Molecular Dynamics Simulations of GlpF in a Micelle vs in a Bilayer: Conformational Dynamics of a Membrane Protein as a Function of Environment

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Octyl glucoside (OG) is a detergent widely employed in structural and functional studies of membrane proteins. To better understand the nature of protein−OG interactions, molecular dynamics simulations (duration 10 ns) have been used to explore an α-helical membrane protein, GlpF, in OG micelles and in DMPC bilayers. Greater conformational drift of the extramembraneous protein loops, from the initial X-ray structure, is seen for the GlpF−OG simulations than for the GlpF−DMPC simulation. The mobility of the transmembrane α-helices is ∼1.3x higher in the GlpF−OG than the GlpF−DMPC simulations. The detergent is seen to form an irregular torus around the protein. The presence of the protein leads to a small perturbation in the behavior of the alkyl chains in the OG micelle, namely an ∼15% increase in the trans−gauche(−)-gauche(+) transition time. Aromatic side chains (Trp, Tyr) and basic side chains (Arg, Lys) play an important role in both protein−detergent (OG) and protein−lipid (DMPC) interactions.

Introduction

Membrane proteins play key roles in many processes in cells, and it is estimated[1,2] that ∼25% of genes code for membrane proteins. Membrane proteins fall into two families: the α-helical family is by far the larger of the two. The folds of its members are made up of bundles of hydrophobic transmembrane (TM) α-helices. The outer membranes of Gram negative bacteria host a second class of membrane protein, based on a *β*-barrel architecture. Despite their functional significance, difficulties in expression and crystallization mean that only ∼50 membrane protein structures have been determined at high resolution. Thus, to accelerate structural studies of membrane proteins, we need to obtain an improved understanding of their interactions with their environment.

Most membrane proteins span a lipid bilayer, and so must contain regions on their surfaces that interact with water (on either side of the membrane), with polar lipid headgroups, and with the hydrophobic core of the lipid bilayer.[3,4] A number of studies have focused on the importance of protein/lipid interactions with respect to the structure and stability of membrane proteins.[5−9]

To purify and characterize membrane proteins, it is necessary to use detergents to disrupt the lipid bilayer. Detergent micelles play an important role in a number of biochemical techniques for membrane proteins, including protein solubilization, purification, and crystallization.[10−13] Mixed detergent/protein micelles have also been used in structural studies of membrane proteins by NMR.[14−21]

1-*O*-Octyl *β*-D-glucopyranoside (commonly referred to as octyl glycoside or OG) is a nonionic detergent consisting of an octane "tail" attached to a glucose "head". It is widely used in studies of membrane proteins[13] and has proved especially useful to solubilize membrane proteins for structure determination by NMR,[21] and for growing crystals for X-ray diffraction studies.[22,23] Indeed, bound OG molecules have been observed in crystal structures of eight membrane proteins (namely, chick cytochrome bc_1, pdb code 1BCC; GlpF, 1FX8; *Rhodopseudomonas acidophila* LH2, 1NKZ; OmpT, 1I78; OMPLA, 1QD5; *Rhodobacter sphaeroides* photosynthetic reaction center, 4RCR; sensory rhodoposin II, 1H2S; Cox-1, 1CVU; and Cox-2, 1EQG). It is therefore of some interest to understand the nature of the interactions of OG with membrane proteins, and to compare this with their interactions with phospholipids.

Molecular dynamics (MD) simulations[24] provide an opportunity to study the conformational dynamics and interactions of membrane proteins. MD simulations have been extended to increasingly complex membrane proteins.[25−28] These simulations are able to provide insights into the nature of the interactions between membrane proteins and their lipid environment.[26,29−33] Current MD simulations of membrane proteins are readily able to address time scales of ∼10 ns. This provides improved sampling of details of protein/lipid interactions[34−38] on a time scale comparable to that observed in NMR studies.[16,18]

MD simulations have been widely used to explore the properties of detergent micelles, including those formed by OG.[39,40] There have been rather fewer studies of mixed protein/detergent micelles, namely: (i) simulations of a ten-residue peptide,[41,42] (ii) simulations of the TM α-helix dimer from glycophorin,[43,44] (iii) simulations of a bacterial reaction center in a "micelle-like environment",[45] and (iv) simulations of the outer membrane protein OmpA.[46]

Here, we describe the first extended (∼10 ns) simulation study of a complex α-helical membrane protein in a detergent micelle compared with simulations of the same protein in a phospholipid bilayer. The main motivation for this study is to explore possible differences the conformational dynamics of the protein as a function of environment, and to compare protein/detergent and protein/lipid interactions.

The protein we have chosen to study is the bacterial aquaglyceroporin GlpF.[47] This has been intensively studied by simulation to explore the mechanism of transport and selectivity.[48−51] GlpF was crystallized from OG, which has also been used to solubilize the protein for functional studies.[52] GlpF

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is tetrameric when crystallized[47] but exists as an equilibrium mixture of tetramers, dimers, and monomers when in membranes[52] and is monomeric when solubilized in OG.[53,54] It therefore provides a good candidate for comparative simulation studies.

Methods

MD Simulations. Simulations were performed using GROMACS 3.0.5[55] (www.gromacs.org). The GROMOS96 force field[56] was used, along with the SPC water model.[57] Partial charges and other parameters for OG were derived from the GROMOS96 parameters, and the initial coordinates were taken from an OG molecule in the 1FX8 (GlpF) crystal structure.

The simulation protocols employed were similar to those in previous studies from this laboratory.[46,58] Long-range electrostatic interactions were calculated using the particle mesh Ewald method[59,60] with a 0.9 nm cutoff for the real space calculation. A cutoff of 1 nm was used for the van der Waals interactions. The simulation was carried out at constant temperature, pressure, and number of particles (NPT). The temperatures of the protein, detergent, and water were each separately coupled using a Berendsen thermostat[61] at 300 K, with a coupling constant $\tau_T = 0.1$ ps. The system pressure was isotropically coupled using a Berendsen barostat at 1 bar with coupling constant $\tau_P = 1.0$ ps and compressibility 4.5×10^{-5} bar^{-1}. The time step for integration was 2 fs and coordinates ware saved every 1 ps for analysis. The LINCS algorithm[62] was used to constrain bond lengths. Molecular graphics images were generated using VMD[63] or RasMol.[64]

Setup of the Simulation Systems. The initial configuration of the GlpF−OG micelle (see Figure 1A) took the form of an expanded micelle-like torus around the protein α-helix bundle, with the detergent molecules radiating like spikes from the protein surface. It was composed of semicircular planes of OG molecules. The total number of detergent molecules was 81, on the basis of experimental estimates given by ref 53. Of course, in reality there must be a range of micelle sizes with different numbers of OG molecules per GlpF monomer, as in pure OG micelles (see below) a range of number of detergent molecules is seen, both in experiments and in simulations (see below). Similar initial micelle configurations have been used in MD studies of DPC detergent molecules[65] and of OmpA/DPC mixed protein/detergent micelles.[46] The OG molecules were randomly rotated about their tail axes and placed so that the terminal methyl groups of their tails were a minimum 0.5 nm from the bundle exterior and from each other, so as to avoid van der Waals overlap. Each detergent glucosyl headgroup was approximately equidistant from its nearest neighbors. The GlpF crystal structure (pdb code 1FX8) was placed at the geometrical center of the above-described configuration, followed by the removal of some detergent molecules that were in steric conflict with protein atoms. The system was energy minimized and then solvated by superimposition of a box pre-equilibrated of SPC water molecules. During the initial solvation stage a slightly higher setting of the van der Waals radius was used when the water−OG overlaps were calculated so as to make sure that no water molecules became "trapped" in the hydrophobic core of the micelle at the start of the simulation. The resultant system was subjected to a further stage of energy minimization. This was followed by 1.5 ns equilibration MD simulation during which with the protein heavy (i.e., non-H) atoms were restrained (using harmonic restraints with force constants of 1000 kJ mol^{-1} nm^{-2}). Finally, an unrestrained 10 ns production simulation was performed.

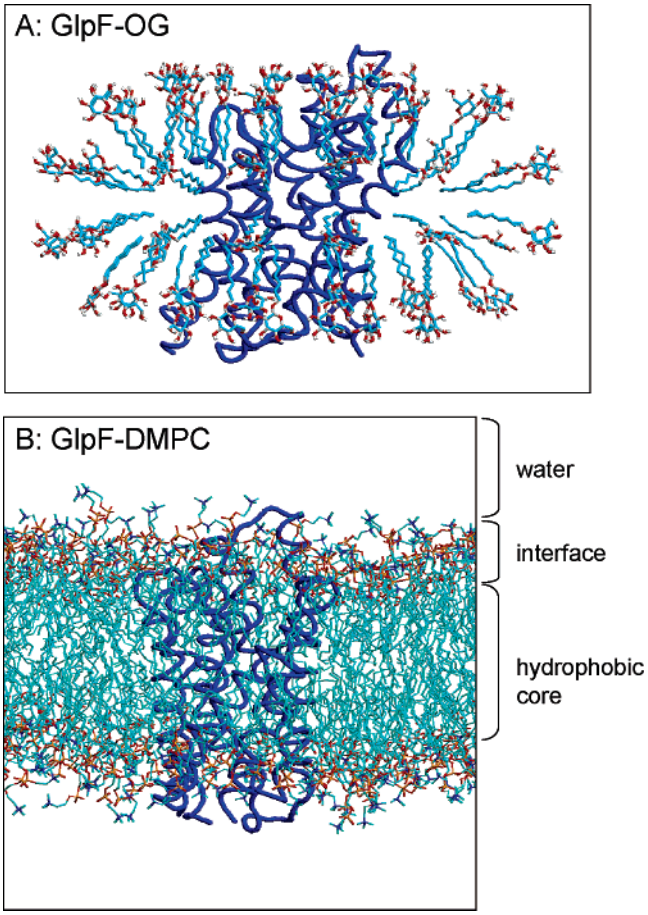

Figure 1. Simulation systems: (A) GlpF in an octyl glucoside (OG) micelle; (B) GlpF in a DMPC bilayer. In both diagrams the protein is shown as a Cα trace blue, and the detergent or lipid molecules are shown in bonds format with C atoms in cyan, oxygen atoms in red, etc. In (B) the location of the bulk water, interface, and hydrophobic core regions are indicated. Diagrams drawn using RasTop.

TABLE 1: Summary of Simulations[a]

simulation name	protein	detergent/ lipid	water	atoms	protein RMSD (nm)
OG		81 OG	7 099	24 241	
GlpF−OG	GlpF	81 OG	10 333	35 366	0.21
GlpF-DMPC	GlpF	264 DMPC	15 050	59 703	0.22

[a] All simulations were run for 10 ns. *Cα RMSD of GlpF from starting structure over last 2 ns of simulation.

A pure OG micelle was also constructed (as a control simulation; see Table 1). The initial configuration was constructed as for the GlpF−OG micelle (i.e., 81 detergent molecules), except that a smaller initial radius was used, such that the geometry was spherical rather than toroidal. We note that previous simulation studies of pure OG micelles suggested that micelles with between 10 and 75 OG molecules were stable,[39] corresponding to experimental estimates of between 20 and 100 OG molecules per micelle.

For the GlpF-DMPC simulation, a GlpF molecule was inserted in a pre-equilibrated 288 DMPC bilayer using an approach described previously.[58] This required the removal of 24 lipid molecules from the bilayer to accommodate the protein. Particular attention was paid to add waters to the central pore so as to secure the formation of a continuous water column. Following solvation with SPC water, the system was energy minimized and subjected to ∼1 ns of MD during which the

positions of the protein heavy atoms were restrained. Finally, a 10 ns unrestrained production simulation was performed.

Results

Simulation Systems. To compare the conformational dynamics of an α-helical membrane protein in a detergent micelle with the same protein in lipid bilayer, two simulation systems, GlpF−OG and GlpF−DMPC, have been constructed (Figure 1). A third simulation system, for purposes of comparison, is provided by a simple octyl glucoside micelle (simulation OG; Table 1). All of the simulations were run for 10 ns, as previous studies[46,66] had suggested that this was sufficient to capture many aspects of protein/detergent or protein/lipid interactions. The simulations were run in the presence of a substantial number of water molecules (water:detergent molar ratio = 128 for GlpF−OG; water:lipid molar ratio = 57 for GlpF-DMPC) to avoid possible artifacts due to incomplete hydration of these complex systems. So as to focus on protein/detergent vs protein/lipid interactions without potential complications from protein/protein interactions, this study of a membrane protein in a micelle has employed GlpF in its monomeric state.

System Stability and Protein Conformational Fluctuations. A measure, albeit crude, of the conformational stability of a protein in a simulation is the root-mean-square deviation (RMSD) of the protein at the end of the simulation relative to the starting structure. For both the GlpF−OG and GlpF−DMPC simulations, the Cα atom RMSD is ∼0.2 nm at the end of the simulation (Table 1). This relatively small conformational drift is comparable to that observed in other simulations on the basis of membrane protein structures of comparable crystallographic resolution.[67]

Structural drift of the protein in the GlpF−OG micelle simulation may be compared with that in the GlpF−DMPC simulation (Figure 2). For both simulations, the Cα RMSD for the core TM α-helices behaves in much the same fashion, rising slowly over the first ∼5 ns of the simulation to a plateau of ∼0.15 nm. In contrast, there are differences between the RMSD profiles for the non-TM loops. Thus, for the GlpF−OG simulation there is rapid (∼1 ns) initial rise in the loop RMSD, which then sits at around 0.3 nm (though with substantial fluctuations) for the remainder of the simulation. In contrast, for the GlpF−DMPC simulation the loop rises more slowly (∼4 ns) to a plateau value of ∼0.22, subsequently fluctuating between 0.2 and 0.3 nm until the end of the 10 ns simulation. This suggests that the micellar environment allows a slightly greater structural drift, on the 10 ns scale, than does the bilayer and that this drift appears to be concentrated in the surface (i.e., nonmembranous) loops of the protein rather than the TM α-helix bundle.

Localization of protein mobility was analyzed by calculating the root-mean-square fluctuation (RMSF) in Cα atoms relative to their time-averaged positions as a function of residue number (see Supporting Information, Figure S1). The profile seen for both simulations mimics that of the crystallographic *B* values (not shown) with peaks corresponding to the extramembranous loops. In both simulations, the greatest fluctuations are seen in the loop connecting the M4 and M5 TM helices (which corresponds to a protein−protein contact in the tetrameric form of GlpF). For most of α-helical residues the RMSF values are a little lower for the GlpF−DMPC simulation than for the GlpF−OG simulation.

A more quantitative comparison of protein mobility can be obtained via block analysis of the MSF values for helices and loops in the two simulations (Figure 3). The nonzero slopes of

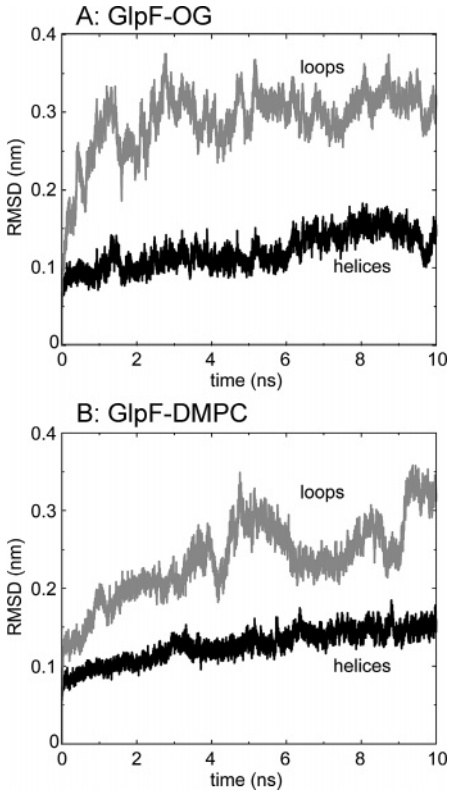

Figure 2. Structural drift as measured by the Cα root-mean-square deviation (RMSD) from the initial structure versus time, for the (A) GlpF−OG and (B) GlpF−DMPC simulations. The lines show the Cα RMSD values for α helices (black) and loops (gray).

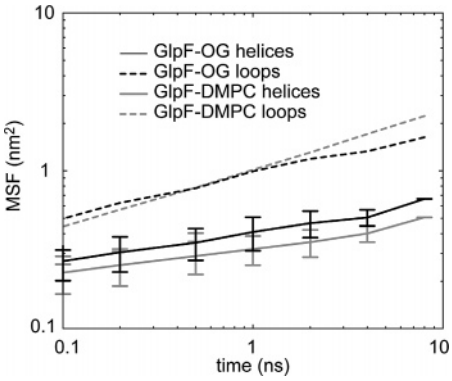

Figure 3. Block analysis of mean square fluctuations (MSFs; calculated for Cα atoms). For the final 8 ns of each simulation, average MSF values were calculated for time windows of 0.1, 0.25, 0.5, 1, 2, 4, and 8 ns. The black lines are for the GlpF−OG simulations and the gray lines for the GlpF−DMPC simulations. In each case the MSF values are evaluated separately for the helices (solid lines) and the loops (broken lines).

the lines indicate that, as expected, the sampling of protein motions in 10 ns is incomplete.[68] There is no consistent trend in the (small) differences in mobility for the loops—they vary according to the size of the time block analyzed. However, for the helices, the mobility in the GlpF−OG simulation is consistently ∼1.3x higher than in the GlpF-DMPC simulation. By way of comparison, for the TM *β*-barrel of OmpA the mobility in micelle simulations was ∼1.5x that in DMPC bilayer simulations.[46]

Micelle Equilibration and Geometry. It is of interest to examine the shape evolution of the micelle in the absence (OG) and presence (GlpF−OG) of the protein. The control micelle (OG) system consisted of 81 detergent molecules. It was set up

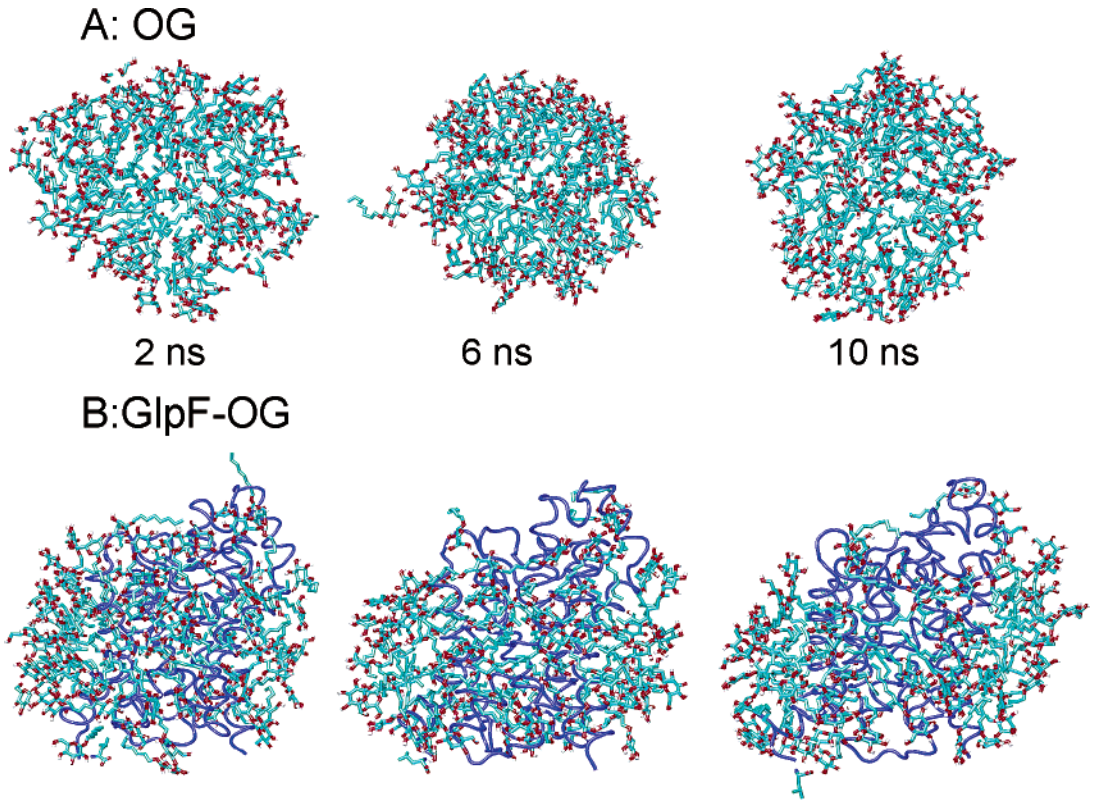

Figure 4. Snapshots of the (A) OG micelle and (B) GlpF−OG mixed micelle simulation, taken at 2, 6, and 10 ns. The detergent molecules are in bonds format (cyan = carbon, oxygen = red) and the protein in helix trace format (blue).

in a similar way to the protein/micelle system but the initial geometry was such that an approximate sphere was generated rather than a torus. Visual inspection of the OG simulation (Figure 4A) shows that the micelle retains an approximately spherical shape over the course of the simulation.

The shape of the OG micelle can be quantified by analyzing the three time-averaged principle moments of inertia, I_1, I_2, and I_3 (i.e., the moments of inertia about the three principal axes). The eccentricity, η, is defined as

$$\eta = 1 - \frac{I_{MIN}}{I_{AVG}}$$

where I_{MIN} is the smallest principal component of inertia and I_{AVG} is the average of all three. Thus, for a perfectly spherical object $\eta = 0$. To evaluate the eccentricity from a simulation, we reformulate it as

$$\eta = 1 - \frac{3\langle R_G^2 \rangle}{\langle R_{G1}^2 \rangle + \langle R_{G2}^2 \rangle + \langle R_{G3}^2 \rangle}$$

where R_{G1}, R_{G2}, and R_{G3} are the three principal radii of gyration corresponding to I_1, I_2, and I_3 and where $\langle\ \rangle$ denotes a time average over the whole trajectory. Applying this to the OG simulation yields $\eta = 0.15$. Thus, although the OG micelle is not completely symmetrical, the deviation from a spherical geometry is relatively small.

Visual inspection of the GlpF−OG mixed micelle simulation (Figure 4B) reveals that the initial torus of detergent molecules around the helix bundle of GlpF adjusts its shape during the course of the simulation. In particular, some detergent molecules move to a location in the vicinity of the periplasmic vestibule of the pore. If we examine the time evolution of the radii of gyration of the protein vs the OG in this simulation (see Supporting Information, Figure S2), we see that there is an adjustment in the distribution of the detergent during the first half of the simulation. The eccentricity of the detergent for this simulation is $\eta = 0.21$, consistent with a shape similar to an oblate ellipsoid. This value is very close to that for an OmpA/DPC mixed micelle simulation.[46] Thus, it may be that a toroidal arrangement of detergent is characteristic of membrane protein/detergent micelles. Of course, comparative analysis of further simulations will be needed to support this tentative generalization.

Interfacial Properties and Hydration. To compare the conformational dynamics of GlpF in a micelle vs a bilayer environment, it is important to analyze the distribution of water, and detergent/lipid polar headgroups and apolar tails relative to the protein. Analysis of radial distribution densities (data not shown) for the GlpF−OG micelles suggested that the detergent-solvent interface was quite broad.

Due to the anisotropic nature of the protein−micelle and the protein−bilayer systems it is perhaps more intuitive to analyze density profiles along approximate the pore axis of GlpF, i.e., along the axis corresponding to the bilayer normal in the GlpF-DMPC system (see Supporting Information, Figure S3). For the bilayer embedded protein we see, as anticipated, a clear separation into an aqueous region ($|z| > 2.5$ nm), an interfacial region (centered around $|z| \sim 1.8$ nm), and a hydrophobic core ($|z| < 1.5$ nm). The protein is largely accommodated within the hydrophobic core plus the interfacial regions. In contrast, the separation for the GlpF−OG micelle is less pronounced, with the hydrophobic tails the highest density for the center of the protein, whereas the headgroups are spread all along the length of the protein. This reflects the torus-like shape of the mixed protein/detergent micelle (as discussed above).

It is of interest to examine the extent to which water penetrates into the micelles, with and without protein. This can be done

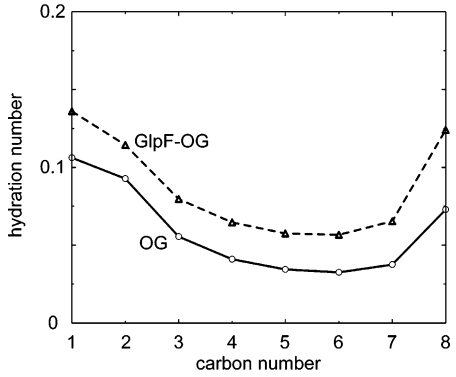

Figure 5. Hydration numbers for the OG hydrocarbon chains in the OG micelle (solid line) and GlpF−OG mixed micelle (broken line) simulations. Hydration numbers were determined by integrating C−O radial distribution functions (i.e., between each carbon atom of the chain and the oxygen atoms of water molecules) out to a distance of 0.35 nm. C1 is next to the glucose group; C8 is the terminal methyl group.

by calculating the hydration numbers for C atoms of the alkyl chains of the detergent molecule (Figure 5). The shapes of the profiles for the OG and GlpF−OG systems are very similar. However, it is evident that the presence of the protein leads to some additional penetration of water into the interior of the micelle. Comparison of the hydration number profiles for the micelle systems and for GlpF−DMPC (data not shown), suggests that the micelle interior admits rather more water than does the bilayer.

Tail Conformation and Dynamics. To provide a more dynamic picture of the hydrophobic interiors of the micelles and the bilayer, one may compare the conformational dynamics of the alkyl chains. To this end we have calculated the trans−gauche(−)-gauche (+) transition times and the trans fraction profiles for the alkyl tails of OG, and for the tails of the hydrophobic core of the DMPC bilayer (as defined by the terminal 8 carbon atoms of the myristoyl chains). The transition time profiles reveal qualitatively similar behavior for the OG and GlpF−OG simulations but suggest that the presence of the protein increases the mean transition time by on average 10−20 ps (an increase of ∼15%; Figure 6A). The protein also decreases the trans fraction by ∼3% relative to the pure OG micelle (Figure 6B). Thus, there is a small but noticeable perturbation of the detergent chain behavior by the protein.

Comparing the GlpF−OG and GlpF−DMPC profiles reveals that the mean transition time for the center of the bilayer is about a third of that for the micelle. One should remember this is comparing the core of the bilayer with somewhat shorter, and hence more restrained, alkyl tails in OG. Thus, perhaps contrary to expectations, the lipid bilayer provides a somewhat more fluid hydrophobic core than does the detergent micelle.

Protein-Detergent vs Protein−Lipid Interactions. The simulations of GlpF also provide an opportunity to examine specific contacts between detergent/lipid and protein. Analysis of other proteins[46,69] indicate that fluctuations in protein/lipid interactions occur on an ∼1 to ∼5 ns time scale; therefore 10 ns simulations should be sufficient to provide comparative data on such interactions in a micelle vs a bilayer. A noticeable difference is observed between the GlpF−OG and GlpF−DMPC simulations in term of the number of atomic contacts between the protein side chains and the lipid/detergent headgroups (Figure 7). In particular, the OG headgroups seem to make ∼4 times as many contacts with the protein as do the DMPC headgroups. This difference may be explained by the fact that the octyl−glucoside molecule, containing four hydroxyl groups

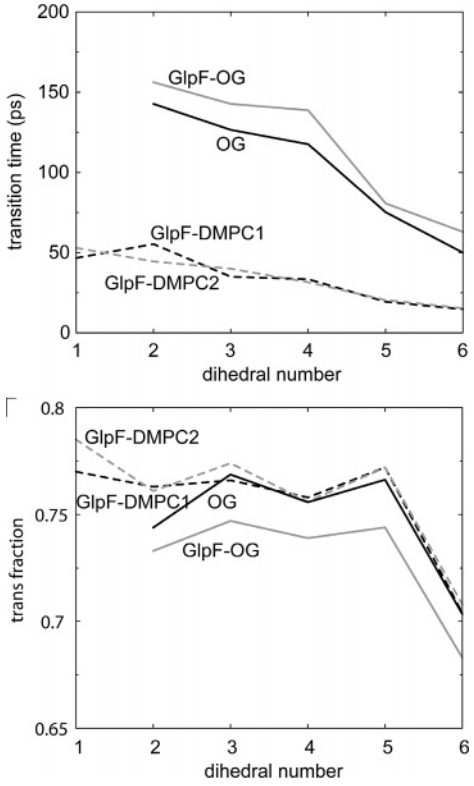

Figure 6. Dihedral angle properties for the eight carbon atoms of OG chain in simulations OG and GlpF−OG, and for the last eight C atoms of the two acyl chains in the GlpF−DMPC system. The (A) average *trans-gauche* (−)-gauche (+) transition times, and (B) average trans fractions, are both shown as a function of dihedral number (where dihedral 6 is defined by C5−C6−C7−C8 and C8 is the terminal methyl of each chain). The solid lines are for the OG simulation (black) and the GlpF−OG simulation (gray); the broken lines are for the GlpF−DMPC simulations (black broken line = sn1 myristoyl chain; gray broken line = sn2 myristoyl chain).

within the glucosyl group, has a somewhat greater potential to form hydrogen bonds with protein side chains compared to the headgroup of DMPC. Specifically, the number of possible H-bonds then for the glucosyl headgroup is 16 (12 to O acceptors, 4 from H donors), whereas for the phosphatidyl choline headgroup it is also 16, but all from O donors. For both of the simulations the number of (apolar) contacts of the protein with the hydrocarbon tails is quite constant, at ∼120 for GlpF−OG and ∼100 for GlpF−DPMC.

Aromatic side chains, especially Trp and Tyr, play a key role in "anchoring" a membrane protein into a lipid bilayer.[70,71] We have examined the temporal evolution and spatial distribution of the interactions of aromatic side chains (Trp, Tyr, Phe) with the headgroups of detergent/lipid molecules in the two simulations (Figure 8AC). In both cases two "bands" of such interactions are seen, as has been observed in other simulations of membrane proteins in PC bilayers.[69] In both cases, the majority of interactions can be seen in the outer (periplasmic) band (centered about $z \sim -1$ nm). The interactions are more extensive for OG than for DMPC. There are also substantial interactions between charged side chains of the protein and headgroups at the lower (intracellular) interface ($z \sim +1$ nm; data not shown). As expected, headgroups do not interact extensively with the protein's hydrophobic residues. The detergent tails seem to interact a little more with the hydrophobic side chains of the protein than do the lipid tails (Figure 8B,C). This may reflect the greater fluidity of the lipid tails in the

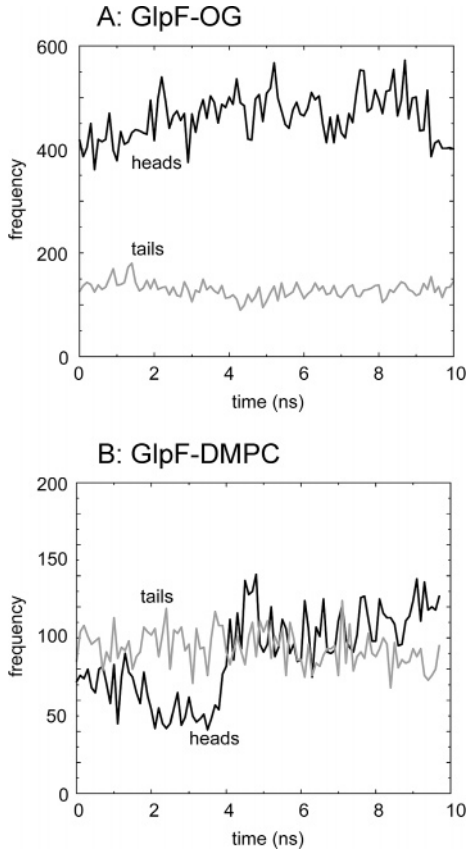

Figure 7. Number of atomic contacts (≤0.35 nm) between GlpF side chains and detergent/lipid headgroup atoms (black line) and tail atoms (gray line) as a function of time for (A) the GlpF−OG and (B) GlpF−DMPC simulations.

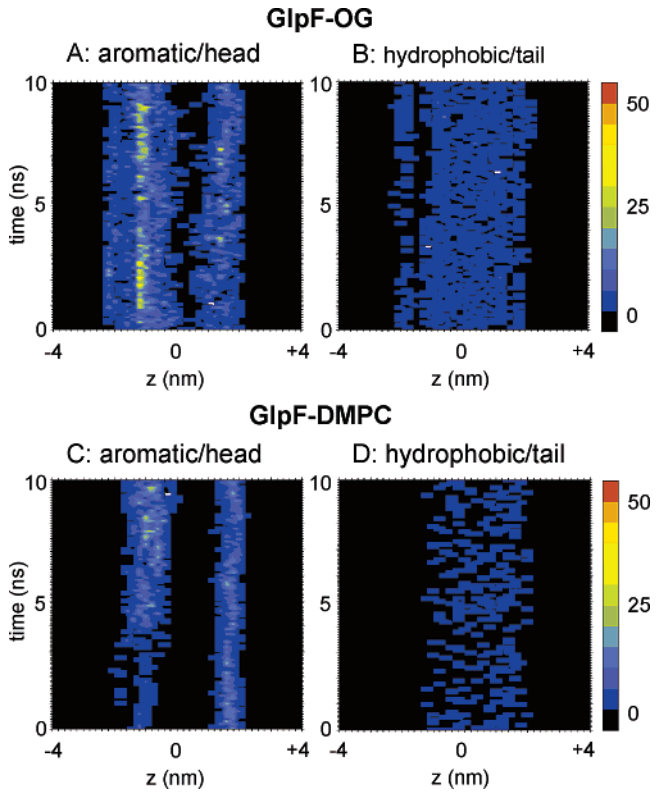

Figure 8. Interactions of (A, C) aromatic side chains with headgroups, and of (B, D) hydrophobic side chains with alkyl/acyl tails for the (A, B) GlpF−OG and (C, D) GlpF−DMPC simulations. In each case, the number of interactions (≤0.35 nm) are shown as a function of position along the bilayer normal (z) and time.

hydrophobic core of the bilayer relative to the tails of the OG molecules (see above).

To illustrate the nature of the interactions observed in these simulations, two selected interactions are shown (Figure 9). In the GlpF−OG interaction (Figure 9A), the glucosyl headgroup forms H-bonding interactions with two basic side chains on the protein surface at the "interfacial" region, namely R247 and K248. The alkyl tail is sandwiched between the side chains of two phenylalanine residues. We note that similar, but nonidentical, interactions of OG with aromatic side chains are seen in the crystal structure of GlpF. In the GlpF−DMPC interaction (Figure 9B), there are two H-bonds to the lipid headgroup: one from a tryptophan, and one from a lysine. Such interactions are typical of those seen in a number of membrane protein simulations.

Pore Properties. Although the primary focus of this paper is on the interactions of the protein with its environment in a micelle vs a bilayer, it is also of interest, from a functional perspective, to examine whether the switch from bilayer to detergent has a major effect on the properties of the transmembrane pore. GlpF is a transport protein that allows water and/or glycerol molecules to cross a membrane via rapid passive transport through a central pore. The average pore radius profiles were calculated over each of the simulations (data not shown). There did not seem to be any significant differences in pore radius profile between the two simulations suggesting that, for this particular protein, the switch in environment would not result in a major perturbation of the functional properties of the protein.

Conclusions

The results from these studies are of some interest, given the widespread use of OG as a detergent in biophysical, structural, and functional studies of integral membrane proteins. In particular, it would appear that, at least on the time scales sampled in these simulations (10 ns), the micelle environment does not seem to significantly perturb the GlpF monomer. However, there were some differences in the behavior of the protein between the micelle and bilayer environments. In particular, in the micelle simulation there was greater conformational drift in the extramembranous loops than in the lipid bilayer simulation. This seems to correlate with the greater potential for H-bond formation by the glucosyl group that forms the OG headgroup. There was also a small enhancement in the flexibility of the core TM α-helix bundle in the micelle relative to the bilayer. This may correlate with differences in the interactions and conformational dynamics of the hydrocarbon chains between the two environments.

It is of interest to compare these results with those of related simulations. Simulations of another α-helix bundle membrane protein, a photosynthetic reaction center in a LDAO micelle[45] did not seem to perturb the structure of the protein. However, the simulations were relatively short (3.5 ns) and were not compared with the same protein in a lipid bilayer. Such a comparison has been made for OmpA,[46] a member of the bacterial β-barrel outer membrane protein family. Although OmpA has a very different architecture from GlpF, and a different detergent was used (DPC vs OG), there seem to be some similarities. In both the OmpA and GlpF simulations, the detergent micelle resulted in enhanced flexibility of the transmembrane domain and greater perturbation of the extramembraneous loops relative to the lipid bilayer. However, more

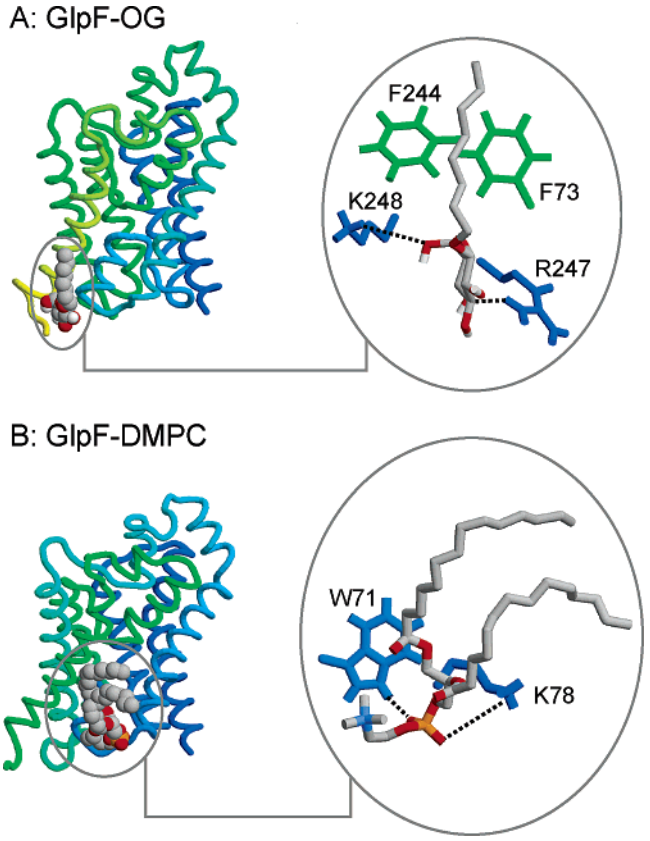

Figure 9. Examples of interactions between detergent/lipid molecules and GlpF: (A) GlpF−OG; (B) GlpF−DMPC. For each simulation, the structure at 10 ns was used and a single GlpF/detergent or GlpF/lipid interaction is highlighted.

extensive comparative simulation studies, covering a wider range of membrane proteins, are needed before one may draw any firm generalizations.

One should consider the limitations of the simulation methodology employed. In particular, the use of PME for the long-range electrostatic interactions may lead to periodicity artifacts.[72,73] This could perhaps be tested by comparison with simulations using a reaction field method for such interactions. Also, the simulations remain quite short relative to the times needed for self-assembly of micelles. This has necessitated ab initio construction of starting geometries for the micelles. Detergent micelles[74] and protein/detergent mixed micelles[75] may be self-assembled from randomly placed detergents, if longer (∼50 ns) time scales are employed. For simulations of micelles of the zwitterionic detergent dodecylphosphocholine (DPC) with either of two proteins (OmpA, a β-barrel, and glycophorin, a TM helix dimer) the self-assembled micelles after ∼50 ns are very similar in a number of their properties to pre-assembled micelles after ∼10 ns. For glycophorin−SDS micelles, a similar situation has been observed by Schulten and colleagues.[76] We are therefore reasonably confident of the protocol we have used for assembly of a starting geometry for the GlpF−OG micelle in this paper. However, it will be of interest to compare the behavior of pre-constructed and self-assembled micelles in simulations of a wider range of membrane proteins.

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Supporting Information Available: Figures of RMSF relative to time-averaged structure, radius of gyration vs time, and density profiles. This material is available free of charge via the Internet at http://pubs.acs.org.

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