Chiral cones and vesicles from Gemini-type fatty acid–heteroditopic amine mixtures†

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A twin-chiral and twin-tailed Gemini-type fatty acid with a rigid spacer, 2,3-bis(decyloxy) succinic acid, formed chiral self-assembled structures in aqueous solution with heteroditopic amines, such as 6-aminohexanoic acid and 4-aminobutyric acid. The morphology of such structures was governed by the stoichiometry between the fatty acid and the amine. Mixtures with 1 : 2 molar ratio formed exclusively spherical vesicles, while the 1 : 1 mixtures resulted in entropically driven chiral cones in addition to spherical vesicles. Surface tension measurements, light scattering, and TEM studies proved the existence of vesicles in aqueous solution. Cones were formed through disclinations introduced in the hexatic chiral membrane with specific angles, in accordance with $\theta = \arcsin(1 - (n/6))$, $[0 < \theta < \pi/2, 1 < n < 5]$. Mixtures prepared with 4-aminobutyric acid formed only spherical vesicles and no conical self-assemblies, emphasizing the geometry of the Gemini–heteroditopic amine pair, which directs the morphology of the final self-assembled structures.

Introduction

With the advent of synthetic vesicles by Kunitake and Okahata,[1] tunable molecular origami is a reality, expanding the newer self-assembling systems.[2] Among several classes of surfactants, the Gemini surfactants,[3] in which the head groups are connected by a spacer, are potential synthetic surfactants with low CMC values, and their the phase behavior has been well exploited.[4,5] The spacer length has shown a profound effect on the aggregation behavior of Gemini-type surfactants, besides the flexibility in tuning their properties by changing the alkyl chain length, spacer length and counter ions.[6,7] While most of the Gemini surfactants studied in the past had a spacer chain between the head groups, Geminis with rigid spacers are scarce.[8] Short spacer chain Geminis have shown specific visco-elastic properties.[9,10] An aqueous solution of a Gemini surfactant with a spacer chain and carboxylate as the head group formed a discrete cubic phase in the presence of a hydrocarbon,[9] while the same molecule without any spacers showed a visco-elastic worm-like micellar solution.[8a] Gemini surfactants with rigid spacers and with phosphate groups have been shown to exhibit stereo-dependent fusion and fission of vesicles in the presence of Ca²⁺ ions.[8d] Gemini surfactants from stilbenes with rigid –C≡C– were found to deviate from the conventional micelle formation, against the one with flexible spacers. The presence of rigid spacers restricted the intra-molecular chain–chain association accounting for the anomalous self-organization behavior.[8d]

While the properties of Gemini surfactants are highly advantageous over conventional single-tail surfactants, their usage is hindered by their high cost owing to synthetic complexity. Also,

biocompatibility or biodegradability ensures their efficient utilization for any eco-friendly process. Tartaric acid-based Gemini surfactants are ideal in such a purview.[11–16] Renewable and biological utilization of surfactants demand their complete solubility in aqueous media.[17] Addition of co-surfactants,[18] hydrogen bonding[19] and catanionic mixtures[20] are the common methods that are adopted for solubilization of a water-insoluble fatty acid into aqueous medium.[21] Bolaamphiples are promising non-conventional-type surfactants[22] that could be exploited in mixed surfactant formulations.[23] They possess two polar head groups on either end of a hydrophobic chain, making them efficient non-covalent solubilizers in aqueous solution, promoted by hydrogen bonding and electrostatic interactions. The unique nature of their molecular skeleton prefers them to form stable monolayers than bilayers.[24] A 'U'-shaped conformation is adopted by them, providing the flexibility of fabricating multifunctional organic self-assemblies[25] by controlling the fatty acid–bola surfactant stoichiometry as 1 : 1 or 1 : 2. Furhop and Mathieu have reported the self-assembling behavior of a cyclohexyl dicarboxylic acid derivative with cyclohexylamine to form vesicular suspensions in aqueous medium.[26] Self-assembly of water-insoluble fatty acids with boladiamines have been reported by Douliez *et al.*[27] Vesicles with superior thermal stability have been achieved with bolamphiphile–surfactant mixtures.[28] Introduction of chirality in the molecular framework is an exciting avenue to form chiral superstructures that are stereoselective towards the guest molecules. This makes the approaches feasible to realize a system that is more akin to real biological systems.

In the present work, we report the formation of chiral cones and spherical vesicular self-assembled structures from a twin-chiral, twin-tailed Gemini-type fatty acid with a rigid spacer in association with heteroditopic amines in aqueous solution. The chiral surfactants are enantiomerically pure (2*R*,3*R*)-(+)-bis(decyloxy)succinic acid and (2*S*,3*S*)-(−)-bis(decyloxy)succinic acid (collectively represented as BDSA), and the hetroditopic

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amines being 6-aminohexanoic acid (6AHA) and 4-aminobutyric acid (4ABA). Carboxyl-terminated amines were the choice in relevance to amino acids, which are the building blocks of biological systems. The fatty acid in the present case is a unique Gemini-type surfactant with head groups connected by a rigid spacer to point chiral centers.

Experimental

Synthesis

$(2R,3R)$-(+)-bis(decyloxy) succinic acid and $(2S,3S)$-(−)-bis (decyloxy) succinic acid were synthesized according to the reported procedure.[29]

Conditions for self-assembly

0.5 M stock solutions of 6AHA and 4ABA were prepared in ultra pure water (Millipore-Academic). Mixtures of 6AHA–BDSA or 4ABA–BDSA were made by ultrasonicating appropriate dilutions of the amino acids from the stock solutions for 20 min. The mixtures were then warmed to 70 °C followed by quenching in an ice bath. Heating and cooling were repeated at least three times to achieve a homogeneous milky translucent solution.

Determination of CAC

The critical aggregation concentration (CAC) of the surfactants was determined by the Wilhelmy method (KSV, Finland) using a platinum plate sensor in a jacketed cell. Temperature was controlled by circulating water from a Julabo thermocontroller with an accuracy of 25 ± 0.5 °C. The solution was stirred and allowed to stand for equilibrium at the set temperature before measuring the surface tension. The mean values of surface tension of the solutions were obtained from three concordant measurements.

Light scattering measurements

Dynamic light scattering measurements were made using a Brookhaven 90 Plus particle size analyzer (Brookhaven Instruments, USA) in a quartz tube.

FTIR measurements were made using a Perkin–Elmer spectrometer. A drop of the solution was pressed between CaF_2 substrates and the spectrum was recorded in transmission mode.

X-Ray measurements were performed using a Rigaku-Miniflex diffractometer. The gel was spread on shallow glass well substrate and data acquisitions were made with a scan step of 0.02 degrees per min using a $CuK\alpha$ source.

Microscopy

Optical microscopy was performed using a Euromex microscope fitted with a color CCD camera (Samsung SD10) and transmission electron microscopy was done using a Philips CM12 electron microscope. The vesicle images were taken after staining with 1% phosphotungstic acid for 2 minutes on a carbon-coated copper grid (200 mesh SPI, Electron Microscopic Sciences, USA).

Results and discussion

The molecular structures of the amphiphiles studied are shown in Chart 1. Mixtures of 6AHA–BDSA or 4ABA–BDSA in water were prepared in the concentration range 0.1 to 50 mM with molar ratios 1 : 1 and 1 : 2. Surface tension measurements for the 1 : 2 solutions (as shown in Fig. 1) revealed the critical aggregation concentration of 0.078 mM for 4ABA and 0.144 mM for 6AHA, respectively. This suggests 4ABA to be more efficient in solubilizing BDSA than 6AHA. Optical micrographs of 1 : 2 mixtures (10 mM) revealed vesicular clusters and spherical vesicles, as shown in Fig. 3a and 3b. TEM micrographs of a 0.25 mM solution of the 1 : 2 mixtures, as shown in Fig. 3c and 3d revealed vesicular structures being formed, while a 50 mM solution upon standing over a week transformed into an opaque gel-like phase. Optical micrographs of the gel under crossed polarizers appeared birefringent with interference hues, characteristic of the crystalline phase. Light scattering measurements of vesicular solutions revealed ensembles of 200–350 nm in size (*vide* Fig. 3) and were in conformity with the TEM results. The FTIR spectrum showed ν_{CH_2} at 2850 cm^{-1} and δ_{CH_2} at 1455 cm^{-1} and 1470 cm^{-1}, while δ_{CH_2} for the pristine AHA was observed at 1411 cm^{-1} (see ESI†). The observed peak positions of the symmetric 2850 cm^{-1} and antisymmetric 2928 cm^{-1} ν_{CH} stretching modes of the methylene groups were close to those observed in crystalline *n*-alkanes having all-*trans* conformation.[30] A similar shift, in layered organic hybrid systems of long chain systems was attributed to
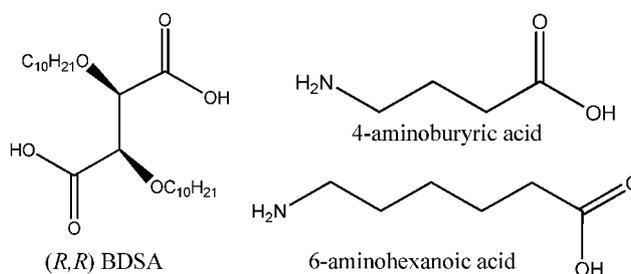

Chart 1 Molecular structures of the Gemini amphiphile and the amino acids under study.
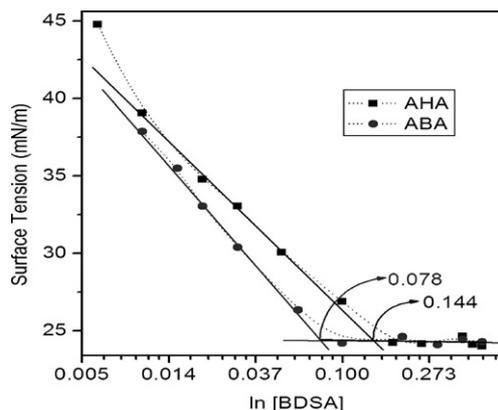

Fig. 1 Surface tension profiles of 1 : 2 BDSA–6AHA and BDSA–4ABA binary solutions showing the CAC values of 0.144 and 0.078 mM (with respect to BDSA), respectively.
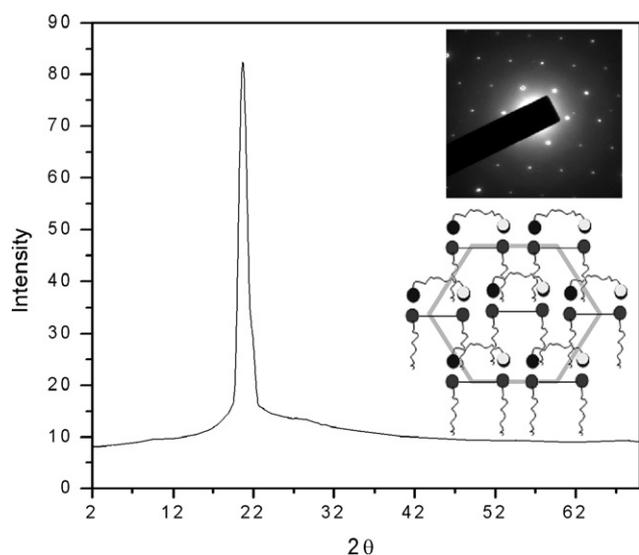
Fig. 2 Wide-angle X-ray diffraction pattern of the BDSA–6AHA gel. The inset shows the SAED pattern and the hexagonal arrangement of BDSA–6AHA pairs.

the presence of all-*trans* conformation of the alkyl chains.[30b] The absence of splitting of the 1470 cm^{-1} band in the present case indicates weak intermolecular interactions between the alkyl chains[30c] suggesting them to be in all-*trans* conformation with few in *gauche* conformation. X-Ray diffraction of the gel phase showed $2\theta = 20.7°$ corresponding to a '*d*' value of 4.2 Å (*cf.* Fig. 2), inferring the formation of a hexatic rotator phase with a loss of order from the regular hexagonal phase.[31,32] The loss of order was ascribed to local distortion fluctuations in the hexagonal phase. The phase transition behavior in the condensed phase region of Langmuir monolayers are similar to the untilted

phases of alkanes.[33] Accordingly, the electron diffraction pattern of BDSA monolayers at the air–water interface and molecular dynamics simulation studies revealed the formation of a hexagonal lattice,[34] as depicted in the inset of Fig. 2. We infer from the above experimental observations that the BDSA–amino acid membranes adopt a hexatic rotator phase with the symmetrical (parallel-packed) or unsymmetrical (antiparallel-packed) membranes.[35] At a molar ratio of 1 : 2, the membrane formed was symmetrical, which resulted in spherical structures.

Formation of conical self-assembled structures

Interestingly at 1 : 1 molar ratios, the 6AHA–BDSA mixtures revealed conical structures in addition to spheres, as shown in Fig. 4. Attempts have been made in the literature to explain the formation mechanism of self-assembled cones.[36] Tsonchev *et al.* developed a geometric packing analysis for the packing of amphiphiles, where the unit structures were assumed to be hard cones.[37] However, the theory was only valid in the limit of very large number of unit structures in a single cluster. A cone could be considered to be formed from a hexagonal lattice by introducing Gaussian curvature through disclinations.[38] Disclinations are topological defects created by cutting a wedge of angle 60° from a hexagonal plane followed by rejoining the cut edges. Douliez explained the formation of conical assemblies from fatty acids through hexagonal disclinations.[39] A conical structure would result on creating such disclinations with different angles in accordance with $\theta = \arcsin [1 - (n/6)]$, where $0 < \theta < \pi/2$ and the number of disclinations '*n*' restricted as $1 < n < 5$. Therefore, in the present case, the formation of conical structures from BDSA–6AHA could be conceived to be formed through the hexagonal disclinations. The calculated angles for the experimentally observed conical self-assemblies illustrated in Fig. 4a were 19.5°, 30° and 56.4° corresponding to $n = 4$, 3 and 1,
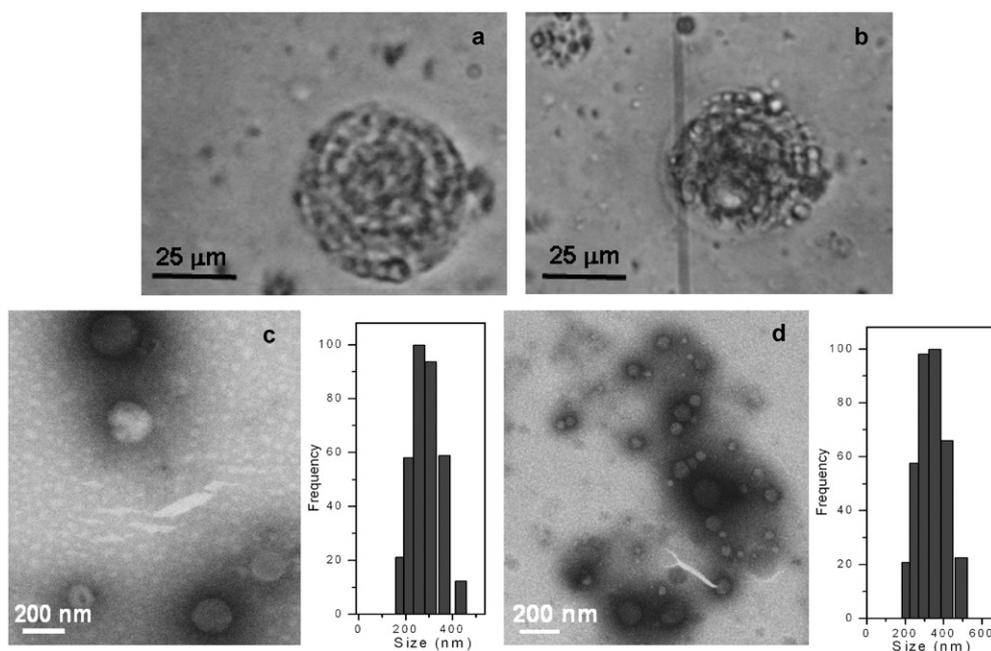

Fig. 3 Optical micrographs of 10 mM 1 : 2 mixtures (a) BDSA–6AHA and (b) BDSA–4ABA showing spherical vesicles and spiral vesicular clusters. (c) TEM micrograph of a 1 : 2 mixture of 0.25 mM BDSA–6AHA and (d) 0.25 mM BDSA–4ABA with the respective size distribution.
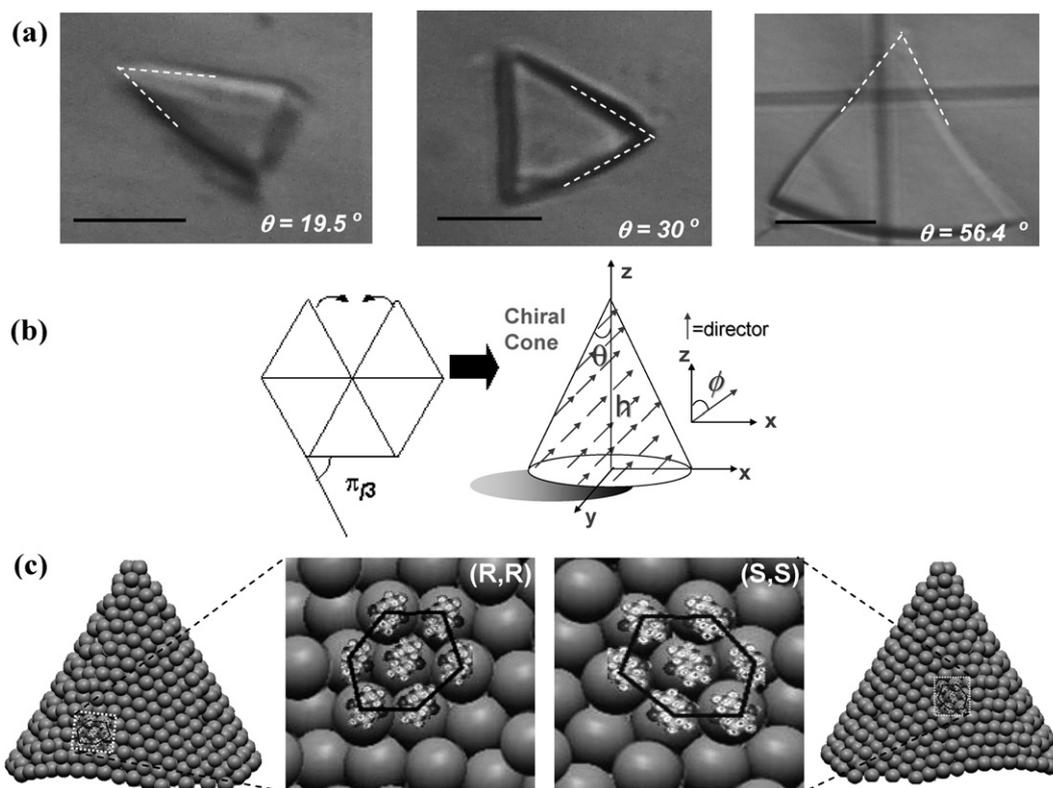
Fig. 4 Optical micrographs under differential interference contrast mode showing conical self-assembly from 0.1 mM, 1 : 1, BDSA–6AHA mixture in aqueous solution (scale bar represents 5 μm). (b) Mechanism of π/3 wedge disclination from a hexagonal lattice to result in a chiral conical structure. (c) Chiral cones resulting from hexagonal disclination from enantiomerically pure BDSA (the amino acids are omitted for clarity).

respectively. We were able to observe conical structures with the above specified angles, however, all possible angles in accordance with $1 < n < 5$ are possible.

Conical self-assembly is an entropically controlled means of forming aggregates without edges through Gaussian curvatures in their supramolecular morphology. The fatty acid BDSA in the present case possesses two chiral centers, attached to the head groups. Therefore, the membranes formed from enantiomerically pure molecules are indeed chiral in nature. The conical structures therefore would possess intrinsic chirality in their membrane structure, schematically shown in Fig. 4b. When a membrane is formed of chiral amphiphiles, the chiral interaction controls the packing with non-zero angle with respect to their nearest neighbours.[40] Such a restriction imposed on the packing will induce an orientation that is energetically stable in its close-packed state, and has been phenomenologically explained by various theories.[41,42] Besides, the membrane from the twin-chiral, twin-tailed BDSA forms are stabilized through hydrogen bonding with the polar heads of the amino acids and the carboxyl groups at one end.

When the BDSA–6ACA mixtures were heated and cooled, disclinations/defect sites were created, resulting in conical structures. The associated chiral elasticity in the membrane favored the bending of the membrane at the disclination point resulting in the formation of conical structures. The BDSA–AHA membranes are fluid enough in solution to undergo disclination-induced folding during repeated freeze–thaw cycles, resulting in self-assembled cones. Furthermore, probable

competition between the intrinsic chiral bending with the vicinal chiral centers and the rigid C–C spacer could exist, making the membrane more fluid against the strictest close packing, as seen in crystalline alkanes. Therefore, cones of enantiomeric membranes are related through mirror plane symmetry with opposite handedness of the twist angle ϕ, as shown in Fig. 4b. Such a phenomenon is akin to the disclination induced conical folding of a graphite sheet.[43]

Racemic mixtures of fatty acids–amino alcohols have been reported to form only tubular self-assembly arising from chiral discrimination.[44,45] A chiral-symmetry breaking is expected from a racemic mixture, where the reflection symmetry is lost even in the fluidic phase. However, in the present study, the racemic BDSA were not able to form conical or tubular assemblies, but only resulted in spherical structures. The absence of specific self-assembled structures other than spheres implies that the BDSA–amino acid pairs exhibit a heterochiral interaction. This is in line with our recent report that a mixture of racemic BDSA–tetraalkyl ammonium surfactant mixture did not form any self-assembled product; however the enantiomeric forms resulted in Gaussian twisted fibres.[46]

Aggregate geometries are governed by molecular shape, concentration and the temperature of self-assembly. In the present case, we found that the primary assembly, i.e. the single BDSA–amino acid pair is controlling the final assembly. The formation of an unsymmetrical membrane could be perceived as a secondary structure, stabilized by non-covalent forces like electrostatic, van der Waals interactions and hydrogen

bonding.[47] The final geometries such as cones and vesicles are the tertiary structures. There exists therefore, a balance of 1° and 2° structures in deciding the final morphology of the self-assembled structures. In conformity with this, the association of BDSA, with 4-aminobutyric acid (with 2C less in the skeleton), did not comply with the conical structures but formed only spherical structures. Therefore, a combination of the appropriate amino acid or any other solubilizer in that case, with a fatty acid would result in conical structures.

Conclusions

The self-assembling characteristics of a twin-chiral, twin-tailed fatty acid in association with heteroditopic amines, such as 4ABA and 6ACA have been reported. The amino acids were able to solubilize the water-insoluble BDSA to initiate spontaneous self-assembly in aqueous solution. The formed self-assembled structures were stoichiometrically dependent, forming spherical vesicles at 1 : 2 molar ratios and cones at 1 : 1 molar ratios. The conical structures were formed as a result of disclinations in the hexagonal, chiral BDSA–amino acid membranes. The chiral cones mimic the real biomembranes that form conical assemblies in viruses[48] and retinal cells. The presence of inherent chirality in the membrane originating from the chiral surfactant attributes stability to such unusual conical structures. The present work has synergy in various biological systems. Living cells in nature adapt conical assembly to perform specific functions. The human eye has three kinds of cone cells possessing different photopsins, with different response curves to electromagnetic wavelengths.[49] Biological patterning of visual cells are the basis of cone-shaped cells to perform photo-responsive functions.[50] It was recently established that the huge Khufu's pyramid of Egypt were built inside-out with a spiral framework tracing from the bottom to the apex[51] and cones are pyramids with a circular base. The above results may enable us to understand and unravel the biomechanisms responsible for the formation of such a special class of self-assembled structures.

Acknowledgements

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