The synthesis and aggregation properties of a novel anionic gemini surfactant

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The aggregation of a peptide-based anionic gemini surfactant has been found to be highly dependent on the environment in which this property is measured; dimensions of aggregates varied from those generally observed in micellar species to those in fibrillar material.

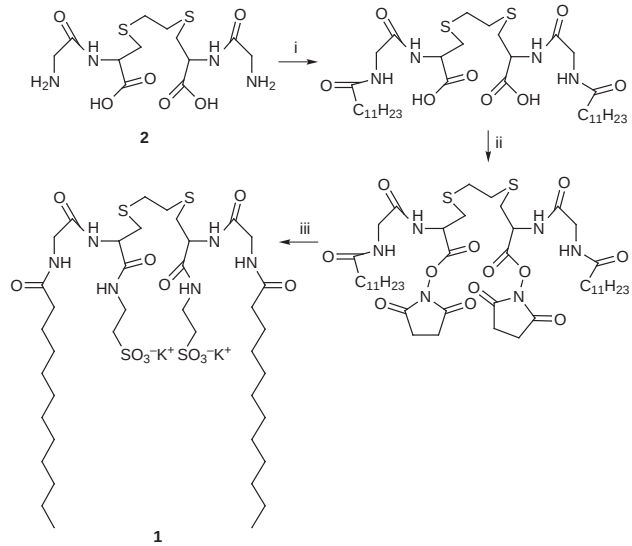
About 25 years ago a rather unusual type of surfactant molecule consisting of dimeric amphiphiles, made up of two long hydrocarbon chains and two ionic groups linked by a spacer, was reported by Bunton *et al.*[1] The name 'gemini' was used by Menger in 1991 to identify these detergents.[2]

The unusual structures of gemini surfactants result in surface and bulk properties that differ from those observed for more conventional single-chain monomeric surfactants having the same number of carbon atoms per polar 'head' group. Thus whereas monomeric surfactants tend to form spherical micelles in dilute solution, geminis usually form thread-like aggregates. Geminis generally have lower critical micelle concentration (cmc) values; their foaming and wetting properties are excellent, resulting in a more efficient lowering of the surface tension of water; they also show unexpected viscosity changes as their concentration is increased.

Most gemini surfactants synthesised over recent years contained bisphosphate or bis(quaternary ammonium) ionic groups linked by spacers ranging from a rigid aromatic group to a more flexible short aliphatic chain. The length of the saturated hydrocarbon tails has ranged from 8 to 18 carbon atoms. The length, flexibility and polarity of the linker group can have a profound effect on the resulting shapes of aggregates formed by gemini surfactants.[2,3]

We have synthesised a peptide-based gemini surfactant **1,** starting from the reaction of L-cysteine with dibromoethane to form the bisthioether. Reaction of the latter with Boc-protected glycine-*O*-succinimide, followed by deprotection, gave compound **2**. This reacted further as shown in Scheme 1 to give **1** as the dipotassium salt. The structure of **1** was confirmed by its ^{1}H and ^{13}C NMR spectra, measured in D_2O. A FAB mass spectrum of **1** in a glycerol matrix, scanning in negative ion mode, gave an isotopic distribution corresponding to the calculated pattern fitting $C_{40}H_{74}N_{6O}O_{12}S_4K$, as shown in Fig. 1.

Gemini **1** was soluble in aqueous media even at neutral pH. The presence of **1** in solutions of the hydrochloride of 1-(1,3-dichloro-6-trifluoromethyl-9-phenanthryl)-3-dibutylaminopropan-1-ol, an anti-malarial drug known as halofantrine and marketed by SmithKline Beecham,[4] caused a substantial change in the fluorescence spectrum of this compound (Fig. 2). From a plot (not shown) of the increase in the intensity of this drug with the increase of the concentration of **1**, an approximate measurement of the cmc was obtained as 10^{-4} mol dm^{-3}. This value is lower than those reported for single hydrocarbon chain surfactants, such as sodium dodecyl sulphate, where the cmc was measured[5] as 7×10^{-3} mol dm^{-3}. Low cmc values have been reported for other gemini surfactants.[2] Fig. 2(*c*) shows that the intensity of the spectrum of halofantrine increases substantially on standing for 24 h, indicating that the organisation of micelles had changed during this period. Electron micros-



Scheme 1 *Reagents*: i, $C_{11}H_{23}COCl$, NaOH, H_2O; ii, *N*-hydroxysuccinimide, DCC, THF; iii, taurine, K_2CO_3, H_2O, THF

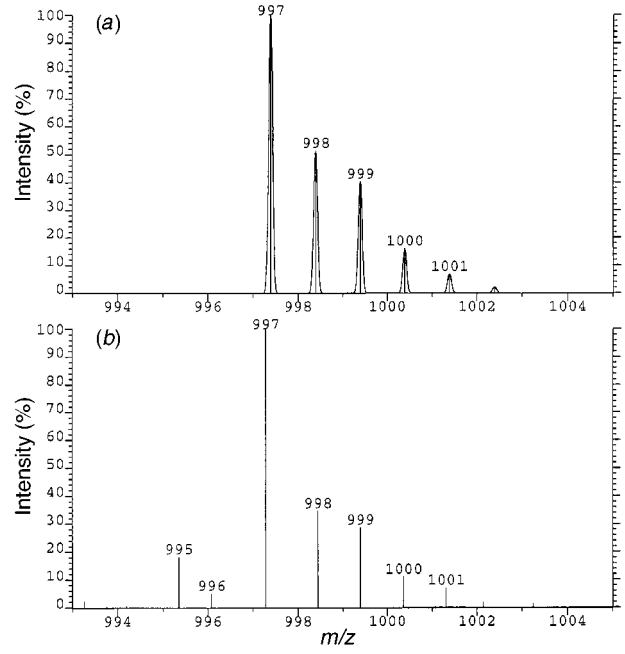


Fig. 1 FAB mass spectrum of **1**: (*a*) calculated and (*b*) experimental isotopic distribution. The spectrum was obtained using a Micromass 70-VSEQ mass spectrometer, negative ionization effected by a primary beam of caesium ions.

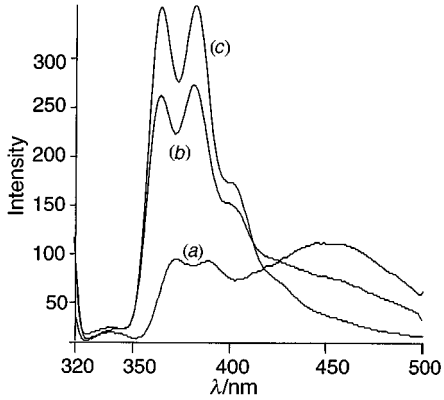


Fig. 2 Emission spectra (λ_{ex} 310 nm) of a 10 μM solution of halofantrine: (*a*) in water, (*b*) in water containing 1 mg ml^{-1} of **1** (fresh solution) and (c) in water containing 1 mg ml^{-1} of **1** (24 h standing). Spectra were recorded on a Perkin-Elmer LS50 spectrofluorimeter.

copy measurements (see later) confirm morphological changes in the organisation of micelles at periods shorter than 24 h.

The diffusion coefficient of **1** was measured as 5.2×10^{-11} m^2 s^{-1} by carrying out diffusion experiments on a sample which was 0.1% (1 mg ml^{-1}) by weight. This value indicated the presence of rather small micelles with a hydrodynamic radius of about 3.5 nm. As the cmc is so low, the diffusion coefficient measured is that of the micelles, with a negligible contribution from the free surfactant.

Attempts to study the morphology of these micelles (1 mg ml^{-1}) in deionised water by transmission electron microscopy (TEM) by negative staining (using 5% w/v ammonium molybdate containing 1% w/v trehalose) at early time points after preparation proved problematic due to surfactant/stain interactions and poor sample spreading; images were obtained that showed discrete globular structures 3 to 5 nm in diameter and globular strings, ~40 nm in length [Fig. 3(A)]. Further analysis of the surfactant solutions after storage at 4 °C for 24 h or longer periods proved easier to prepare by spreading on a grid and staining.

TEM analysis of this aged solution by negative staining gave distinct fibrillar/ribbon structures with periodic 'twist-like' features [Fig. 3(B)]. Fibrils appeared in excess of 10 μm long and ranged between 6 ± 1.5 (n = 23) and 40 ± 13 nm (n = 11) in width. Three populations were identified based on the periodic features exhibited. Low numbers of flat ribbon-like fibrils with irregular spaced twists were seen interspersed among fibrils with 145 ± 23 nm (n = 37) periodicity and a smaller population of shorter 63 ± 8nm (n = 23) period fibrils.

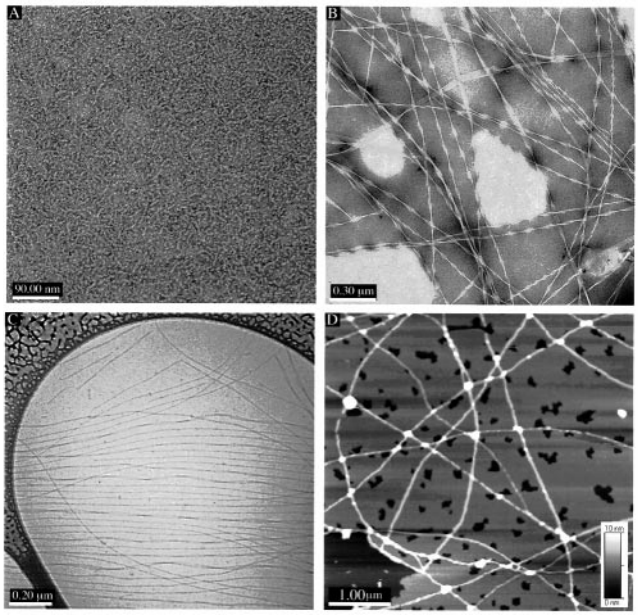


Fig. 3 (A) Negative stained TEM images of **1** immediately after dissolution; (B) negative stained TEM images recorded after 21 h; (C) cryogenic TEM images recorded after 9 days dissolution; and (D) AFM images of surfactant dried onto mica (sample taken from a solution which was 6 h old). TEM and AFM images were obtained using a Hitachi H7100 microscope and a TopoMetrix Explorer microscope, respectively.

Cryogenic transmission electron microscopy (TEM) of samples incubated at 4 °C for 9 days confirmed the presence of fibrils similar to those seen by negative staining after 24 h [Fig. 3(C)]. Fewer fibrils showed periodic features than were seen by negative staining—those present also appeared longer at 211 ± 4 nm (n = 20). Other fibrils (10–20 nm wide) appeared to lack any periodicity. A noticeable inter-fibrillar regular parallel spacing of 20 to 40 nm observed may reflect electrostatic repulsion between surfactant anionic head groups in aqueous solution.

Atomic force microscopy (AFM) images of **1** were also obtained for samples, dried on mica from filtered deionised water, immediately after dissolution and 6 h after dissolution. Images of surfactant immediately after dissolution showed orientated fibrils on top of a relatively flat substructure. Fibrils showed heights of 0.3–0.5 nm above the underlying structure and widths of 37 ± 8 nm. Spreading artefacts due to drying would be expected to result in underestimates of fibril height and overestimates of fibril width (due to tip-sample interactions).

At 6 h after dissolution [Fig. 3(D)] three distinct morphologies were observed by AFM for dried surfactant: sheets, *ca.* 3–4 nm in thickness; globules, 44 nm ± 14 nm (n = 12) in cross-section and 1–5 nm in height; and fibrils, 48 nm ± 10 nm (n = 8) in cross-section and 2–5 nm in height. Fibrils were found to settle predominantly on top of the underlying sheets: areas of exposed mica were largely free of fibrils or contained fibril fragments. This may reflect the strong electrostatic repulsion between the anionic head groups, which would be expected to lie on the outer surface of fibrils in aqueous solution, and the negatively charged mica surface. Approximately 80% of fibrils displayed clear periodicity (mean periodic length = 106 ± 18 nm; n = 8). Periodic features were conserved when the scan direction was rotated through 90° (data not shown) suggesting that it was not caused by a scanning artefact.

The disappearance of smaller fibrils and the appearance of larger, periodic fibrils with time is consistent with a time-dependent aggregation/twisting of early fibrils into larger structures. The fibrilisation phenomena, observed in the TEM and AFM analysis of aggregates of **1,** are reminiscent of those observed in the aggregation of β-amyloid protein (β-AP),[6] which has been implicated in the development of Alzeimer's disease. β-AP contains both hydrophobic and hydrophilic peptide sequences, and has been reported to have micellar properties.[7] Further studies are in progress aimed at identifying the structural features of **1** that lead to the observed aggregation properties.

Notes and References

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