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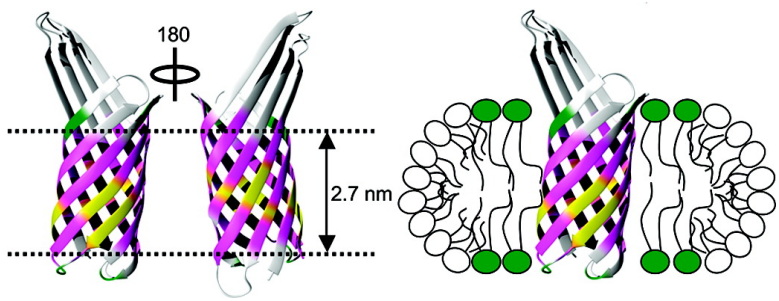

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Bilayer in Small Bicelles Revealed by Lipid−Protein Interactions Using NMR Spectroscopy

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Micelles formed by a variety of detergents are successfully used to study membrane proteins by solution NMR.[1,2] However, structures determined under these conditions have been questioned by the argument that micelles cannot mimic a natural membrane environment because of the strong curvature at their surface and the different lateral pressure compared to lipid bilayers.[3,4] Furthermore, membrane proteins embedded in the micelles often loose their activities.[5] Small bicelles composed of a mixture of short-chain and long-chain phospholipids, usually dihexanoyl phosphatidylcholine (DHPC) and dimyristoyl phosphatidylcholine (DMPC), are presumed to be superior in these regards.[5,6] From deuterium spectroscopy, it was derived that DMPC in bicelles makes up the flat surface of a bilayered disk, and DHPC covers its rim irrespective of whether more discoid or "swiss cheese"-like bicelles are formed.[7−9] Because bicelles are more similar in structure to an actual membrane, yet still relatively small in size, they have the potential to serve as replacements for micelles in NMR studies of membrane proteins. The properties of any system for solubilizing membrane proteins will depend primarily on its interactions with the protein. For bicelles, previous NMR studies on the interactions between lipids and protein were performed using peptides.[10] Here, we present a study of the interaction between the intact integral outer membrane protein OmpX from *Escherichia coli*[11] and small bicelles formed by a DMPC/DHPC mixture.

For the study, [∼85% ^{2}H,^{13}C,^{15}N]-labeled OmpX in bicelles with molar ratio [DMPC]/[DHPC] = 0.5 was prepared (Supporting Information). Formation of bicelles in the presence of OmpX was verified as follows: In agreement with the fact that DMPC and DHPC experience different magnetic environments in bicelles,[7] distinct ω-methyl proton resonances of DMPC and DHPC (Figure S1) and distinct phosphorus resonances (Figure S3) were observed that have the same chemical shift as in protein-free bicelles[7] and that are in contrast to indistinguishable peaks in 1D ^{1}H spectra (Figure S4) and in 1D ^{31}P spectra (Figure S3) when DMPC and DHPC are dissolved in methanol. Similar to protein-free bicelles,[8] NOE cross-peaks between ω-methyl protons of DMPC and DHPC were not observed in 2D ^{1}H,^{1}H-EXSY spectra[12] recorded with mixing times ranging from 3 to 40 ms. Thus, there is no exchange between DMPC and DHPC on this time scale and the result further supports that OmpX is reconstituted in bicelles.

To directly observe contacts between lipid molecules and the protein, we have measured a 3D ^{15}N-resolved ^{1}H,^{1}H-NOESY spectrum with a mixing time of τ_m = 150 ms (Figure 1). On the basis of almost complete backbone resonance assignment of OmpX in bicelles (>95%; will be published elsewhere), intermolecular NOEs between amide protons of OmpX and the hydrophobic tails of DMPC were assigned. The strongest and weakest intensities of these intermolecular NOEs were observed for residues located

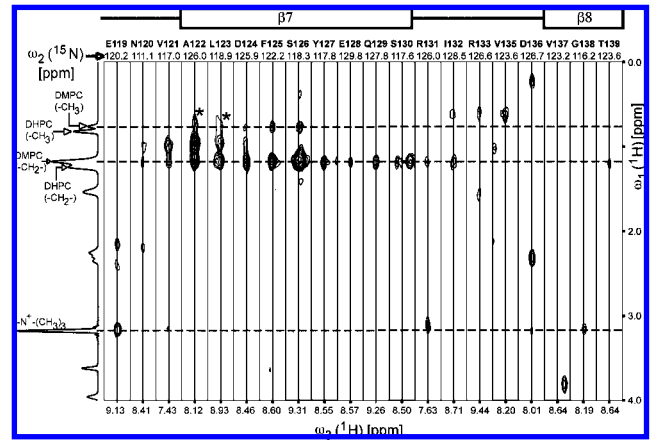

Figure 1. Selection of ω_1(^{1}H)/ω_3(^{1}H) strips from an 800 MHz 3D ^{15}N-resolved ^{1}H,^{1}H NOESY spectrum measured at 30 °C with a sample of [∼85% ^{2}H,^{13}C,^{15}N]-labeled OmpX in protonated DMPC/DHPC bicelles (molar ratio [DMPC]/[DHPC] = 0.5). The strips were taken from the polypeptide segment of residues 119−139. The residues and their ^{15}N chemical shifts are indicated at the top of the spectrum. On the top, the secondary structure elements are also indicated.[11] On the left, the 1D ^{1}H NMR spectrum of DMPC/DHPC bicelles, is shown. The positions of the signals arising from the hydrophobic end (−CH_3 and −CH_2−) of DMPC and the choline N^+-bound methyls (−N^+−$(CH_3)_3$) are marked with broken lines. Assignments of the hydrophobic end of DHPC are also indicated in the 1D ^{1}H NMR spectrum of DMPC/DHPC. On the strips of A122 and L123, NOEs from residual methyl protons from OmpX are marked with an asterisk (∗).

centrally on the barrel surface, and near the edges of the barrel surface, respectively (Figure S5). No intermolecular NOEs to lipids were detected in the loops and turns of the protein. The intermolecular NOEs to lipid molecules cover the surface of OmpX over a range of approximately 2.7 nm centered about the middle of the barrel (Figure 2A). Solvent-accessible surfaces of OmpX were identified by paramagnetic relaxation enhancement (PRE) by the titration of gadolinium−diethylene triamine pentaacetic acid into the sample, similar to Hilty et al.[13] (Figure S6). Solvent-inaccessible surface derived from PRE agrees well with the intermolecular NOE data. The hydrophobic area of OmpX covered by lipids is very similar to that of OmpX in DHPC micelles,[2] which implies that the hydrophobic interactions between the lipids and the protein are preserved in both micelles and bicelles.

However, all of the intermolecular NOEs in the bicelles are from DMPC and none are from DHPC (Figure 1) giving further evidence for incorporation of OmpX in the middle of the bicelle. If there were a statistical distribution of lipids on OmpX, the NOEs from ω-methyl groups of DHPC would be twice as strong as those from DMPC due to their intensities and line widths based on a 1D spectrum (Figure S1). Most of the lipid−protein NOEs are from methylene protons of the hydrophobic tails and only few from

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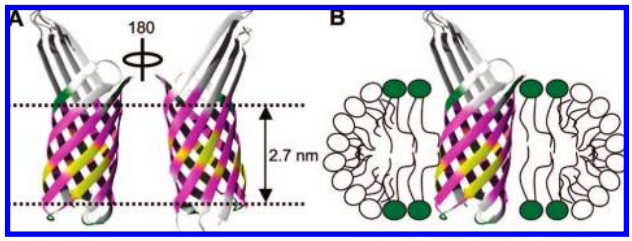

Figure 2. Ribbon drawing of OmpX (PDB access code: 1QJ8[11]) showing intermolecular NOEs. (A) The residues showing NOEs to both methyl and methylene groups of the hydrophobic tails of DMPC in the NOESY spectrum are colored yellow. The residues showing NOEs only to methylene groups of hydrophobic tails of DMPC are colored magenta. The residues showing NOEs to the polar headgroup methyl protons are colored green. Grey residues did not show intermolecular NOEs. The horizontal broken lines indicate the boundaries between the central hydrophobic and the peripheral hydrophilic areas of OmpX, and the approximate height of the hydrophobic cylindric jacket is indicated. (B) Model of a DMPC/DHPC bicelle and OmpX, based on observed intermolecular NOEs. Color-coding of OmpX is the same as in panel A. Spatial distribution of DMPC (green) and DHPC (white) in bicelles is schematically drawn.

ω-methyl protons of DMPC (Figure 2A). The residues showing NOEs from ω-methyl protons are located centrally on the barrel surface of OmpX. Because of the large size of the OmpX-bicelle complex (overall rotational tumbling time $\tau_c = 35$ ns from 1D TRACT;[14] Figure S7), it could be argued that these NOEs originate from spin-diffusion between the lipid methylene and ω-methyl protons. However, similar NOE patterns in a NOESY spectrum with a three times shorter mixing time ($\tau_m = 50$ ms) were observed, ruling out the possibility of spin diffusion (Figure S8). On the other hand, it may also be argued that NOEs from ω-methyl groups are underestimated because of their weak intensities (6) in the 1D spectrum, compared with NOEs from methylene groups (40). However, intensity ratios of NOEs from ω-methyl and methylene groups vary between 0.12 and 0.65 (Figure S8) and do not correspond to a statistical distribution of 6/40 = 0.15. Thus, NOEs from ω-methyl groups of DMPC indicate direct contacts to OmpX. These observed NOE patterns can then only be explained by a model where the hydrophobic tails of DMPC make parallel contacts to the surface of OmpX (Figure 2B) in the DMPC/DHPC bicelles. This observation stands in contrast to the previously reported perpendicular contacts between the hydrophobic tail of DHPC and OmpX in DHPC micelles[2] but is in agreement with the current models for protein-free bicelles.[9,15] In particular, this study shows that OmpX locates in the middle of the bicelle and does not segregate laterally to the rim of the bicelle where it would interact with DHPC. Thus, DMPC molecules in the small bicelles appear to form a bilayer not only in the absence but also in the presence of the membrane protein.

Intermolecular NOEs between polar head groups of lipids and the amide protons of OmpX were also observed (Figure 1 and Figure 2A). As suggested previously,[2] these NOEs may be rationalized by amino acid type-specific interactions. Several additional NOEs of this type were observed in bicelles (residues 21, 22, 75, 118, 119, and 136), when compared with micelles (residues 118 and 119).[2] This difference may be due to the closely packed head groups and favorable dynamic effects of the bilayer compared to the micelles.[4] Note that the number of residues showing these interactions is increased in the bicelles, while almost identical residues show the hydrophobic interactions between lipids and the protein in bicelles and micelles. This observation implies that the differences related to the structure and function of bicelle- or micelle-solubilized membrane proteins are related to interactions between the polar head groups of lipids and the protein. It should also be noted that the "yellow" region in Figure 2 is not a regular ring around the barrel. Moreover, contacts between α- and β-methylene groups of lipids and OmpX show also similar irregularity (Figure S9). These irregularities may suggest that not only the protein is influenced by the bilayer but also the bilayer by the protein. This aspect needs further exploration that is currently being conducted.

In summary, we show that in small DMPC/DHPC bicelles, only DMPC molecules contact the solubilized protein, and that they are arranged in parallel to the hydrophobic surface of the protein. Thus, DMPC molecules form a bilayer environment for the membrane protein and the protein does not diffuse to the rim of the bilayer. The interactions between the polar head groups of lipids and the protein are increased in number in the bicelles. Although these changes are subtle, the presence of these interactions together with the irregularity in the interactions between the protein and the end of the hydrophobic tails of lipids provides a possible rationale for the observation that membrane proteins often behave differently in bilayers compared to micelles and may further indicate that bilayers loaded with protein differ from protein-free ones.

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Supporting Information Available: Sample preparation, lipid aggregation number and model of protein-loaded bicelle, ^{1}H spectra, ^{1}H,^{1}H TOCSY, ^{31}P spectrum, 1D TRACT, NOE build-up curves, and ribbon drawing of OmpX showing intermolecular NOEs between α- and β-methylene groups of lipid and the protein and PRE. This material is available free of charge via the Internet at http://pubs.acs.org.

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