Gas-Phase Micelles

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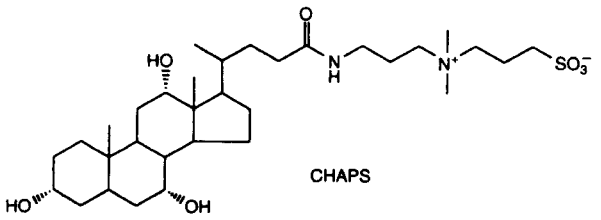
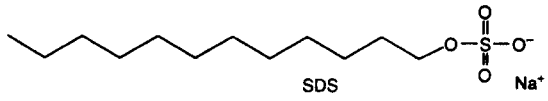
Surfactant micelles represent primitive vesicles associated with the origin of life. Their formation preceded the development of biochemical interactions, membrane assembly, and the beginning of cellular evolution. Micelles are used extensively in biochemistry to facilitate the transport of hydrophobic material and as a site for chemical reactions, thus mimicking their utility in the primordial pools. They are studied through a variety of methods, including gel permeation chromatography,[1] light scattering,[2] and interfacial tensiometry.[3, 4] In this study, pneumatically assisted electrospray (ionspray) ionization (ESI) was used to transfer micelles from the condensed phase into the gas phase. Previous electrospray studies[5] on glycolipids resulted in the observation of Ca^{2+}-dependent glycolipid dimers. Evidence suggests that the origin of these dimers was, in part, due to the noncovalent disruption of glycolipid micelles. We now focus on the detection of the intact micelle.

The electrospray ionization technique (ESI) enables charged molecules to be transferred from a liquid solution to the gas phase by creating a fine spray of highly charged droplets. The sample solution is sprayed from the tip of a metal syringe maintained at approximately 5000 V. Dry gas is fed to the stream of droplets before they enter the vacuum of the mass spectrometer, which facilitates solvent evaporation. As each droplet decreases in size, the electric field density on its surface increases. The mutual repulsion between like charges on this surface becomes so great that it exceeds the forces of surface tension and results in the ion's ejection from the droplet. The ions are then directed into the mass analyzer by electrostatic lenses. This type of ionization is conducive to the formation of highly charged molecules and noncovalently bound molecular complexes.[6-9]

This study focused on CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate) and SDS (sodium dodecylsulfate). The surfactants sodium octylsulfate, octylamine,



CHAPS



SDS

dodecylamine, sodium taurocholate, sodium taurodeoxycholate, and cholic acid were also analyzed. Experiments were performed with an API III Perkin Elmer Sciex triple-quadrupole mass spectrometer having an upper m/z range of 2400 and capable of analyzing for positive and negative ions. An electrospray interface potential of 5.0 kV was used for sample introduction. The declustering potential was varied between 30–250 V to control the orifice collisional energy. Samples with concentrations from 0.01 to 1.00 mM were dissolved either in water or 50:50 methanol:chloroform and introduced at a rate of 3.0 $\mu L\,min^{-1}$. Both positive and negative ions were monitored in these experiments and gave analogous results, although the positive ions generally gave a greater signal intensity.

Since ESI is an evaporation process, it is important to minimize the sample concentration and thus maximize evaporization efficiency to maintain a strong ion signal. To facilitate ionization in these experiments, the concentration of surfactants (1.0 mM) was kept below the critical micelle concentration (cmc; 6–10 mM for CHAPS and 7–10 mM for SDS).[10] However, since desolvation occurs more slowly[11] than micelle formation,[12] it can readily be assumed that the critical micelle concentration is been reached prior to ion ejection, and micelles are formed. Micelle formation in the droplet is likely, since the concentration of the solute in the droplet increases by a factor of 100[11] before ion ejection.

Micelle observation by mass spectrometry may be affected by a number of factors: 1) the size of the micelle, 2) the stability of the charged micelle in the gas phase, and 3) the conditions during evaporation of the droplets (temperature and pressure fluctuations). The first factor was addressed by using a variety of large and small micelle systems. An important feature of mass spectrometers is that they measure the mass-to-charge ratio, m/z, making it possible to observe very large molecules or molecular complexes with an instrument having a relatively small m/z range if the molecules are multiply charged. Micelles typically have counterions at the head group to minimize head group repulsion, and while removal or addition of these counterions (for example, H^+, Na^+, or Cl^-) is necessary in mass analysis in order to create a charged species, this may result in the destabilization and fragmentation of the micelle. Larger micelles would require many charges in order to be observed and could be destabilized by the numerous charges. The second factor was addressed with the choice of surfactants, some of which would potentially undergo intramicellar hydrogen bonding. Micelle formation in solution is driven by an entropy gain of the solvent; therefore, it is important that the molecules forming the micelle have strong intramicellar binding to preserve structure in the gas phase. Lastly, the inability to control droplet conditions precisely (third factor) could be detrimental to micelle formation; however, performing these experiments under identical conditions minimized these fluctuations.

The surfactants CHAPS, sodium taurocholate, sodium taurodeoxycholate, and cholic acid were selected because they have relatively low aggregation numbers and contain intermolecular binding sites[13] that could potentially stabilize association into the gas phase. The electrospray ionization source allows an ion's kinetic energy to be adjusted by means of the declustering potential; declustering potentials on the order of 70 V or lower are conducive to the observation of noncovalent complexes, while greater potentials usually promote the dissociation of noncovalent complexes and even covalent bonds. All of the experiments in which micelles were observed were performed at low declustering potentials, typically at or below 50 V. Figure 1 shows the mass spectra of CHAPS at declustering potentials of 50 V and 150 V. The spectrum acquired at 50 V contains oligomers that correlate with the expected micelle aggregation number (4–14).[10] Experiments performed at a declustering potential of 150 V gave no evidence of micelle formation. Similar results were obtained for sodium taurocholate and sodium taurodeoxy-

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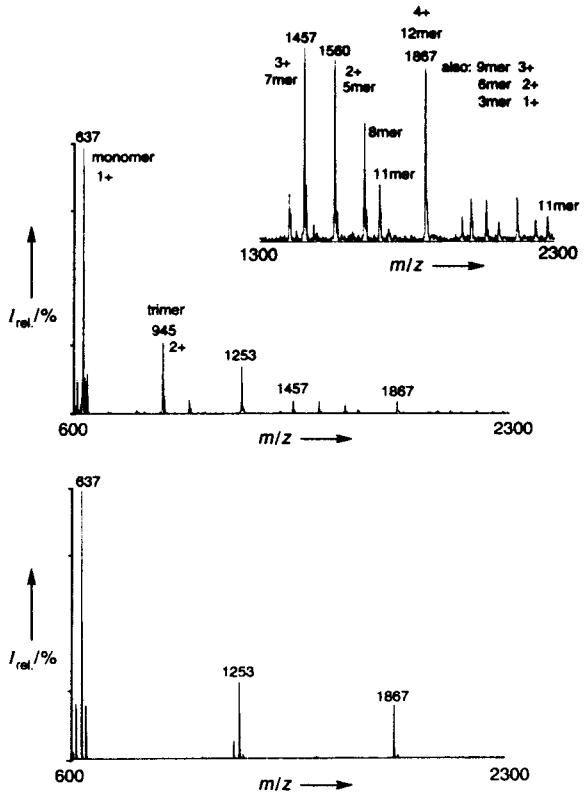

Fig. 1. Positive ion electrospray mass spectra of CHAPS micelles obtained from water at declustering potentials of 50 (a) and 150 V (b). At 50 V the micelles (as Na^+ adducts) vary in size and charge; for example, the ion m/z 1457 represents the $[(CHAPS)_7 + 3Na]^{3+}$. At 150 V only the singly charged monomer, dimer, and trimer were observed. Charge states were based on isotope distribution, cation addition, and MS/MS data.

cholate; however, cholic acid produced no evidence of micelle formation. From these results it is concluded that the zwitterionic nature of CHAPS, sodium taurodeoxycholate, and sodium taurocholate, and the hydrogen bonding interactions between their monomers may have facilitated the observation of gas phase micelles by stabilizing the charged (micelle) structure. Typically, CHAPS, sodium taurodeoxycholate, and sodium taurocholate produced micelles containing at least three charges.

The measured charge states were based on isotope patterns, cation addition, and MS/MS data. The spacing between isotope lines provides a means of determining charge state (Fig. 2); the

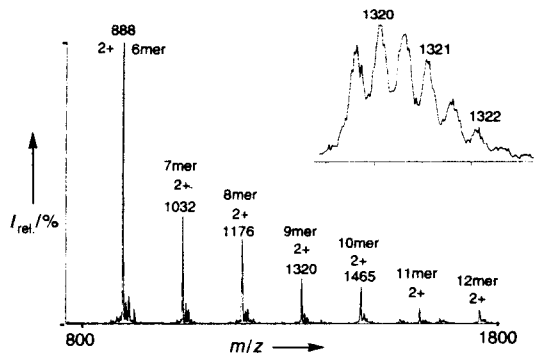

Fig. 2. Aggregates $[(SDS)_n + 2Na]^{2+}$ ($n = 6-12$) observed by positive ion electrospray mass analysis. Charge states determination can be based on spacing of the isotopes; for example, ^{13}C isotopes spaced 1.0 mass unit apart correspond to the 1 + charge state, those 0.5 mass units apart to the 2 + charge state, etc. This spectrum demonstrates the uniform stepwise addition typical of salt aggregates and nonspecific molecular aggregations.

resolving power of the quadrupole instrumentation allowed the determination of up to the 3 + charge state. Cation addition also served to identify the charge states (Fig. 3). The spacing between the cation adducts provided the same information as the isotope pattern without the need for high resolving power.

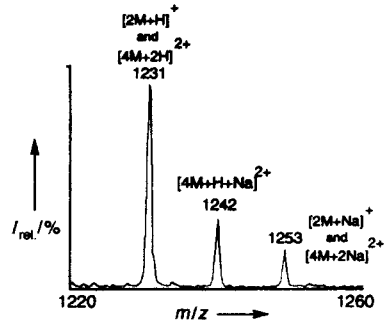

Fig. 3. Mass spectrum of CHAPS obtained at a declustering potential of 75 V. Cation addition is another way of determining an ion's charge state, analogous to examining the isotope distribution. The formation of cation adducts at half the mass of the ion is indicative of a 2 + charge state, at one third the mass of the ion of a 3 + charge state, and so on.

Cation addition was also used to test the effect different cation adducts may have on observing micelle formation. Here, Na was replaced with other alkali metals, Li, K, and Cs; however, no other significant changes were observed in the charge states or ion intensitites with the different cations. MS/MS data provided information about the charge states, since fragment ions were indicative of the minimum aggregation number.

SDS and other alkyl surfactants were also analyzed. In order to observe SDS micelles (aggregation number of 62)[10] with this instrumentation, they would require a positive or negative charge of at least 7. However, the mass spectrum of SDS shows no association that could correlate with micelle formation (Fig. 2), but is more consistent with spectra obtained from salt aggregates.[14] The same experiments were performed on structurally related surfactants, including sodium octyl sulfate, octylamine, and dodecylamine and gave results similar to those observed for SDS. No micelles were observed with these systems regardless of their size. The inability to observe micelles may be associated with significant head group repulsion on the highly charged micelles, as only charges of less than or equal to 2 + were observed for the aggregates. It was theorized that attempts to observe micelles with relatively high aggregation numbers may be difficult due to their instability when highly charged. Furthermore, the relatively weak van der Waal interactions of these molecules may be insufficient to stabilize micelles for transfer to the gas phase.

Tandem mass analysis was used along with the declustering potential to identify the micelle ions further. Tandem mass analysis through collision-induced dissociation (CID or MS/MS) is used to effect fragmentation. An ion of interest is selected with the mass analyzer and introduced into a collision cell, where, typically, argon is used as collision gas. The selected ion will collide with an inert gas molecule to give fragment ions, which are recorded with a second analyzer. Tandem mass spectrometry experiments were performed on the cholic acid derivatives at two different declustering potentials (50 V and 150 V) again to establish micelle formation at the low declustering potentials and a minimal aggregation number for the observed ions. The fragmentation data of the cholic acid derivatives under low declustering potentials (50 V) produced fragment ions (hep-

tamer, decamer, and undecamer) consistent with a dodecamer precursor ion. Figure 4 also illustrates the fragmentation of CHAPS at a declustering potential of 150 V to form only the monomer and dimer, fragments that are more consistent with the trimer precursor ion.

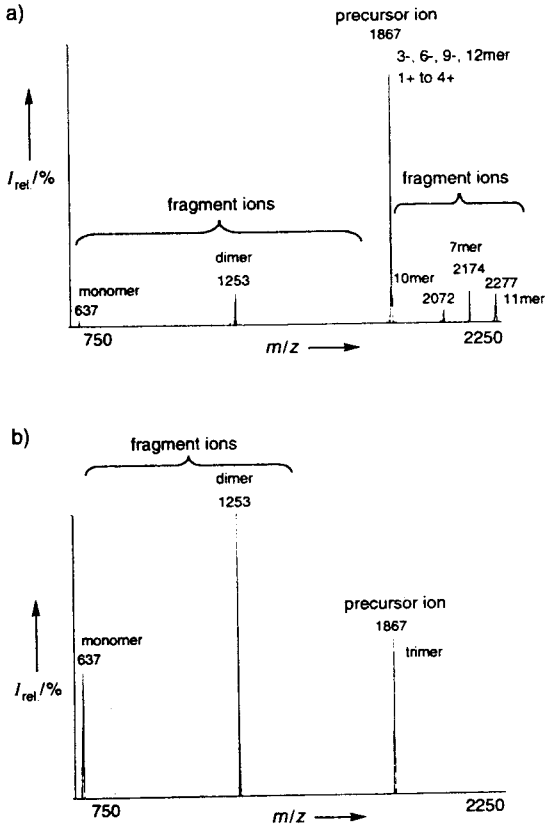

Fig. 4. Positive ion electrospray MS/MS analysis of the ion, m/z 1867, at declustering potentials of 50 V (a) and 150 V (b). The data at 50 V are consistent with the following structures: $[(CHAPS)_{12} + 4Na]^{4+}$, $[(CHAPS)_9 + 3Na]^{3+}$, $[(CHAPS)_6 + 2Na]^{2+}$, and $[(CHAPS)_3 + Na]^{1+}$. The data at 150 V are consistent with $[(CHAPS)_3 + Na]^{1+}$.

SDS and CHAPS were also evaluated in methanol:chloroform (50:50), a solvent system that is conducive to reverse micelle formation. Under these conditions the ion distribution for SDS closely resembled that under aqueous conditions, which further suggested that the clusters observed for these compounds were the result of nonspecific aggregation. However, CHAPS in methanol:chloroform (50:50) produced significantly different results in terms of ion distribution and relative ion intensity than those obtained in aqueous solution. This change could be attributed to the structural difference between reverse micelle and micelle formation.

Additional evidence of micelle formation in the gas phase was obtained from experiments monitoring the critical micelle concentration (cmc). The cmc is defined as the surfactant concentration above which micelles are formed.[10] The cmc's of CHAPS, taurocholate, and taurodeoxycholate are 6–10 mM, 3–11 mM, and 1–4 mM, respectively. The cholesterol-like surfactants were especially ideal for this study, because their similar structures are likely to produce similar evaporation conditions. In these experiments, the concentration of each surfactant was increased from a concentration of 0.01 mM in 0.01 mM increments, and the onset of micelle formation determined by the observation of the heptamer in the 2+ and 3+ charge states. The heptamer was chosen because it is consistent with the aggregation number of all three surfactants, and its signal does not overlap with that of any other oligomer ions in the 1+, 2+, or 3+ charge states.

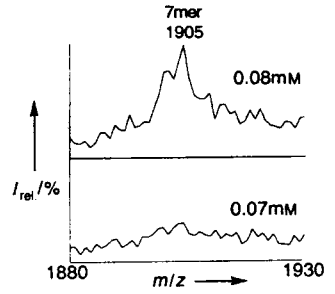

Fig. 5. Onset of micelle formation for taurocholate, monitored with the signal of the heptamer $[(TC)_7 + 2Na]^{2+}$ at 0.08 mM (top) and 0.07 mM (bottom).

The cmc for CHAPS, taurocholate (TC), and taurodeoxycholate (TDC) was found to be 0.06 mM, 0.08 mM, and 0.02 mM, respectively (Fig. 5, Table 1). Assuming evaporation increased the droplet surfactant concentration by a factor of approximately 100,[11] the results correlate with the absolute cmc values and are also consistent with respect to the relative cmc's of the surfactants. These experiments were also performed on SDS, cmc 7–10 mM, where SDS produced significant aggregation only at concentrations greater than 0.20 mM. The aggregation was deemed to be significant if the signal-to-noise ratio of the heptamer was greater than 2. The relatively high concentrations needed to observe aggregation of SDS is further evidence of the nonspecific nature of its aggregation, while the absolute and relative cmc correlations between the steroid surfactants further suggest that micelles were formed.

Table 1. A comparison of critical micelle concentrations (cmc) determined in solution and obtained with ESI for the surfactants CHAPS, TC, TDC, and SDS.

Surfactant	CHAPS	TC	TDC	SDS
cmc (solution) [mM]	6–10	3–11	1–4	7–10
cmc (ESI) [mM]	0.06	0.08	0.02	0.20
c [mM] [a]	6	8	2	20

[a] Estimated concentration of surfactant upon ion ejection.

The "soft" ionization/vaporization process of electrospray has allowed the transfer of micelles into the gas phase and thus the ability to analyze micelles by mass spectrometry. CHAPS, sodium taurodeoxycholate, and sodium taurocholate have provided useful models, probably because they form relatively small micelles and stable, multiply charged complexes.[15]

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