Supplementary Materials

Estimation of free and protein-bound micelle fractions

The observed diffusion coefficient, \( D_{C\text{observed}} \), of a pure micelle sample is the weighted average of free and bound detergent/lipid species and can be written, using a two-site model (45), as

\[
D_{C\text{observed}} = F_{\text{micelle}}D_{C\text{micelle}} + (1 - F_{\text{micelle}})D_{C\text{free}}
\]

where \( F_{\text{micelle}} \) and \( F_{\text{free}} \) (\( = 1 - F_{\text{micelle}} \)) are the fractions of the molecules in micelle-bound and free forms, respectively, and \( D_{C\text{micelle}} \) and \( D_{C\text{free}} \) are the diffusion coefficients of the micelles and free molecules, respectively. Inclusion of the protein molecules, keeping the ratio of micelle to protein > 1, requires another component for the protein–micelle complexes to be included and the observed diffusion coefficient, determined using the micelle peaks, would correspond to a three-site situation

\[
D_{C\text{observed}} = F_{\text{complex}}D_{C\text{complex}} + F_{\text{micelle}}D_{C\text{micelle}} + [1 - (F_{\text{complex}} + F_{\text{micelle}})]D_{C\text{free}}
\]

where \( F_{\text{complex}}, F_{\text{micelle}} \) and \( F_{\text{free}} \) (\( = 1 - (F_{\text{complex}} + F_{\text{micelle}}) \)) are the fractions of the detergent/lipid molecules in complex-bound, pure micelle-bound and free forms, respectively, and \( D_{C\text{complex}}, D_{C\text{micelle}} \) and \( D_{C\text{free}} \) are the diffusion coefficients of the complexes, micelles and free molecules, respectively. A much more complicated situation would arise when two proteins are added to the micelles and the proteins interact heterogeneously (e.g., SP-A and Mini-B in SDS). This work was performed using detergent/lipid concentrations well above the critical micelle concentration (CMC) values. Therefore,

\[
F_{\text{free}} \ll F_{\text{complex}} + F_{\text{micelle}}
\]

and hence, the observed diffusion coefficient of a protein–micelle sample essentially corresponds to the weighted average from the subpopulations of protein-bound complexes and protein-free micelles

\[
D_{C\text{observed}} = S_{\text{complex}}D_{C\text{complex}} + (1 - S_{\text{complex}})D_{C\text{micelle}}
\]
where $D_{C(\text{complex})}$ and $D_{C(\text{micelle})}$ are the diffusion coefficients and $S_{\text{complex}}$ and $S_{\text{micelle}} (= 1 - S_{\text{complex}})$ are the subpopulations of protein–micelle complexes and protein–free micelles, respectively.

For SDS and DPC samples, the use of deuterated detergents allowed us to determine the diffusion coefficient of the protein–micelle complexes using the protein peaks, which can be considered to represent $D_{C(\text{complex})}$ (all of the protein molecules are likely bound as the diffusion data corresponds to a single component fit), in addition to the diffusion coefficient determined using the detergent peaks, which represents $D_{C(\text{observed})}$ (Table 1). The diffusion coefficient of the micelles, $D_{C(\text{micelle})}$, was determined from the pure detergent sample (Table 1). Thus, rearranging the terms in the above equation as

$$S_{\text{complex}} = (D_{C(\text{observed})} - D_{C(\text{micelle})})/(D_{C(\text{complex})} - D_{C(\text{micelle})})$$

we estimated the fractions of SDS and DPC micelles bound to SP-A and Mini-B. The values are presented in Table S1. However, similar estimations could not be performed for LMPC, LMPG and LMPC+LMPG samples since the much more intense non-deuterated lipid peaks overwhelmed the protein peaks and diffusion measurements based on the protein peaks were not obtained.

**Table S1:** Subpopulations of protein-bound and free SDS and DPC micelles ($S_{\text{complex}}$ and $S_{\text{micelle}}$, respectively)

| Composition | $D_{C(\text{observed})}$ (m²/s) | $D_{\text{complex}}$ (m²/s) | $D_{\text{micelle}}$ (m²/s) | $S_{\text{complex}}$ (~ %) | $S_{\text{micelle}}$ (~ %) |
|-------------|-------------------------------|------------------|------------------|---------------------|---------------------|
| SP-A–SDS    | 2.091 ± 0.595                 | 1.055 ± 0.158    | 5.395 ± 0.101    | 76                  | 24                  |
| SP-A–DPC    | 1.837 ± 0.110                 | 1.566 ± 0.077    | 3.362 ± 0.008    | 85                  | 15                  |
| Mini-B–SDS  | 3.460 ± 0.052                 | 2.696 ± 0.267    | 5.395 ± 0.101    | 72                  | 28                  |
| Mini-B–DPC  | 2.621 ± 0.051                 | 2.591 ± 0.359    | 3.362 ± 0.008    | 96                  | 4                   |
Estimation of hydrodynamic diameters for the SP-A–micelle complexes

We estimated the expected hydrodynamic diameters of the SP-A–micelle complexes for the same oligomeric form of SP-A as alone in water. First, the volumes of SP-A in water \((V_{SP-A})\) and pure micelle \((V_{micelle})\) were determined from the corresponding apparent hydrodynamic diameters \((d_{HA})\), calculated from the observed diffusion coefficients \((D_C)\), using the equations

\[
V = \frac{4}{3}\pi \left(\frac{d_{HA}}{2}\right)^3 \quad \text{and} \quad d_{HA} = \frac{k_B T}{3\pi \eta D_C}
\]

The volumes of SP-A in water and individual micelles were then added to obtain the expected volumes of the SP-A–micelle complexes.

\[
V_{SP-A+micelle} = V_{SP-A} + V_{micelle}
\]

Finally, the expected hydrodynamic diameters of the SP-A–micelle complexes were calculated from the volumes of the complexes. Table S2 shows the expected hydrodynamic diameters along with the hydrodynamic diameters actually observed for the SP-A–micelle complexes.

**Table S2:** Expected hydrodynamic diameters of the SP-A–micelle complexes based on the same oligomeric state of SP-A when alone in water versus the observed apparent values

| Micelle system | Micelle volume (cm³) | SP-A volume in water (cm³) | Expected SP-A–micelle volume (cm³) | Expected hydrodynamic diameter (nm) | Observed hydrodynamic diameter (nm) |
|---------------|----------------------|-----------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| SDS           | 9.51E-22             | 7.18E-19                    | 7.19E-19                          | 11.12                             | 3.27                              |
| DPC           | 3.94E-21             | 7.18E-19                    | 7.22E-19                          | 11.13                             | 3.57                              |
| LMPC          | 2.04E-19             | 7.18E-19                    | 9.22E-19                          | 12.08                             | 10.37                             |
| LMPG          | 3.00E-19             |                             | 1.02E-18                          | 12.49                             | 11.27                             |
| LMPC+LMPG     | 3.10E-19             |                             | 1.03E-18                          | 12.53                             | 10.83                             |
Comparison of the NMR signal intensities of SP-A and SP-A + Mini-B with Mini-B

We compared the backbone HN signal intensities of SP-A and SP-A + Mini-B with Mini-B for all micelle systems. The height of the tallest peak in the HN region of the corresponding 1D $^1$H NMR spectrum was used. All intensities were normalized with respect to the DSS peak. Table S3 shows the comparison of the NMR signal intensities.

Table S3: Comparison of the backbone HN signal intensities of SP-A and SP-A + Mini-B with Mini-B in individual micelle systems (rounded to the nearest full number)

| Micelle system | Mini-B | SP-A | SP-A (50%) + Mini-B (50%) |
|----------------|--------|------|---------------------------|
| SDS            | 1      | 22   | 8                         |
| DPC            | 1      | 8    | 4                         |
| LMPC           | 1      | 6    | 4                         |
| LMPG           | 1      | 5    | 2                         |
| LMPC+LMPG      | 1      | 4    | 2                         |
Prediction of NMR parameters for different oligomeric forms of SP-A

We calculated the theoretical values of rotational correlation time ($\tau_c$), proton linewidth ($\Delta\nu_{1/2}$) and relative intensity ($I$) for six different oligomeric forms of SP-A from monomer to octadecamer. First, the hydrodynamic radius ($r_H$) was calculated from the equation

$$r_H = [(3VM)/(4\pi N_A)]^{1/3} + r_w$$

where $V$ is the specific volume of the protein (taken to be 0.73 cm$^3$.g$^{-1}$), $M$ is the molecular mass of the protein (29000 g.mol$^{-1}$ and its multiples), $N_A$ is Avogadro’s number (6.022 x 10$^{23}$ mol$^{-1}$) and $r_w$ is the thickness of one hydration shell (taken to be 3.2 Å) (57). The rotational correlation time ($\tau_c$) was determined from Stoke’s law

$$\tau_c = (4\pi\eta_w r_H^3)/(3k_B T)$$

in which $k_B$ is the Boltzmann constant (1.38 x 10$^{-23}$ J/K), $T$ is the absolute temperature (taken to be 310 K) and $\eta_w$ is the viscosity of the solvent (taken to be 6.92 x 10$^{-4}$ kg/m.s) (57). The proton resonance linewidth ($\Delta\nu_{1/2}$) was determined from the equation

$$\Delta\nu_{1/2} = 1.2\tau_c + 0.3$$

which was an empirical parameterization of Figure 1.6 in Ref. 57. The transverse relaxation time ($T_2$) was determined from the equation (57)

$$\Delta\nu_{1/2} = 1/\pi T_2$$

However, reduction of the signal intensity, i.e., $I(t)$ at time $t$ from the initial intensity $I_0$, follows relaxation processes characterized by a first-order rate equation with a time constant $T$ (58),

$$I(t) = I_0\exp(-t/T)$$

Thus the relative signal intensity of SP-A$_{\text{multimer}}$ ($I_{\text{multimer}}$), with respect to SP-A$_{\text{monomer}}$ ($I_{\text{monomer}}$), was calculated from,

$$I_{\text{multimer}}/I_{\text{monomer}} = T_2(\text{multimer})/T_2(\text{monomer})$$

The estimated values of the NMR parameters are shown in Table S4.
Table S4: Estimates of rotational correlation times, proton linewidths and relative signal intensities for SP-A oligomers at 37 °C

| SP-A oligomeric form (number of molecules) | Rotational correlation time (ns) | Proton linewidth (Hz) | Relative intensity (%) |
|-------------------------------------------|---------------------------------|-----------------------|------------------------|
| Monomer (1)                               | 8.8                             | 10.9                  | 100                    |
| Dimer (2)                                 | 16.2                            | 19.7                  | 55                     |
| Trimer (3)                                | 23.3                            | 28.3                  | 39                     |
| Hexamer (6)                               | 43.8                            | 52.9                  | 21                     |
| Dodecamer (12)                            | 83.4                            | 100.3                 | 11                     |
| Octadecamer (18)                          | 122.0                           | 146.7                 | 7                      |

Estimation of masses and oligomeric forms of micelle-bound SP-A

The molecular masses and the corresponding oligomeric forms of micelle-bound SP-A species were estimated from the experimentally determined translational diffusion coefficients. The structure of a pure micelle, as well as an SP-A/micelle complex, was considered to be spherical. The volume of the sphere (V) was determined using the hydrodynamic diameter ($d_{HA}$) calculated from the translational diffusion coefficient ($D_C$)

$$d_{HA} = \frac{k_BT}{3\pi\eta D_C} \quad \text{and} \quad V = \frac{4}{3}\pi(d_{HA}/2)^3$$

The volume of SP-A ($V_{SP-A}$) was determined from its contribution to the SP-A–micelle complex,

$$V_{SP-A} = V_{SP-A+micelle} - V_{micelle}$$
The molecular mass of SP-A ($M_{SP-A}$) was calculated from its volume using the average protein density ($\rho_{protein}$, taken to be 1.37 g/cm$^3$)

$$M_{SP-A} = \rho_{protein} \times V_{SP-A}$$

The corresponding oligomeric form of SP-A was calculated by dividing the mass by 29,000 Da, the mass of an SP-A monomer. The estimated mass and the matching oligomeric state of SP-A in individual micelle systems are shown in Table S5.

**Table S5:** Molecular masses of SP-A species and their matching oligomeric forms in water and different micelle systems, calculated using the apparent hydrodynamic diameters obtained from the experimentally observed translational diffusion coefficients

| Composition          | Complex volume (cm$^3$) | Micelle volume (cm$^3$) | SP-A volume (cm$^3$) | Estimated SP-A mass (g) | Estimated SP-A mass (Da) | Matching oligomeric state |
|----------------------|-------------------------|-------------------------|----------------------|-------------------------|--------------------------|--------------------------|
| SP-A in water        | ---                     | ---                     | 7.18E-19             | 9.84E-19                | 592,382                  | > 18                     |
| SP-A in SDS          | 1.31E-19                | 9.51E-22                | 1.30E-19             | 1.78E-19                | 107,230                  | ~ 3                      |
| SP-A in DPC          | 3.88E-20                | 3.94E-21                | 3.48E-20             | 4.77E-20                | 28,752                   | ~ 1                      |
| SP-A in LMPC         | 5.84E-19                | 2.04E-19                | 3.80E-19             | 5.21E-19                | 313,676                  | ~ 10                     |
| SP-A in LMPG         | 7.49E-19                | 3.00E-19                | 4.49E-19             | 6.15E-19                | 370,454                  | ~ 12                     |
| SP-A in LMPC+LMPG    | 6.65E-19                | 3.10E-19                | 3.55E-19             | 4.86E-19                | 292,679                  | ~ 9                      |