Mixing and Matching Detergents for Membrane Protein NMR Structure Determination

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Abstract: One major obstacle to membrane protein structure determination is the selection of a detergent micelle that mimics the native lipid bilayer. Currently, detergents are selected by exhaustive screening because the effects of protein—detergent interactions on protein structure are poorly understood. In this study, the structure and dynamics of an integral membrane protein in different detergents is investigated by nuclear magnetic resonance (NMR) and electron paramagnetic resonance (EPR) spectroscopy and small-angle X-ray scattering (SAXS). The results suggest that matching of the micelle dimensions to the protein’s hydrophobic surface avoids exchange processes that reduce the completeness of the NMR observations. Based on these dimensions, several mixed micelles were designed that improved the completeness of NMR observations. These findings provide a basis for the rational design of mixed micelles that may advance membrane protein structure determination by NMR.

Introduction

Integral membrane proteins comprise ≈25% of most proteomes and facilitate transport and signaling across cell membranes. Despite their importance, less than 1% of known protein structures are of membrane proteins. One major obstacle to membrane protein structure determination is the selection of a detergent that mimics the native lipid bilayer and stabilizes the protein fold.1–5

Detergents are small amphipathic molecules that are used to solubilize membrane proteins for structural and functional investigations. However, unlike phospholipid bilayers, detergents form micelles, which are spheroid and have a core composed of the detergent hydrophobic tails. Micelles have different shapes and sizes depending on the detergent chemical structure. For structural investigations, a multitude of detergents is screened until a condition that provides high-quality crystals6 or NMR spectra7 is found. However, a correlation between the physical properties of the detergent micelle and the likelihood of obtaining a membrane protein structure is currently not known.

In this study, we present data on the model polytopic α-helical membrane protein TM0026. TM0026 is a membrane protein of unknown function from the thermophile Thermotoga maritima and was initially characterized as part of the high-throughput structure determination pipeline of the Joint Center for Structural Genomics.1,7 The data presented demonstrate a correlation between protein conformations, micelle size and thickness, and quality of nuclear magnetic resonance (NMR) spectra. The structure and dynamics of TM0026 in different detergents are investigated by NMR and electron paramagnetic resonance (EPR) spectroscopy and small-angle X-ray scattering (SAXS). The results suggest that matching of the micelle dimensions to the protein’s hydrophobic surface avoids exchange processes that reduce the completeness of the NMR observations. Based on these observations, mixed micelles are designed that improve the completeness of NMR observations. These findings provide a basis for the rational design of mixed micelles.

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that have the potential to advance membrane protein structure
determination.

**Experimental Section**

**Cloning, Expression, and Purification.** N-terminal His-tagged
TM0026 was cloned as previously published. Individual cysteine
mutants were produced by standard polymerase chain reaction
(PCR) protocols. Protein expression was performed with Luria—Bertani
(LB) medium containing 1% glycerol (v/v) and 50 mg/mL
ampicillin. Expression was induced by the addition of 0.20% arabinose
for 3 h. For deuterated, 15N-labeled proteins, published protocols using
conventional shakers and minimal medium in D2O supplemented with
15NH4Cl were used. TM0026 was purified in each detergent (decel maltoside, DM; dodecyl maltoside, DDM; decylphosphocholine, FC-10; and
dodecaphosphocholine, FC-12 from Anatrace, Inc., Maumee, OH; 1-palmitoyl-2-hydroxy-sn-
glycero-3-[phospho-rac-(1-glycerol)], LPPG, and 1,2-dihexanoyl-
-sn-glycero-phosphocholine, DHPC, from Avanti Polar Lipids, Inc.,
Alabaster, AL) as previously described by Co2+-affinity chroma-
tography. For cysteine mutants, the lysis and purification buffers
contained 0.2 mM tris(2-carboxyethyl)phosphine (TCEP). Protein solutions
were then concentrated and unreacted spin label
absorbance at 280 nm in 6 M guanidine hydrochloride and by
SDS-PAGE. TM0026 was purified in 6 M guanidine hydrochloride and by
TCEP and imidazole were removed by use of
a 20 mM phosphate buffer (pH 6.2) with 150 mM NaCl and either 5 mM
TCEP, and imidazole were removed by use of
a 20 mM phosphate buffer (pH 6.2) with 150 mM NaCl.

**Spin Labeling of TM0026 Cysteine Mutants.** Immediately
before spin labeling, TCEP and imidazole were removed by use of
a PD-10 desalting column (GE Healthcare Bio-Sciences Corp.,
Piscataway, NJ) and a 20 mM phosphate buffer (pH 6.2) with 150 mM
NaCl and either 5 mM DM, 5 mM DDM, or 15 mM FC-10.

**Modeling of TM0026 in Micelles.** The protein backbone atoms
were assigned by use of HNCA and HN(CO)CA 3D spectra. The
Detergent Interactions

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because of extensive line broadening. The observed line broadening, which impedes NMR structure determination, is attributed to conformational heterogeneity and exchange processes and not to aggregation, unfolding, or overall size of the protein–detergent complex.1,15

Structural Changes of TM0026 in Different Detergents. To investigate the physical origin of the line broadening, site-directed spin labeling (SDSL) was employed to study the structure and dynamics of TM0026 under different detergent conditions. A nitroxide probe (R1, Figure S1 in Supporting Information) was introduced at four sequential sites (Figure 2). These four residues were chosen because they compose a full α-helical turn at the center of the first hydrophobic sequence and at least one of the residues is likely to be involved in tertiary interactions with the second α-helix. The introduction of the nitroxide side chain does not significantly perturb the overall structure of TM0026, as the $^{15}$N,$^1$H-TROSY spectrum of the protein labeled with R1′ (a diamagnetic analogue) is identical to that of wild type (Figure S2 in Supporting Information). The spectral parameters $\Delta H_{pp}$ and $2A_z$ (Figure 2) provide an assessment of mobility (the central line width, $\Delta H_{pp}$, is determined by the g-tensor anisotropy; that is, nitroxide mobility modulates the averaging of the g-tensor elements and reduction in mobility resolves the anisotropies and the line width is broadened) and can be used to determine the topology16 and backbone dynamics17 of the α-helix.

In DM, the EPR spectra of residues L14–L16 are very similar to those observed for R1 at lipid/detergent-exposed sites.18,19

In contrast, the EPR spectral line shape of A13R1 represents a highly restricted nitroxide side chain, based on the evaluation of $\Delta H_{pp}$ and $2A_z$ (Figure 2 and Table S1 in Supporting Information), indicating a direct interaction with the second transmembrane α-helix. In contrast, in FC-10 the EPR spectrum of A13R1 has two components, indicating that R1 samples two conformations. One spectral component is similar to that observed in DM and the other represents a more mobile R1 (Figure 2) and is similar to spectra observed in highly dynamic sequences.17,20 An increase in mobility for a fraction of the protein population is observed at each labeled position throughout the α-helical turn. These data suggest that the tertiary interaction between the two α-helices is lost in a substantial fraction (≈10–20%) of the protein population. The structural heterogeneity and potential exchange between the two populations provide a possible explanation for the observed NMR line broadening in FC-10 detergent micelles. In DDM, the tertiary contact at A13R1 is maintained (Figure 2). However, compared to DM, the EPR spectra for the lipid-exposed residues exhibit extensive line broadening as assessed by $\Delta H_{pp}$ (the most dramatic difference is observed at L16R1) (Table S1 in Supporting Information). These data demonstrate that the structure of TM0026 is perturbed in FC-10 and DDM; in order to understand how the detergent influences the protein structure, the characteristics of the detergents were investigated.

Comparison of DM, FC-10, DDM, and FC-12 Micelles. A comparison of the detergent properties (Table 1, Figure S3 and
Table 1. Properties of Select Detergents

<table>
<thead>
<tr>
<th>detergent (abbreviation)</th>
<th>cmc (mM)</th>
<th>$N^*$</th>
<th>$L^i$ (Å)</th>
<th>$r_{HC}$ (Å)</th>
<th>$V_{HC}$ (nm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-decyl β-D-maltoside (DM)</td>
<td>1.8</td>
<td>80–90</td>
<td>34</td>
<td>12.5, 23</td>
<td>26</td>
</tr>
<tr>
<td>n-dodecyl β-D-maltoside (DDM)</td>
<td>0.17</td>
<td>135–145</td>
<td>39</td>
<td>14, 29</td>
<td>49</td>
</tr>
<tr>
<td>n-decylphosphocholine (FC-10)</td>
<td>11</td>
<td>45–53</td>
<td>28</td>
<td>21, 13</td>
<td>15</td>
</tr>
<tr>
<td>n-dodecylphosphocholine (FC-12)</td>
<td>1.5</td>
<td>65–80</td>
<td>34</td>
<td>26, 16</td>
<td>27</td>
</tr>
<tr>
<td>1,2-dihexanoyl-sn-glycero-3-phosphocholine (DHPC)</td>
<td>15</td>
<td>30–35</td>
<td>23</td>
<td>21, 10</td>
<td>8.8</td>
</tr>
<tr>
<td>1-palmitoyl-2-hydroxy-sn-glycero-3-[phospho-rac-(1-glycerol)] (LPPG)</td>
<td>0.018</td>
<td>160–170</td>
<td>46</td>
<td>19, 30</td>
<td>72</td>
</tr>
<tr>
<td>DDM/FC-10 (molar ratio 4:7)</td>
<td>nd$^a$</td>
<td>77</td>
<td>34</td>
<td>14, 21</td>
<td>26</td>
</tr>
<tr>
<td>DHPC/LPPG (molar ratio 1:1)</td>
<td>nd$^a$</td>
<td>80</td>
<td>35</td>
<td>17, 20</td>
<td>29</td>
</tr>
</tbody>
</table>

"Aggregation number determined by SAXS.$^{21}$ $^a$ Dominant distance from headgroup to headgroup across the short dimension of the micelle core. $^b$ Radii defining spheroid with one axis $a$ and two other axes $b$. $^c$ Hydrophobic volume calculated from $r_{HC}$. $^d$ Not determined.

Table S2 in Supporting Information) indicates that the detergents that produce resolved NMR spectra (DM and FC-12) do not have similar head groups, ionic properties, or alkyl chain lengths. To characterize the micelles formed by the different detergents, we have determined their shape and size using SAXS. In particular, we obtain the aggregation number ($N$) and hydrophobic core volume ($V_{HC}$) from fits of a two-component spheroid model, and the dominant headgroup separation ($L_i$, the distance between the head groups centers across the short diameter of the spheroid) from the position of the second maximum in the scattering intensity (see ref 21 and Figure S4 in Supporting Information). We find that DM and FC-12 form micelles of similar size with respect to $N$, $V_{HC}$, and $L_i$ (Table 1).$^{21}$ In contrast, the detergents for which poor NMR spectra were observed form either smaller and thinner (FC-10) or larger and thicker (DDM) micelles compared to DM and FC-12.

**Dimensions of Mixed Micelles.** In order to further probe the relationship between micelle size and thickness and membrane protein conformational homogeneity, it is desirable to be able to systematically influence micelle geometries. Therefore, we explored whether engineering mixed micelles by mixing detergents at different ratios might be a way to systematically change the size and shape of detergent micelles. To this end, we obtained SAXS data for a comprehensive set of mixed micelles, including detergents with varying hydrophobic tails and head groups, including nonionic, zwitterionic, and ionic species (Figure 3, Figures S4–S6 and Tables S2 and S3 in Supporting Information).

The dependence of $L_i$ (determined from the position of the second peak in the scattering intensity) on the mixed micelle composition for two detergents $A$ and $B$ was fit by the relationship

$$L_i(X_A) = L_B + (L_A - L_B)X_A$$  \hspace{1cm} (1)

with the mixing ratio $X_A = ([A])/([A] + [B])$ (Figure 3, solid lines). $[A]$ and $[B]$ are the detergent concentrations that have been corrected for the monomeric detergent concentration by use of the relationship $X_{i,monomer} \approx X_i$ cmc, where $X_i$ and cmc are the mole fraction and critical micelle concentration of detergent species $i$.\textsuperscript{22} The linear dependence of $L_i$ on the mixing ratio $X_A$ appears to hold for a wide range of detergent mixtures (Figure 3). The data set includes mixtures of detergents that differ only by their alkyl chain length (DDM/DM mixtures) and that feature different nonionic head groups (DDM/OG mixtures, a mono- and a disaccharide) and combinations of nonionic and zwitterionic head groups (DDM/FC-10 and NG/DHPC mixtures), as well as combinations involving an ionic detergent species (LPPG). Furthermore, the relationship of thickness to mixing ratio is largely independent of whether the concentration of detergent A was held fixed and varying concentrations of detergent B were added or whether the reverse strategy was employed. Finally, the relationship seems to be largely independent of the total (absolute) concentration of detergents employed (data not shown), in agreement with the finding that the position of the second peak is independent of detergent concentration for micelles formed by a single detergent species.$^{21}$

**Design of Mixed Micelles for NMR Structure Determination.** The linear dependence of the characteristic micelle thickness on detergent mixing ratios (eq 1) provides a straightforward method to engineer micelles of a particular thickness...
Figure 4. Optimization of mixed micelles for TM0026 NMR measurements. Characteristic micelle thickness $L$ is plotted as a function of the mixing ratio of detergents $\chi$ for DDM/FC-10 (red circles) and LPPG/DHPC (green squares) mixed micelles. A horizontal line is drawn at the $L$ value measured for DM and FC-12 (34 Å).

Figure 5. NMR $^{15}$N,$^1$H-TROSY spectra of $^2$H,$^{15}$N-labeled TM0026 in (a) DDM/FC-10 mixed micelle ($\chi_{\text{DDM}} \approx 0.4$, red) and (b) DHPC/LPPG mixed micelle ($\chi_{\text{LPPG}} \approx 0.5$, green). In both panels, the spectrum of TM0026 in DM is shown in black.

Protein–Detergent Interactions. NMR NOEs and chemical shift perturbation mapping have been widely used to investigate protein–protein and protein–ligand interactions and can provide information about protein–detergent interactions. The backbone assignments of TM0026 in DM and DDM/FC-10 allow a direct comparison of the chemical shifts in each detergent condition. The chemical shift differences $\Delta \delta = \left[\left(\Delta \delta_{\text{H}} / 5\right)^2 + \left(\Delta \delta_{\text{N}}\right)^2\right]^{1/2}$ between the DM and DDM/FC-10 detergent conditions were calculated and, as expected from the overall similarity of the spectra, the majority of the chemical shift differences (Figure S8 in Supporting Information) are small ($\Delta \delta < 0.4$ ppm, the average chemical shift difference). However, two regions of the protein exhibit greater differences in chemical shifts: L48–R54 and E61–R68 (the protein sequence is shown in Figure S9 in Supporting Information). The variability in the chemical shifts in the C-terminus (E61–R68) might stem from different interactions of the highly charged C-terminus with the zwitterionic FC-10 headgroups compared to the neutral DM head groups. The residues C-terminal of P46 in the second transmembrane helix (L48–R54) exhibit an increased variability in chemical shift. It is plausible that the proline residue results in a kink that uncouples the motion of these residues from the N-terminal region of the helix. The C-terminal region may adopt slightly different conformations in DM and FC-10/DDM micelles, possibly coupled to the interactions of the C-terminus.

To investigate the protein conformation in the other detergent conditions, backbone assignments in DM and DDM/FC-10 micelles were compared to the chemical shifts observed in the $^{15}$N,$^1$H-TROSY spectra of detergent conditions in which line broadening impeded obtaining 3D spectra for assignments and structure determination. If it is assumed that cross peaks with similar chemical shifts are the same in different detergent conditions, the observable resonances in DM and FC-10 are distributed throughout the protein sequence. Cross peaks in the entire C-terminus and both $\alpha$-helices were observed under all detergent conditions, consistent with previous evidence that TM0026 is $\alpha$-helical, monomeric, and not aggregated in DDM and FC-10 and that the observed line broadening is due to exchange processes. However, resonances in the loop, at the C-terminal end of the first $\alpha$-helix, and the tertiary contact (A13 determined from the EPR data) were not identified and/or observed under DDM and FC-10 detergent conditions. Therefore, the exchange effects contributing to line broadening do not seem to be localized to one region of TM0026.

In addition, the interactions between the protein and detergents can be mapped by use of the $^{15}$N-resolved $^1$H,$^1$H-NOESY for the DM and DDM/FC-10 mixed micelles (Figure S9 in Supporting Information). For DDM/FC-10, NOEs with the detergent alkyl chain were observed for both transmembrane $\alpha$-helices (L7–R27 and F34–V53) and rapid exchange with water was observed for residues in the loop and C-terminus. The cartoon of TM0026 in Figure 6 is colored according to the observed NOEs between the backbone and the alkyl chain of the detergents. A similar trend was observed with DM; however, the assignment was not as comprehensive (see Experimental Section).

Figure 6. Models of matching micelle size and shape to the hydrophobic surface of TM0026. (a) Model demonstrating hydrophobic matching between the hydrophobic dimensions of the protein (hydrophobic length of the α-helices is ≈29–31 Å) and the detergent alkyl chains such as in the case of DM, FC-12, and the 4:7 DDM/FC-10 mixed micelle. (b) Model demonstrating a hydrophobic mismatch between the surface area of the protein and the micelles, which are too small, such as in the case of FC-10, causing the α-helices to separate in order to bury more surface area in the interior of the micelle. (c) Possible model for the heterogeneity observed in the DDM larger micelle. The tertiary fold of the protein is maintained, but there are many regions of the micelle that may accommodate the protein. Another protein molecule is shown in gray for which the hydrophobic surface area of the protein is buried within the micelle, and an arrow indicates exchange between the two conformers. Approximately 25% of the detergent micelle is removed in order to view the protein and the interior of the micelle. The micelle is rendered as a surface and colored yellow. The protein is displayed as a cartoon model; residues for which NOEs between the amide proton and the alkyl chain of detergent molecules are observed are colored red, and residues that were unassigned or lacked NOEs with the detergent are colored gray. The A13R1 side chain is rendered as blue sticks. The dominant headgroup separation L is labeled in each panel.

Influence of Micelle Dimensions on Protein Conformations.

The results for TM0026 protein−detergent complexes suggest that the protein conformations are strongly influenced by the size and thickness of the detergent micelle. In interpreting these results, we need to consider that the aggregation number N can be different for “empty” micelles and micelles in protein−detergent complexes.15 Similarly, we expect the micelle thickness L to show some variability in the presence of a protein due to the fluid nature of detergent micelles. Nonetheless, the characteristic headgroup−headgroup dimension L measured for micelles in the absence of proteins appears to reflect an intrinsic packing preference of the detergent and we observe a similar thickness in TM0026 complexes that yield well-resolved NMR spectra (Figure S6 in Supporting Information).

Taken together, the results suggest that well-resolved NMR spectra are observed when the hydrophobic component of L (which is equal to L minus the thickness of the headgroup, ≈29–31 Å; see Table S3 and Figure S5c in Supporting Information) matches the length (≈30–33 Å) of the hydrophobic part of the α-helices of TM0026 (Figure 6a). We note that this hydrophobic dimension matches the average hydrophobic thickness of the T. maritima bilayer (≈30 Å27,28). In the case of the FC-10 micelle, L is too small (Table 1). A possible explanation for the observed loss of tertiary interaction and structural heterogeneity in FC-10 is that the mismatch between the hydrophobic surface and the micelle dimensions may result in a perturbation of the protein structure to avoid hydration of the hydrophobic α-helices (Figure 6b). This structural perturbation is reminiscent of that observed in the case of “hydrophobic mismatch” observed in lipid bilayers29 and interhelical packing disruption observed in detergents.30–32 For the case of DDM, the dominant headgroup separation is larger than the length of the hydrophobic α-helices, but there is sufficient hydrophobic core volume to accommodate the protein hydrophobic surface area. The protein tertiary structure is maintained in DDM, but line broadening in both the NMR and EPR suggests structural heterogeneity and/or conformational exchange. One of many possible explanations is exchange between protein conformers (for which the tertiary structure is maintained) within a micelle (Figure 6c). For instance, if it is assumed that the protein does not perturb the micelle, the protein cannot traverse the center of the micelle. Instead, the protein may pack in regions of the large micelle that better match the hydrophobic regions of the protein. These allowed environments may be heterogeneous and/or two or more conformers could be in exchange, which would give rise to the observed line broadening in the NMR and EPR spectra. More experiments need to be performed to understand the effects of the detergent on the protein structure that give rise to the observed line broadening in the DDM micelle.

Conclusion

Using NMR, EPR, and SAXS, we demonstrate a correlation between the dimensions of detergent micelles and the structure of a membrane protein, which leads to the rational design of mixed micelles that facilitates NMR structure determination. In particular, we found that the micelle thickness of mixed micelles depends linearly on the mixing ratio for a significant range of detergent mixtures and that this thickness appears to correlate strongly with the quality of NMR observations.

The generality of the dependence of tertiary contact stability on micelle thickness is difficult to assess at this point, as only very few polytopic membrane protein NMR structures have been determined (seven structures, out of which only one is α-helical).33 However, our observations are in qualitative agreement with previous findings. The structure of the glycoporphin A dimer (with a similar hydrophobic thickness as TM0026) was solved in FC-12.34 In sodium dodecyl sulfate (SDS), which has a slightly larger L value of ≈36 Å (J.L. and S.D., unpublished results), dimerization is still possible but significantly reduced compared to FC-12.31 Therien and Deber30 found that a helix−loop−helix construct from cystic fibrosis transmembrane conductance regulator, which has a shorter hydrophobic thickness compared to glycoporphin A and TM0026, dimerizes in micelles with short alkyl chains, while the dimer interaction is predominantly lost in SDS and other micelles of similar thickness.

Future work will explore whether similar relationships can be observed for different membrane proteins varying by size (in particular, larger helical bundles with larger hydrophobic

References

surface area), fold, and origin. Likely, other micelle properties (such as the total micelle volume) will have to be taken into account to fully understand protein–detergent interactions.

Nonetheless, our data suggest that, rather than exhaustively screening a multitude of detergents, it might be possible to rationally engineer appropriate mixed micelles for NMR structure determination following simple principles and from a limited set of detergents.

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Supporting Information Available: Further details of SDSL, SAXS, and NMR results. This material is available free of charge via the Internet at http://pubs.acs.org.

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