with accepted views of partitioning of amphiphiles. Using the experimentally available energies, −2.6 kcal/mol and −1.4 kcal/mol, we would choose a value of 1.01 to

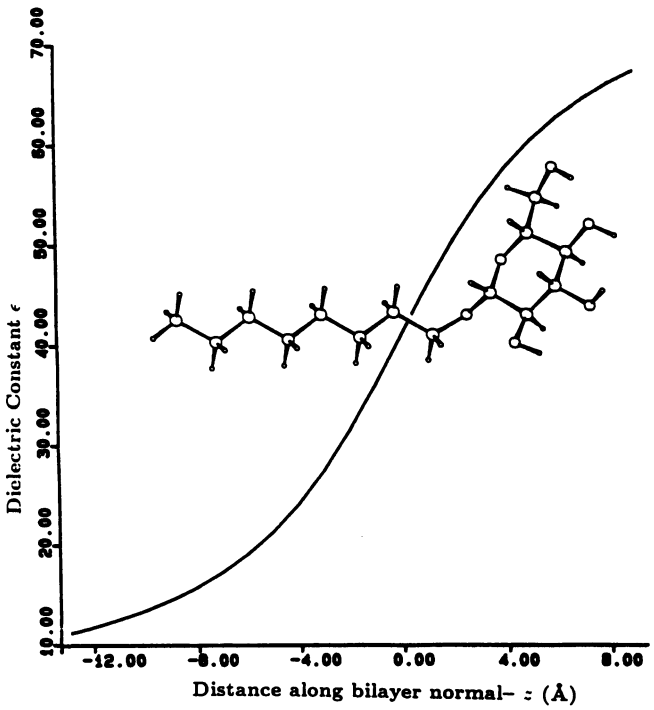

FIGURE 2 Final conformation of OG.

represent the hydrophobic interactions of these amphipathic molecules. During the course of the calculations we came across a local minimum structure which was ~10 kcal higher in energy. The results presented in Table 2 correspond to calculations performed on the lowest energy conformer found. However, minimizations performed with the higher energy conformation and the same value of C also resulted in a very similar binding energies, −2.9 kcal for OG and −1.5 kcal for HG.

The structural implications of calculations performed with $C = 1.01$ and $n = 0.2$ for OG are depicted in Fig. 2 for the most stable conformer. The structure and orientation of the molecule is largely as expected, with the carbohydrate headgroup in the high dielectric phase and the hydrocarbon tail in the lower dielectric phase, and the midpoint of dielectric transition at about the second carbon in the hydrocarbon chain. OG is found to penetrate deeper into the bilayer than HG. The fact that appropriate orientation and levels of insertion into the membrane are maintained, indicates that use of the membrane interaction energy function can effectively exclude unreasonable geometries of membrane bound molecules when modeling is used to seek structures in agreement with experimental data. The energies contributed by the membrane interaction function are not large, they are a few kcal/mol for the molecules discussed here. But this is comparable to variations in individual torsion potentials, and most pairwise non-bonded contributions represented in the normal force field of a molecular mechanics program. They obviously can influence minimum energy conformers predicted by these programs. We therefore expect empirical representations of interactions with a membrane surface to form a useful part of future efforts to model glycolipid surface behavior.

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