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MD Simulations of Spontaneous Membrane Protein/Detergent Micelle Formation

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Detergent/protein micelles are central to studies of the chemistry of membrane proteins[1] and are relevant to biological processes such as folding[2] and transport.[3] Although it is presumed that detergents solubilize membrane proteins by mimicking the natural lipid bilayer environment, little is known about protein−detergent micelle formation at an atomistic level. Molecular dynamics (MD) simulations have been used to study the aggregation of surfactants,[4] as well as to analyze the properties of micelles containing membrane proteins.[5,6] Recently, the formation of a sodium dodecyl sulfate micelle around the simple α-helical membrane protein glycophorin A (GpA) has been simulated, using a two-step procedure.[7]

Here, we report two 50 ns MD simulations of spontaneous dodecylphosphocholine (DPC) micelle formation (self-assembly) around representatives of the two major families of membrane proteins. The outer membrane protein OmpA from *Escherichia coli* consists of a β-barrel, as revealed by crystallography[8] and by NMR.[9] GpA from red blood cells is a homodimer of transmembrane (TM) α-helices, as shown by solution-[10] and solid-state NMR.[11] For comparison, we have also performed 25 and 50 ns simulations of preformed micelles containing OmpA and GpA, respectively. To generate the starting configurations for the self-assembling simulations, DPC molecules (∼0.2 M) were placed at random around each protein (80 and 60 detergents for OmpA and GpA, respectively). Simulations were performed using the GROMACS simulation package[12] (see Supporting Information).

Figure 1 illustrates critical events during the two 50 ns micelle formation simulations. Over just a few nanoseconds, individual detergent molecules rapidly fuse to form small micelle-like aggregates of ∼10 DPC molecules each, thereby reducing the exposed solvent accessible surface (SAS) of the hydrophobic detergent tails. Within ∼5 ns in both simulations, only four or five pure detergent micelles remain, consistent with a reduction in the average SAS per detergent from ∼6 to ∼3 nm^2, primarily as a result of burial of the DPC tails. At the same time, a number of initial protein−detergent interactions are created. These include bilayer-like interactions of detergent tails with exposed, hydrophobic protein surfaces on a section of the OmpA β-barrel and on one of the GpA α-helices, along with two patches of electrostatic interaction between detergent headgroups and polar regions of OmpA (i.e., interstrand loops and turns). Subsequently, the small micelle-like aggregates fuse with one another, and with the detergent molecules already bound to the protein. These fusion events proceed primarily via headgroup−headgroup interactions of the detergents, and are cooperative. After ∼10 to 20 ns, the systems each effectively consist of a loosely packed protein−DPC micelle (in which detergents cover much of the protein surface) plus a weakly interacting DPC aggregate, or "globule". Micelle formation kinetics can be followed by measuring the radius of gyration of the detergent molecules (Figure 2A), which decays approximately exponentially in each system, with a time constant of ∼5 ns for GpA and ∼10 ns for OmpA. This decline results from a gradual reduction in protein and detergent SAS, and from a large increase in the number of protein−DPC interactions (Figure 2B).

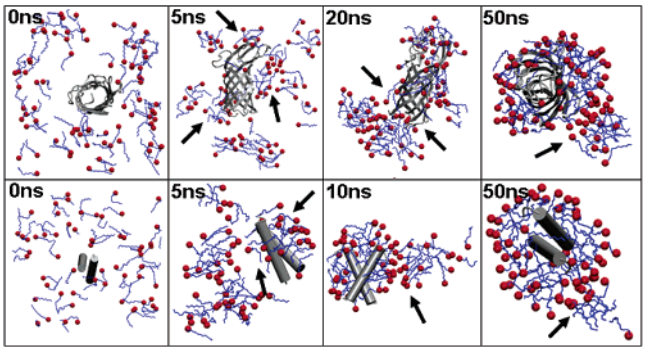

Figure 1. Snapshots of self-association simulations for OmpA (top) and GpA (bottom). Arrows represent sites of interaction: 5 ns, protein-bound detergent; 10/20/50 ns, main micelle and "globules".

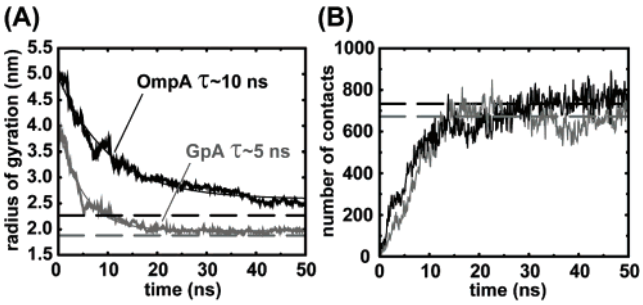

Figure 2. (A) Radius of gyration versus time for detergent molecules in self-association simulations. Exponential fits (thin lines) yielded rate constants of 4.9 ns for GpA and 10.4 ns for OmpA. (B) For the self-association OmpA simulation, detergent−protein interatomic contacts (within 0.4 nm) for DPC tails (black lines) and headgroups (gray lines). In both (A) and (B), the respective average values from the last 10 ns of the preformed micelle simulation are shown (horizontal dashed lines).

At this intermediate stage (∼10−20 ns), the separate "globules" observed in both simulations take slightly different forms, probably reflecting stochastic fluctuations, rather than being a function of the class of membrane protein present. In the case of GpA, a single, diffuse globule composed of ∼15 detergents makes headgroup−headgroup interactions with other protein-adsorbed DPC molecules. For OmpA, an extended "globule network" is bound to the polar periplasmic turns, but effectively consists of two conjoined detergent micelles, bound together by headgroup−headgroup interactions. Following binding to the protein, the globules undergo slow structural transitions over tens of nanoseconds, allowing "fine-tuning" transfer of DPC molecules into the main protein−detergent micelle. Additionally, while the stronger interactions between DPC headgroups and polar residues are established reasonably quickly, a longer-time scale process in micelle formation is the slow relaxation of detergent tails against the protein surface upon binding.

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This is corroborated by the gradual increase in the number of detergent tails in contact with each membrane protein, between ~10−30 ns for GpA and between ~20−45 ns for OmpA, despite the fact that the number of *interatomic* contacts changes very little after ~15 ns (Figure 2B). Hence, micelle equilibration is presumably driven by the need to cover exposed hydrophobic surfaces. Indeed, while the mean DPC headgroup SAS has reached an equilibrium, the mean tail SAS reduces by a further ~0.5 nm^2, and the hydrophobic protein SAS by ~5 nm^2, over the remaining tens of nanoseconds. This relaxation of detergent around each protein is further illustrated by the detergent radius of gyration values, which only stabilize after ~25 ns for GpA and ~40 ns for OmpA (Figure 2A).

For both of the final (50 ns) aggregate structures, detergent molecules cover most of the protein surface. The arrangement largely supports the traditional view of micelle structure, with detergent tails contacting the TM sections of each protein, radiating outward so that DPC headgroups contact the polar extramembraneous regions and aqueous solution. This organization effectively mimics a biological membrane environment, and the patterns of protein−detergent interaction (data not shown) resemble those observed with phospholipid in protein−bilayer simulations.[13] Meanwhile, the adsorbed globules are tightly packed and stably bound to the protein−detergent micelle surface via headgroup−headgroup interactions. For GpA, the globule consists of a small micelle containing nine DPC molecules. For OmpA, half of the "globule network" has fused with the main protein−detergent aggregate, leaving a stably adsorbed globule of 14 DPC molecules.

To investigate the effect of starting configuration on the evolution of system properties, we compared data from the final 10-ns period of the self-association simulations with similar 10-ns periods from simulations of *preformed* micelles (generated by packing detergents around the hydrophobic surface of the protein in a toroidal fashion[6]). For GpA, based on the ratios of principle moments of inertia (data not shown), the self-assembled and preformed aggregates were both approximately spherical. Moreover, the radial densities of each system component closely superimpose (see Supporting Information), while the proportions of relative detergent-buried SAS are very similar (each ~80%). In contrast, because slightly more detergents are localized toward the non-TM regions of OmpA in the self-assembled micelle, its shape is rather more oblate than the spherical preformed OmpA micelle. Hence, the detergent-buried SAS values are ~80% for the β-barrel and ~40% for non-TM regions in the preformed micelle, in comparison with respective values of ~70% and ~60% in the self-assembled micelle. Nevertheless, the total buried surface area for the protein is very similar between simulations. Moreover, the water radial density minima are coincident with those of the detergent headgroups, while the interfacial water widths are only ~5% different. This indicates a similar local packing arrangement of detergent at the protein surface, as confirmed by the match between number of OmpA−DPC tail and headgroup atomic contacts in the self- and preformed simulations (Figure 2B). This is also the case for GpA. Thus, for OmpA, small differences in aggregate geometry are tolerated as long as system properties such as SAS and detergent−protein interactions are maintained.

To summarize, we have demonstrated the spontaneous formation of detergent micelles around a model α-helical protein and a simple β-barrel protein. Many mechanistic similarities in aggregation were apparent. The need for preformed detergent micelles (rather than monomeric detergent) prior to substantial protein−detergent association is in accordance with experimental folding studies of OmpA.[14] On the basis of the detergent radius of gyration, the time constant for micelle formation for OmpA was found to be about double that of GpA; interestingly, the ratio of effective protein adsorption surface area for OmpA versus GpA is ~2, suggesting that a simple diffusive, stochastic adsorption model may explain these kinetics. The end-structures for self-assembled OmpA and GpA micelles are remarkably similar to their preformed counterparts, justifying current protocols for generation of protein−micelle models[6] and supporting the view of detergent micelles as mimics of the biological membrane, as suggested by OmpX−detergent interactions measured using NMR.[15] Finally, our results suggest that "brute force" MD simulations are able to capture large-scale rearrangements in protein−detergent, and by extension protein−lipid, packing interactions.

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Supporting Information Available: Methodological details; time-dependent graphs of detergent diffusion coefficients, protein and detergent SAS, detergent phosphate−phosphate radial distribution numbers; and time-averaged system radial densities. This material is available free of charge via the Internet at http://pub.acs.org.

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