

The fluidity of phosphocholine and maltoside micelles and the effect of CHAPS

Marissa Kieber[†], Tomihiro Ono[†], Ryan Oliver[†], Sarah B. Nyenhuis[†], D. Peter Tieleman[‡], Linda Columbus[†]

[†]Department of Chemistry, University of Virginia, Charlottesville, Virginia 22904, United States

[‡]Department of Biological Sciences and Centre for Molecular Simulation, University of Calgary, Calgary, Alberta T2N 1N4, Canada

Abstract

The dynamics of phosphocholine and maltoside micelles, detergents frequently used for membrane protein structure determination, were investigated using electron paramagnetic resonance of spin probes doped into the micelles. Specifically, phosphocholines are frequently used detergents in NMR studies and maltosides are frequently used in X-ray crystallography structure determination. Beyond the structural and electrostatic differences, this study aimed to determine if there are differences in the local chain dynamics (i.e. fluidity). The nitroxide probe rotational dynamics in longer chain detergents is more restricted than in shorter chain detergents and maltosides micelles are more restricted than phosphocholine micelles. Furthermore, the micelle microviscosity can be modulated with mixtures as demonstrated with mixtures of 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) with n-dodecylphosphocholine (FC12), n-tetradecylphosphocholine (FC14), n-decyl- β -D-maltoside (DM), or n-dodecyl- β -D-maltoside (DDM). These results indicate that observed differences in membrane protein stability in these detergents could be due to fluidity in addition to the already determined structural differences.

Introduction

Detergent micelles are used to mimic properties of the lipid bilayer to solubilize and stabilize membrane proteins. However, the physical properties of the bilayer that are important to stabilizing a membrane protein fold are not fully known. Systematically manipulating the mimics (e.g. detergent mixed micelles) allows for the possibility of exploring the physical forces and properties that are important to stabilizing membrane protein fold and function. In general, micelles are thought to be both translationally and rotationally more dynamic than bilayers (1-3). Reports of micelle microviscosities range from 4 - 50 cP (2-4) depending on the detergent micelle (and ionic strength), and range from 63 – 223 cP for a synthetic DMPC bilayer depending on the position within the bilayer (2) and ~50 – 140 cP in biological membranes (5). Several scattering (6-10), spectroscopy (1, 11-17), and molecular dynamics (18-24) studies have investigated micelles; however, most of these studies have focused on the micellar structure, polarity or water penetration, and critical micelle concentration rather than chain dynamics. Molecular dynamics studies report that the chain dihedral and CH₂ order parameters in dodecylphosphocholine micelles were comparable to that of the bilayer (24) and that the dihedral transition rate was slower in micelles than that of a DPPC bilayer (24, 25). A comparison of EPR studies of bilayers (26-29) and micelles (30-35) suggest that the rotational dynamics of the alkyl chains can be similar or different depending on the detergent micelle and bilayer. Micelle dynamics could be modulated by numerous intermolecular forces between the detergent monomers. Head group – head group interactions mediated by hydrogen bonds or electrostatic interactions could mediate packing and, thus, translational and rotational dynamics at the surface of the micelles. Restrictions in the internal rotational dynamics of the hydrophobic tail could be modulated by van der Waals interactions between the hydrophobic tails and could vary depending on structure. Similar to lipid bilayers,

steroid-like hydrophobic tails may have similar impacts to those of cholesterol on the rotational dynamics of alkyl chains of other detergent molecules. In this study, the dynamics of phosphocholine and maltoside micelles, detergents frequently used for membrane protein structure determination (36-40), were investigated using electron paramagnetic resonance of spin probes doped into the micelles. Specifically, phosphocholines are frequently used detergents in NMR studies and maltosides are frequently used in X-ray crystallography structure determination. In NMR experiments, the protein-detergent complexes must not interact, or exchange processes and increases in the size of the particles will contribute to undesirable line broadening. Therefore, detergents that reduce interactions between protein-detergent complexes, such as ionic and zwitterionic detergents, will result in quality NMR spectra. In contrast, protein-detergent complexes must interact with each other to form crystals and, thus, having head groups that lack electrostatic repulsion could explain the observed preference towards nonionic detergents. However, more recently, phosphocholine micelles were proposed to be denaturing and destabilizing to many membrane proteins (41-44). In addition, proteins can have different structures in different membrane mimics. For example, mitochondrial calcium uniporter structures determined with x-ray crystallography and cryo-EM structures in nanodiscs and maltosides (45, 46) are different from structures determined with NMR in FC14 (47), although it is important to note that the protein used for NMR studies was also truncated and refolded protein from inclusion bodies, which could also significantly impact the protein fold. Membrane fluidity impacts membrane protein folding (48-50) and function (51, 52); however, the impact detergent fluidity has on stabilizing membrane proteins has not been well investigated, in part because the microfluidity of micelles is thought to be much higher than the lipid bilayer (2, 3).

In this study, we determined that the local chain dynamics (and, thus, fluidity) of detergent micelles varies significantly. The nitroxide probe rotational dynamics (which reports on fluidity) in longer chain detergents is more restricted than in shorter chain detergents and maltosides micelles are more restricted than phosphocholine micelles. Furthermore, the micelle fluidity can be modulated with mixtures, as demonstrated with mixtures of 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) with n-dodecylphosphocholine (FC12), n-tetradecylphosphocholine (FC14), n-decyl- β -D-maltoside (DM), or n-dodecyl- β -D-maltoside (DDM).

Experimental Methods

Materials. Doxyl-labeled PC lipids were purchased from Avanti Polar Lipids, Ltd. (Alabaster, AL). The lipids arrived as a powder, and were stored at -20°C until use. Doxyl-labeled stearic acid was purchased from Sigma-Aldrich. The detergents FC12, FC14, DM, DDM, and CHAPS were obtained from Anatrace and stored at -20 °C until use. The chemical structures of these lipids and detergents are shown in Figure 1. All other chemicals were purchased from Sigma-Aldrich or Fisher. All samples were prepared in an aqueous buffer solution (20 mM sodium phosphate pH 6.8, 150 mM NaCl, and 10% D2O).

Micelle Preparations. For EPR power saturation experiments, ~2 mM detergent micelles (152 mM FC12, 200 mM FC14, 202 mM DM, and 290 mM DDM) were prepared containing 0.1 mM doxyl stearic acid label (to ensure that there is more than one probe in a micelle) at the 5, 12, or 16 position or 0.1 mM doxyl PC label at the 5, 7, 12, or 16 position in 20 mM sodium phosphate pH 6.8, 150 mM NaCl, and 10% D2O (EPR buffer). Micelle concentrations were calculated using the following equation

$$[\text{micelle}] = ([\text{det}] - \text{cmc})/N \quad \text{Eq. 1}$$

where [det] is the concentration of the detergent monomer, cmc is the critical micelle concentration, and N is the aggregation number. Values of N for the different detergents were obtained from ref (8). Each sample was prepared in triplicate, and power saturation experiments were collected under 3 conditions: under N₂, under air (O₂), and under N₂ with 10 mM nickel-ethylenediaminediacetic acid (NiEDDA) added. For EPR continuous wave studies of mixed micelles, the doxyl-labeled PC lipids were solubilized in chloroform to a concentration of 2 mM. The labeled lipid in chloroform solution was aliquoted into sample vials at the appropriate volume, dried under N₂ stream, and placed under high vacuum overnight. Appropriate volumes of 200 mM detergent micelle and CHAPS were diluted with EPR buffer and added directly to the vials to solubilize the dried lipid. The amounts to maintain the estimated 2 mM micelle concentrations were calculated using the following relationships

$$\frac{1}{CMC_{mix}} = \sum \frac{x_i}{CMC_i} = \frac{x_{det}}{CMC_{det}} + \frac{x_{CHAPS}}{CMC_{CHAPS}} \quad \text{Eq. 2}$$

where x_i represents the mole fraction of the species in the micelle. The aggregation number of the mixture is calculated by weighting the pure aggregation numbers (7, 8) with the mole fraction of the component:

$$N_{mix} = N_{det}x_{det} + N_{CHAPS}x_{CHAPS} \quad \text{Eq. 3}$$

With the aggregation number and the cmc of the mixture, the micelle concentration can be estimated using Equation 1. The glass vials were vortexed briefly to ensure thorough mixing. FC12 micelles with SA and PC derivatives were prepared in triplicate from the aliquot stage to analyze the error associated with power saturation measurements.

EPR Spectroscopy. A Bruker EMX X-band CW-EPR spectrometer was used. All spectra were recorded at room temperature. The micelle samples were loaded into 0.6 mm I.D. capillary tubes

with a sample volume of around 10 μ l. The EPR spectra were processed with the packaged Bruker EMX software, and analyzed with LabView programs provided by Christian Altenbach and Wayne Hubbell.

For power saturation experiments, previously described methods were used (53-55). Approximately 10 μ l of sample was loaded into a TPX capillary (Molspec) and equilibrated with air or nitrogen gas. Amplitudes of the center manifold were measured at either 12 or 13 power steps for most samples (in some cases a few additional power steps were required), starting from an attenuation of 29.0 dB and step of -2.0 dB (corresponding to a power range of 0.25 up to 158 mW). These amplitudes and powers were plotted to determine $P_{1/2}$ values for each condition. $\Delta P_{1/2}$ values for oxygen and NiEDDA were calculated by subtracting $P_{1/2}$ values under nitrogen from the $P_{1/2}$ values of the sample equilibrated with air or 20 mM NiEDDA and N_2 . Concentrations less than or equal to 10 mM NiEDDA did not saturate with the power range used. The $\Delta P_{1/2}$ values were normalized by dividing by the central line width ΔH^* measured at an attenuation of 20.0 dB. To obtain the collisional frequency, Π , for O_2 or NiEDDA, the normalized $\Delta P_{1/2}$ value was divided by the normalized $P_{1/2}$ value for a standard, diphenyldipicrylhydrazine. A contrast function, Φ , was calculated using: $\Phi = \ln[\Pi(O_2)/\Pi(NiEDDA)]$. Inverse central line width and $2A_{zz}'$ were measured directly from the CW EPR line shapes.

Results and Discussion

Evaluation of spin labeled probes for assessing micelle dynamics and packing. Two types of nitroxide probes were investigated with FC12 micelles in order to assess the difference between the probes and the errors of the measurements. Spin labels varying in the position of the nitroxide were used to investigate the different micellar environments. As was previously done in lipid

bilayers (54), accessibility, Π , of the spin label to an apolar (O_2) or polar (NiEDDA) relaxation-enhancing reagent was measured. Standard deviations for the SA and PC power saturation data were on average 3% but in some cases could be up to 13% (Figure S1). The only significant difference in O_2 accessibility between SA and PC was observed at position 12 with the probe in a more apolar environment in PC than in SA (Figure S1A). The only significant difference observed for the NiEDDA accessibility is at position 5 with the probe having more NiEDDA accessibility in SA than PC (Figure S1B). Within the bilayer, Π values with 20 mM NiEDDA are very low and differences can be difficult to interpret. Differences in NiEDDA accessibility values between lipid exposed and tertiary contacts of a α -helical membrane protein in a synthetic lipid bilayer are no greater than ~ 0.15 (for 20 mM NiEDDA) (56) compared to differences up to ~ 1 for 3 mM NiEDDA between solvent-exposed and buried sites in soluble proteins (57). Thus, the differences of ~ 0.03 observed in position 5 do not reflect large differences in NiEDDA accessibility between the PC and SA probes. The contrast function, Φ , combines both NiEDDA and O_2 accessibility, with high Φ values indicating the nitroxide is buried (i.e. excluded from aqueous solvent). The Φ values for PC are higher than SA for all three positions (5, 12, and 16) (Figure S1C) suggesting the PC probe is overall positioned deeper into the micelle.

Observed differences in the accessibility are due to differences in the diffusion and/or local concentration of the reagent, as well as, potential differences in the micelle localization of the nitroxide probe. Since the micelle is the same for the SA and PC probes, the local concentration and diffusion of O_2 and NiEDDA is likely the same for FC12/PC and FC12/SA. The micelle localization of the nitroxide at each position could be different because SA and PC properties are dramatically different. PC has a large zwitterionic head group and two alkyl chains and SA has a population of negatively charged head groups (pKa of ~ 4.5 in bulk aqueous solvent and ~ 7.0 in a

zwitterionic DMPC bilayer (58)), small head group and a single alkyl chain (Figure 1). In addition, the chain length difference may impact the localization of the 16-doxyl due (i) to the shorter radius of the micelle ellipsoid for FC12 compared to FC14 (7, 8) and (ii) the previously reported localization of 16-doxyl towards the head group in lipid bilayers (54) and in FC12 micelles (59). Considering these contributors, the accessibility differences between PC and SA indicate that the PC nitroxide probes at position 5 and 12 are deeper inside the micelle than the SA probe.

One striking observation is the overall small difference in Φ between positions for each of the probes (the largest difference observed is ~ 0.7), including the 16-position. In lipid bilayers, Φ increased linearly for positions 7, 10, and 12 with the maximum $\Delta\Phi \approx 2.5$ (54). In contrast, the depth parameter, Φ , for positions 5 and 16 was similar to that of position 7 in lipid bilayers (54). However, the trend in the contrast function Φ in FC12 for both PC and SA (Figure S1C) indicates that the magnitude of the gradient in accessibility observed in bilayers is not observed.

For both the SA and PC probes, the spin label dynamics increases as the nitroxide moiety is positioned further away from the head group (Figure S2). Despite the different positioning of the probes as observed in the accessibility measurements, the overall trends are similar between the two probes. There are small differences in dynamics (as evaluated by $2A_{zz}'$ and ΔH_{pp}^{-1} , Figure S2) between the SA and PC derivatives; the 5-doxyl PC is more restricted than the 5-doxyl SA and the 12-doxyl SA is more restricted than the 12-doxyl PC. The dynamics of the nitroxides are dominated by internal rotational modes about the bonds in the alkyl chain that increase as the nitroxide is positioned further from the head group. The difference observed in the localization based on the accessibility measurements may account for the dynamic differences between the SA and PC labels. Because (i) the PC and SA probes reported similar trends in the micelles, (ii) the 7-doxyl position was available commercially in the PC-derivative, and (iii) the additional chain in

PC may allow the dynamics at the 5- and 7-doxyl positions to have greater sensitivity to detergent packing, the PC doxyl probes were used to investigate four different pure micelles and CHAPS mixtures of each.

FC14 micelles are more apolar than FC12 micelles and DM micelles are similar in polarity to DDM micelles. The accessibility of the nitroxide to relaxation-enhancing reagents depends on the diffusion and solubility of the probe environment. Although it is difficult to imagine a mechanism that would significantly alter O₂ diffusion in the micelle, if diffusion was the determining factor for the O₂ accessibility differences between detergents with the same head group, then nitroxides in the longer chain detergent would have less or similar accessibility to O₂ as the shorter chain. FC14 and DDM have higher aggregation numbers (Table S1) than FC12 and DM and, thus, could have a higher packing density (if the micelles were the same volume as FC12 or DM, respectively) or the same packing density (if FC14 and DDM micelles were larger than FC12 and DM, respectively). The longer chain detergents with the same head group cannot have packing densities less than the shorter chain micelles based on the experimentally determined micelle dimensions and aggregation numbers (Table S1) (7, 8). Thus, O₂ accessibility difference of detergents with the same head group are predominantly due to O₂ solubility in each environment and, thus, polarity. The O₂ accessibility data (Figure 2) indicates that at all positions, except the 16-doxyl, FC12 is more polar than FC14 and DM and DDM do not differ in polarity. The contrast function Φ values (Figure S3B) overall represent the polarity differences and similarities observed with the O₂ accessibility. However, at position 12, the Φ value is higher for DM than DDM. The high value is also observed in FC14 and is due to very low NiEDDA accessibility (Figure S3A). In proteins, NiEDDA accessibility values in the range of 0.1 are attributed to “steric exclusion”, that is, at

deeply buried sites the NiEDDA local concentration approaches zero due to packing of the protein that excludes molecules the size of NiEDDA. In the case of micelles, the NiEDDA accessibility of position 12 may approach zero at the deepest penetrating probe in the series (not the 16-doxyl as discussed above). Further discussion of the trends at position 12 requires a comparison of the maltosides to the phosphocholines. Despite all of the potential caveats, the O_2 accessibility indicates that FC14 is more hydrophobic than FC12 at positions 5, 7, and 12.

Maltoside and phosphocholine micelles have different O_2 and NiEDDA accessibility. The oxygen accessibility at all positions is lower for the maltoside micelles than for the phosphocholine micelles (Figure 2). The Φ values (Figure S3B) at positions 5 and 7 are lower for the maltosides than the phosphocholines. At position 12, FC14 and DM have higher Φ values because of the very low NiEDDA accessibility (Figure S3A) observed for FC14 and DM. The diffusion, local concentration, and different micelle localization of relaxation-enhancing probes are all potential explanations for these accessibility differences between the maltoside and phosphocholine maltosides.

Different localization of the probe inside the detergent micelle can be due to different interactions between the PC head group and the phosphocholine or maltoside head group. To explain the observed accessibility data, the PC probe has to be localized more deeply in the phosphocholine micelles than in the maltoside micelles. One possibility is that in phosphocholine micelles the charged phosphate and amine groups of the probe have ionic interactions with the charged groups in the phosphocholine detergent, while the large maltoside head group has more polar interactions with the PC head group and the charged groups in PC are solvated (Figure 3). As a result of these different interactions, the PC probe would be deeper in the phosphocholine micelles than the

maltoside micelle (Figure 3). For the most part, the trends in Φ (Figure S3B) support this explanation, with the probe in FC14 having the highest Φ for positions 5, 7 and 12 and DDM the lowest. For both FC12 and DM, the interpretation is complicated by the fact that the probes at position 12 and 16 are longer than the detergent chain and likely can sample a large space of the micelle that is not representative of a localized reporter.

In addition to the probe depth, the observed accessibility differences could be due to differences in the packing of the detergent monomers which could (i) modulate the microviscosity, (ii) change the hydrophobicity, and/or (iii) sterically exclude the relaxation-enhancing reagent. If steric exclusion was significantly contributing to the observed accessibility, then O_2 and NiEDDA accessibility would be less for the more tightly packed micelle. The micelle shapes, sizes and aggregation numbers (number of monomers per micelle) of these micelles differ (Table S1) (7-9) because of the differences in the monomer shape, the length of the alkyl chain, and the head group charge and polarity. As explained earlier, the longer chain detergents have the potential to have tighter packing than the shorter chains; however, the impact of the head group is more difficult to compare. The extent of detergent packing can be determined by calculating the surface area or volume per monomer by dividing the experimentally determined volumes by the aggregation number and comparing the value to the volume derived from the structure of the monomer detergent. The larger the volume per monomer, the more loosely packed the micelle. In all cases, the average monomer volume calculated from the volume of the micelle is greater than the actual monomer volume; yet, the difference between the calculated and actual monomer volume for DDM is much less (Figure S5). Thus, DDM would be more tightly packed than the other detergent micelles; however, the micelle models derived from small angle x-ray scattering experiments have ranges of dimensions and aggregation numbers (7, 8) that limit the analysis. Regardless of the

precision, packing density due to the head groups would impact the 5, 7, and 12 positions and have a consistent trend in the accessibility and contrast function, which is not observed.

The O₂ accessibility data (Figure 2) implies that the interior of the phosphocholine micelles is more hydrophobic than the maltoside micelles. One explanation for the difference in hydrophobicity is that the detergent packing in phosphocholines is tighter than in maltosides. The microviscosities for these four detergents have not been reported; however, the values reported for both lipid bilayers (2, 5) and micelles (2-4) indicate potential differences that would certainly impact the diffusion of the relaxation-enhancing reagent. Because there are many variables that contribute to O₂ and NiEDDA accessibility, it is likely impossible to uniquely determine the physical micelle properties that contribute to the observed accessibility differences. Therefore, the dynamics of the nitroxide probes, which reflect packing and microviscosities more directly, were investigated by analyzing the CW EPR spectra.

Longer chain detergents are more tightly packed and have higher microviscosities compared to shorter chain detergents with the same head group. To investigate the variability in the fluidity of different detergents, the dynamics of doxyl probes in four different micelles (FC12, FC14, DM, and DDM) were investigated. The nitroxide motions in the micelle are predominantly determined by the internal bond rotations of the alkyl chain (with faster dynamics down the alkyl chain, increasing the probes rate and amplitude). The extent of interactions and packing of the detergent monomers modulates the probe dynamics and, thus, can report on the fluidity. The EPR spectra of the nitroxide at all positions along the chain differ between the different detergents (Figure S4). In both the maltoside and phosphocholine micelles, the nitroxides at all four positions are less mobile in the longer chain micelles (FC14 and DDM) compared to the shorter chain micelles with the

same head group (FC12 and DM, respectively) (Figure S4). These dynamic differences can be compared using the spectral parameters inverse central linewidth, ΔH_{pp}^{-1} (Figure 4), and the rotationally averaged hyperfine splitting, $2A_{zz}'$ (Figure 4), which are more sensitive to rates and amplitudes of motion, respectively (60). In three of the four positions, ΔH_{pp}^{-1} is lower and $2A_{zz}'$ is higher for FC14 compared to FC12, indicating that FC14 is more tightly packed than FC12 micelles. The same trend is observed comparing DM to DDM, with DDM having the more restricted environment at most positions. Thus, longer chain detergent micelles have more tightly packed interior with higher microviscosities compared to shorter chain detergent micelles (with the same head group). The difference in interior micelle microviscosity as estimated based on standard curves determined for SA at 5, 12, and 16 positions in glycerol-ethanol (2) between FC12 and FC14 is approximately 11 cP and between DM and DDM is approximately 12 cP. Because of the differences observed between SA and PC dynamics, the microviscosity was calculated from the linewidths of the 12- and 16-doxyl positions only.

Maltoside micelles have higher microviscosities than phosphocholine micelles. The spectra in the maltoside micelles (Figure S4) indicate the nitroxide probes at all four positions are less mobile than those in the phosphocholine micelles. The 5- and 7-doxyl nitroxides in the maltoside micelles are indicative of an anisotropic motion with a restoring potential. Spectral parameters indicate that the nitroxide in the phosphocholine micelles are more dynamic (greater values of ΔH_{pp}^{-1} and lower $2A_{zz}'$ values) than in the maltoside detergents (Figure 4). The $2A_{zz}'$ values of the nitroxide in DDM (Figure 4B) is the closest to that in the lipid bilayer (26-28) at all positions and is very similar in value at the 5- and 7-doxyl positions (Figure 4B). The close agreement between the maltosides and bilayer $2A_{zz}'$ values compared to the phosphocholine values could be explained by the differences in the nitroxide positions in the different micelles (Figure 3). However, the mobility

differences between the two head groups is observed at all positions, which implies that the restriction in motion is not solely due to a direct interaction between the nitroxide and the head group or differences in position of the nitroxide probes (Figure 3). Direct comparison of the positions should not be used to compare localized microviscosities because of the possibility the probes are at different depths in the micelle. However, overall, at all positions, the phosphocholines are more mobile than the maltosides. Thus, maltoside micelles are more tightly packed than phosphocholine micelles, resulting in a higher microviscosities in maltoside micelles than phosphocholine micelles. The average interior micelle microviscosities calculated from the EPR lineshapes (using only the 12 and 16-position) (2) for FC12 and FC14 are ~54 cP and ~65 cP, respectively, and for DM and DDM are ~69 cP and ~81 cP, respectively. In comparison, DMPC liposomes have a microviscosity of ~63 cP (as probed by the 12- and 16-positions).

Effect of sterol-like detergents on detergent micelle dynamics. In biological bilayers, fluidity is modulated through mixtures of different lipids; thus, the fluidity of detergent mixtures was investigated. CHAPS is a zwitterionic detergent with a rigid ring structure that may modulate micelle packing and microviscosity. There is a more polar side of the rigid ring system that has three hydroxyl groups that impact how the molecules interact in the micelle (15, 19, 30). Nonetheless, CHAPS forms micelles and the rigid ring structure is predicted to modulate the fluidity of the four detergents FC12, FC14, DM, and DDM investigated. EPR spectra of 5-, 7-, 12- and 16-doxyl PC were recorded for binary mixtures of 8-75% CHAPS (Figure S6). Based on the evaluation of the line shapes and the inverse central linewidth, the 12- and 16-doxyl positions decrease in mobility with an increase in CHAPS concentration, while the 5- and 7-doxyl nitroxide dynamics are less impacted by CHAPS (Figure 5 and S6 and S7). The inverse central linewidth (ΔH_{pp}^{-1}) is dominated by the rate of motion of the nitroxide probe, while the spectral parameter

$2A_{zz}'$ is more sensitive to changes in the amplitude of motion (60). In phosphocholine micelles, the 5- and 7-doxyl nitroxides become less mobile (as assessed with $2A_{zz}'$; Figure 6) with increasing concentrations of CHAPS up to ~35%. Above concentrations of 50%, the nitroxides become more mobile with increasing concentrations of CHAPS. For DDM and DM micelles, the dynamics of the 5- and 7-doxyl does not change for CHAPS concentration less than 35%. The mobility of the 12- and 16-doxyl labels decreases with increasing CHAPS throughout the entire range of CHAPS concentrations investigated for all four detergents. A rearrangement of the micelles at higher CHAPS concentrations is implied based on the EPR line shapes and in the $2A_{zz}'$ measurement; however, this rearrangement was not as clear from the central line width data. Concentration-dependent structural transitions were previously reported for pure CHAPS micelles (15, 19), and shape transitions in binary detergent mixtures were observed when mixing prolate and oblate micelles (9).

Although the localization of the probes could be different with the different detergent/CHAPS mixtures, the similar trends observed for all four mixtures (with the same head group and with different head groups) at all four positions indicates that the effect is independent of the potential differences in probe position. The average micelle interior microviscosities (calculated from standard curves for the 12- and 16-positions (2)) vary from ~54 cP in pure FC12 to ~79 cP in 36% CHAPS and 64% FC12 mixture, ~65 cP in pure FC14 to ~86 cP in 36% CHAPS and 64% FC14 mixture, ~69 cP in pure DM and ~109 cP in 36% CHAPS and 64% DM mixture, and ~81 cP in pure DDM and ~93 cP in 36% CHAPS and 64% DDM mixture (only position 16 was used in calculation for DM and DDM). Thus, the microviscosities of micelles, in addition to previously determined properties(6, 9), can be modulated with binary mixtures of detergents.

Conclusions

The fluidity of detergent micelles depends on the head group and chain length of the detergent monomers. The more tightly packed micelles have reduced rotational dynamics and approach the fluidity of a bilayer. DDM and DM are strikingly less fluid than phosphocholine micelles. Fluidity of micelles can be modulated with CHAPS. However, micelles with greater than 50% CHAPS likely have structures significantly different than the pure detergent micelle.

Author Contributions

TO and MK and RO performed the experiments and contributed to the writing of the paper. SBN helped acquire and analyze data. DPT and LC designed the experiments and wrote the paper.

Acknowledgements

Tracy Caldwell and Nicole Swope provided useful feedback and edits to the manuscript. This work was funded by the National Science Foundation (MCB 0845668, L.C.), by the National Institute of Health under Grant (2R01GM087828-06, L.C.), the Research Corporation for Scientific Advancement (L.C.), the Natural Sciences and Engineering Research Council of Canada (D.P.T.), the Canada Research Chairs Program (D.P.T.) and Alberta Innovates Technology Futures through the Strategic Chair in (Bio)Molecular Simulation (D.P.T.).

References

1. Nery, H., O. Soderman, D. Canet, H. Walderhaug, and B. Lindman. 1986. Surfactant Dynamics in Spherical and Nonspherical Micelles - a Nuclear-Magnetic-Resonance Study. *J Phys Chem-Us* 90(22):5802-5808.
2. Bahri, M. A., B. J. Heyne, P. Hans, A. E. Seret, A. A. Mouithys-Mickalad, and M. D. Hoebeke. 2005. Quantification of lipid bilayer effective microviscosity and fluidity effect induced by propofol. *Biophys Chem* 114(1):53-61.
3. Fowler, M., V. Hisko, J. Henderson, R. Casier, L. Li, J. L. Thoma, and J. Duhamel. 2015. DiPyMe in SDS Micelles: Artifacts and Their Implications in the Interpretation of Micellar Properties. *Langmuir* 31(44):11971-11981.
4. Shinitzky, M., A. C. Dianoux, C. Gitler, and G. Weber. 1971. Microviscosity and Order in Hydrocarbon Region of Micelles and Membranes Determined with Fluorescent Probes .1. Synthetic Micelles. *Biochemistry* 10(11):2106-+.
5. Vanderkooi, J. M., and J. B. Callis. 1974. Pyrene. A probe of lateral diffusion in the hydrophobic region of membranes. *Biochemistry* 13(19):4000-4006.
6. Columbus, L., J. Lipfert, K. Jambunathan, D. A. Fox, A. Y. Sim, S. Doniach, and S. A. Lesley. 2009. Mixing and matching detergents for membrane protein NMR structure determination. *J Am Chem Soc* 131(21):7320-7326.
7. Lipfert, J., L. Columbus, V. B. Chu, S. A. Lesley, and S. Doniach. 2007. Size and shape of detergent micelles determined by small-angle X-ray scattering. *J Phys Chem B* 111(43):12427-12438.
8. Oliver, R. C., J. Lipfert, D. A. Fox, R. H. Lo, S. Doniach, and L. Columbus. 2013. Dependence of micelle size and shape on detergent alkyl chain length and head group. *PLoS One* 8(5):e62488.
9. Oliver, R. C., J. Lipfert, D. A. Fox, R. H. Lo, J. J. Kim, S. Doniach, and L. Columbus. 2014. Tuning micelle dimensions and properties with binary surfactant mixtures. *Langmuir* 30(44):13353-13361.
10. Dupuy, C., X. Auvray, C. Petipas, R. Anthore, F. Costes, I. RicoLattes, and A. Lattes. 1996. Small angle X-ray and neutron scattering study of the micellization of (N-alkylamino)-1-deoxylactitols in water. *Langmuir* 12(13):3162-3172.
11. Rawat, S. S., and A. Chattopadhyay. 1999. Structural transition in the micellar assembly: A fluorescence study. *J Fluoresc* 9(3):233-244.
12. Lebedeva, N., and B. L. Bales. 2006. Location of spectroscopic probes in self-aggregating assemblies. I. The case for 5-doxylstearic acid methyl ester serving as a benchmark spectroscopic probe to study micelles. *J Phys Chem B* 110(20):9791-9799.
13. Lebedeva, N., R. Zana, and B. L. Bales. 2006. A reinterpretation of the hydration of micelles of dodecyltrimethylammonium bromide and chloride in aqueous solution. *J Phys Chem B* 110(20):9800-9801.
14. Peric, M., M. Alves, and B. L. Bales. 2006. Combining precision spin-probe partitioning with time-resolved fluorescence quenching to study micelles - Application to micelles of pure lysomyristoylphosphatidylcholine (LMPC) and LMPC mixed with sodium dodecyl sulfate. *Chem Phys Lipids* 142(1-2):1-13.
15. Qin, X. G., M. L. Liu, D. W. Yang, and X. Zhang. 2010. Concentration-Dependent Aggregation of CHAPS Investigated by NMR Spectroscopy. *J Phys Chem B* 114(11):3863-3868.

16. Becerra, N., C. Toro, A. L. Zanocco, E. Lemp, and G. Gunther. 2008. Characterization of micelles formed by sucrose 6-O-monoesters. *Colloid Surface A* 327(1-3):134-139.
17. Alvares, R., S. Gupta, P. M. Macdonald, and R. S. Prosser. 2014. Temperature and Pressure Based NMR Studies of Detergent Micelle Phase Equilibria. *J Phys Chem B* 118(21):5698-5706.
18. Faramarzi, S., B. Bonnett, C. A. Scaggs, A. Hoffmaster, D. Grodi, E. Harvey, and B. Mertz. 2017. Molecular Dynamics Simulations as a Tool for Accurate Determination of Surfactant Micelle Properties. *Langmuir* 33(38):9934-9943.
19. Herrera, F. E., A. S. Garay, and D. E. Rodrigues. 2014. Structural properties of CHAPS micelles, studied by molecular dynamics simulations. *J Phys Chem B* 118(14):3912-3921.
20. Kraft, J. F., M. Vestergaard, B. Schiott, and L. Thogersen. 2012. Modeling the Self-Assembly and Stability of DHPC Micelles Using Atomic Resolution and Coarse Grained MD Simulations. *J Chem Theory Comput* 8(5):1556-1569.
21. Lebecque, S., J. M. Crowet, M. N. Nasir, M. Deleu, and L. Lins. 2017. Molecular dynamics study of micelles properties according to their size. *J Mol Graph Model* 72:6-15.
22. Roussel, G., C. Michaux, and E. A. Perpete. 2014. Multiscale molecular dynamics simulations of sodium dodecyl sulfate micelles: from coarse-grained to all-atom resolution. *J Mol Model* 20(10).
23. Sayyed-Ahmad, A., L. M. Lichtenberger, and A. A. Gorfe. 2010. Structure and Dynamics of Cholic Acid and Dodecylphosphocholine-Cholic Acid Aggregates. *Langmuir* 26(16):13407-13414.
24. Tieleman, D. P., D. van der Spoel, and H. J. C. Berendsen. 2000. Molecular dynamics simulations of dodecylphosphocholine micelles at three different aggregate sizes: Micellar structure and chain relaxation. *J Phys Chem B* 104(27):6380-6388.
25. Mackerell, A. D. 1995. Molecular-Dynamics Simulation Analysis of a Sodium Dodecyl-Sulfate Micelle in Aqueous-Solution - Decreased Fluidity of the Micelle Hydrocarbon Interior. *J Phys Chem-Us* 99(7):1846-1855.
26. Hubbell, W. L., and H. M. McConnell. 1971. Molecular motion in spin-labeled phospholipids and membranes. *J Am Chem Soc* 93(2):314-326.
27. Griffith, O. H. a. J., P.C. 1976. Lipid Spin Labels in Biological Membranes. *Spin Labeling Theory and Applications*. L. J. Berliner, editor. Academic Press, New York, pp. 454-523.
28. Gaffney, B. J., and H. M. McConnell. 1974. Paramagnetic-Resonance Spectra of Spin Labels in Phospholipid Membranes. *J Magn Reson* 16(1):1-28.
29. Bratt, P. J., and L. Kevan. 1993. Electron-Spin-Resonance Line-Shape Analysis of X-Doxylstearic Acid Spin Probes in Dihexadecyl Phosphate Vesicles and Effects of Cholesterol Addition. *J Phys Chem-Us* 97(28):7371-7374.
30. Rodi, P. M., M. D. B. Gianello, and A. M. Gennaro. 2014. Insights About CHAPS Aggregation Obtained by Spin Label EPR Spectroscopy. *Appl Magn Reson* 45(12):1319-1332.
31. Kawamura, H., Y. Murata, T. Yamaguchi, H. Igimi, M. Tanaka, G. Sugihara, and J. P. Kratochvil. 1989. Spin-Label Studies of Bile-Salt Micelles. *J Phys Chem-Us* 93(8):3321-3326.
32. Motyakin, M. V., L. L. Yasina, A. M. Wasserman, L. Z. Rogovina, and V. N. Matveenko. 2010. Local Dynamics of Micelles of New Long-Chain Surfactants in Aqueous Media. *Colloid J* 72(1):31-39.

33. Wasserman, A. M., M. V. Motyakin, L. L. Yasina, Y. A. Zakharova, V. N. Matveenko, Y. V. Shulevich, and L. Z. Rogovina. 2010. EPR Spin Probe Study of New Micellar Systems. *Appl Magn Reson* 38(1):117-135.
34. Wasserman, A. M., L. L. Yasina, M. V. Motyakin, I. I. Aliev, N. A. Churochkina, L. Z. Rogovina, E. A. Lysenko, and V. Y. Baranovsky. 2008. EPR spin probe study of polymer associative systems. *Spectrochim Acta A* 69(5):1344-1353.
35. Bales, B. L., and C. Stenland. 1993. Statistical Distributions and Collision Rates of Additive Molecules in Compartmentalized Liquids Studied by Epr Spectroscopy .1. Sodium Dodecyl-Sulfate Micelles, 5-Doxylstearic Acid Ester, and Cobalt(II). *J Phys Chem-Us* 97(13):3418-3433.
36. Columbus, L., H. Klock, S. Lesley, and K. Wuthrich. 2005. Preparation and characterization of alpha-helical membrane proteins from *Thermotoga maritima* for solution NMR structural studies. *Biophysical Journal* 88(1):188a-188a.
37. Nollert, P. 2005. Membrane protein crystallization in amphiphile phases: practical and theoretical considerations. *Prog Biophys Mol Bio* 88(3):339-357.
38. Prive, G. G. 2007. Detergents for the stabilization and crystallization of membrane proteins. *Methods* 41(4):388-397.
39. Warschawski, D. E., A. A. Arnold, M. Beaugrand, A. Gravel, E. Chartrand, and I. Marcotte. 2011. Choosing membrane mimetics for NMR structural studies of transmembrane proteins. *Bba-Biomembranes* 1808(8):1957-1974.
40. Wiener, M. C. 2004. A pedestrian guide to membrane protein crystallization. *Methods* 34(3):364-372.
41. Chipot, C., F. Dehez, J. R. Schnell, N. Zitzmann, E. Pebay-Peyroula, L. J. Catoire, B. Miroux, E. R. S. Kunji, G. Veglia, T. A. Cross, and P. Schanda. 2018. Perturbations of Native Membrane Protein Structure in Alkyl Phosphocholine Detergents: A Critical Assessment of NMR and Biophysical Studies. *Chem Rev* 118(7):3559-3607.
42. King, M. S., P. G. Crichton, J. J. Ruprecht, and E. R. S. Kunji. 2018. Concerns with yeast mitochondrial ADP/ATP carrier's integrity in DPC. *Nat Struct Mol Biol* 25(9):747-749.
43. Kurauskas, V., A. Hessel, F. Dehez, C. Chipot, B. Bersch, and P. Schanda. 2018. Dynamics and interactions of AAC3 in DPC are not functionally relevant. *Nat Struct Mol Biol* 25(9):745-747.
44. Kurauskas, V., A. Hessel, P. Ma, P. Lunetti, K. Weinhaupl, L. Imbert, B. Brutscher, M. S. King, R. Sounier, V. Dolce, E. R. S. Kunji, L. Capobianco, C. Chipot, F. Dehez, B. Bersch, and P. Schanda. 2018. How Detergent Impacts Membrane Proteins: Atomic-Level Views of Mitochondrial Carriers in Dodecylphosphocholine. *J Phys Chem Lett* 9(5):933-938.
45. Fan, C., M. Fan, B. J. Orlando, N. M. Fastman, J. Zhang, Y. Xu, M. G. Chambers, X. Xu, K. Perry, M. Liao, and L. Feng. 2018. X-ray and cryo-EM structures of the mitochondrial calcium uniporter. *Nature* 559(7715):575-579.
46. Yoo, J., M. Wu, Y. Yin, M. A. Herzik, Jr., G. C. Lander, and S. Y. Lee. 2018. Cryo-EM structure of a mitochondrial calcium uniporter. *Science* 361(6401):506-511.
47. Oxenoid, K., Y. Dong, C. Cao, T. Cui, Y. Sancak, A. L. Markhard, Z. Grabarek, L. Kong, Z. Liu, B. Ouyang, Y. Cong, V. K. Mootha, and J. J. Chou. 2016. Architecture of the mitochondrial calcium uniporter. *Nature* 533(7602):269-273.
48. Danoff, E. J., and K. G. Fleming. 2015. Membrane Defects Accelerate Outer Membrane beta-Barrel Protein Folding. *Biochemistry* 54(2):97-99.

49. Dewald, A. H., J. C. Hodges, and L. Columbus. 2011. Physical Determinants of beta-Barrel Membrane Protein Folding in Lipid Vesicles. *Biophysical Journal* 100(9):2131-2140.
50. Hong, H. D., and L. K. Tamm. 2004. Elastic coupling of integral membrane protein stability to lipid bilayer forces. *Proceedings of the National Academy of Sciences of the United States of America* 101(12):4065-4070.
51. Sun, X. L., Y. Fu, M. X. Gu, L. Zhang, D. Li, H. L. Li, S. Chien, J. Y. J. Shyy, and Y. Zhu. 2016. Activation of integrin alpha 5 mediated by flow requires its translocation to membrane lipid rafts in vascular endothelial cells. *Proceedings of the National Academy of Sciences of the United States of America* 113(3):769-774.
52. Gustavsson, M., N. J. Traaseth, and G. Veglia. 2011. Activating and Deactivating Roles of Lipid Bilayers on the Ca^{2+} -ATPase/Phospholamban Complex. *Biochemistry* 50(47):10367-10374.
53. Altenbach, C., S. L. Flitsch, H. G. Khorana, and W. L. Hubbell. 1989. Structural studies on transmembrane proteins. 2. Spin labeling of bacteriorhodopsin mutants at unique cysteines. *Biochemistry* 28(19):7806-7812.
54. Altenbach, C., D. A. Greenhalgh, H. G. Khorana, and W. L. Hubbell. 1994. A collision gradient method to determine the immersion depth of nitroxides in lipid bilayers: application to spin-labeled mutants of bacteriorhodopsin. *Proc Natl Acad Sci U S A* 91(5):1667-1671.
55. Altenbach, C., T. Marti, H. G. Khorana, and W. L. Hubbell. 1990. Transmembrane protein structure: spin labeling of bacteriorhodopsin mutants. *Science* 248(4959):1088-1092.
56. Gross, A., L. Columbus, K. Hideg, C. Altenbach, and W. L. Hubbell. 1999. Structure of the KcsA potassium channel from *Streptomyces lividans*: a site-directed spin labeling study of the second transmembrane segment. *Biochemistry* 38(32):10324-10335.
57. Isas, J. M., R. Langen, H. T. Haigler, and W. L. Hubbell. 2002. Structure and dynamics of a helical hairpin and loop region in annexin 12: a site-directed spin labeling study. *Biochemistry* 41(5):1464-1473.
58. Horvath, L. I., P. J. Brophy, and D. Marsh. 1988. Influence of lipid headgroup on the specificity and exchange dynamics in lipid-protein interactions. A spin-label study of myelin proteolipid apoprotein-phospholipid complexes. *Biochemistry* 27(14):5296-5304.
59. Sommer, L. A., J. J. Janke, W. F. Bennett, J. Burck, A. S. Ulrich, D. P. Tieleman, and S. A. Dames. 2014. Characterization of the immersion properties of the peripheral membrane anchor of the FATC domain of the kinase "target of rapamycin" by NMR, oriented CD spectroscopy, and MD simulations. *J Phys Chem B* 118(18):4817-4831.
60. Columbus, L., and W. L. Hubbell. 2004. Mapping backbone dynamics in solution with site-directed spin labeling: GCN4-58 bZip free and bound to DNA. *Biochemistry* 43(23):7273-7287.

Figure captions

Figure 1. Names, abbreviations, and structures of detergents and lipids used in this investigation. Only the 5-doxyl spin labeled molecules are shown; however, additional spin labeled positions at carbons 7, 12, and 16 were also used in this study.

Figure 2. O₂ accessibility, $\Pi(\text{O}_2)$, of PC doxyl labels at positions along the alkyl chain in four different detergent micelles: FC12 (black squares), FC14 (black circles), DM (gray squares), and DDM (gray circles). The data for FC12 is identical to that in Figure S1 and error bars represent standard deviations of three measurements of three different samples. Dashed lines are only shown to guide the eye.

Figure 3. Potential differences in the localization of the PC probes in phosphocholine and maltoside micelles. The two extreme positions are presented with the alignment of the head groups between PC and FC12 or FC14 possibly driven by ion-ion interactions and the alignment between maltoside and PC driven by polar interactions and solvating the charged groups of PC. The black line indicates alignment of the head group, with the positioning of PC on the left deeper than the position on the right. Orange lines indicate the 5, 7, 12, and 16 positions with the deeper penetrating PC and blue lines indicate the same positions for the less penetrating PC localization.

Figure 4. Inverse central linewidth, ΔH_{pp}^{-1} , (A) and the averaged hyperfine splitting, $2A_{zz}'$, (B) of the EPR spectra for the doxyl spin labeled PC at different positions in FC12 (black squares), FC14 (black circles), DM (gray squares), DDM (gray circles). An increase in ΔH_{pp}^{-1} or a decrease in $2A_{zz}'$ corresponds to an increase in mobility. Red horizontal lines mark the $2A_{zz}'$ observed in an egg phosphocholine lipid bilayer for each doxyl position at room temperature (54.6 G, 5-SA; 54.2 G, 7-SA; 42.4, 12-SA, and 34.0 G, 16-SA) (27).

Figure 5. Dependence of nitroxide mobility on the mole fraction of CHAPS in DM and FC12 detergent micelles. The inverse central linewidth, ΔH_{pp}^{-1} , of the EPR spectra for the PC doxyl moiety at positions 5 (teal inverted triangles), 7 (blue triangles), 12 (red circles), and 16 (black squares) in FC12 (panel A) and DM (panel B). FC14 and DDM are shown in Figure S6.

Figure 6. Dependence of nitroxide mobility on the mole fraction of CHAPS in FC12 (black squares), FC14 (black circles), DM (gray squares), and DDM (gray circles) detergents micelles. The averaged hyperfine splitting, $2A_{zz}'$, is plotted versus CHAPS mole fraction, χ , for the PC doxyl moiety at positions 5 (A), 7(B), 12(C), and 16 (D). The text of the position labels in each panel corresponds to the color of the corresponding symbols in Figure 4. Red horizontal lines mark the $2A_{zz}'$ observed in an egg phosphocholine lipid bilayer for each doxyl position at room temperature (54.6 G, 5-SA; 54.2 G, 7-SA; 42.4, 12-SA, and 34.0 G, 16-SA) (27).