Vesicular Adhesion

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Adhesive Vesicles through Adaptive Response of a Biobased Surfactant**

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Adaptive response in functional systems of nature is best exemplified by the homeostasis (homeoviscous alterations) or the tropism observed in flora and fauna. The term "homeoviscous alteration" describes the process whereby the fluidity of the membrane is adjusted in response to a perturbation such as temperature, pressure, etc.[1,2] Most of the natural lipids utilize their characteristic unsaturations as a tool to execute such elegant processes. The biophysical properties of the membranes thus depend on the subtle adjustments in the structure and composition of the alkyl chains that are attached to glycerol backbones.[3] Chilling sensitivity in plants is the direct repercussion of the membrane dynamics in plants that involves conformational changes and variations in unsaturation component of the lipid membranes.[4] Deciphering the stimuli-responsive character in such biological systems not only sheds light on the underlying mechanism in the race for survival of the fittest, but also provides clues to generate unique functional materials in the laboratory. Amphiphilic molecules rich in unsaturations in their side chain are expected to display such interesting phenomena and provide vistas for new soft materials.[5] Taking cue from this, we designed an amphiphile from a naturally available raw material—cardanol—that possesses structural features akin to natural lipidic systems.

Cardanol is a biobased non-isoprene lipid obtained from cashew nut shell liquid (CNSL). It consists of a rich mixture of phenolic lipids: 5% of 3-(pentadecyl)phenol, 49% of 3-(8*Z*-pentadecenyl)phenol, 17% of 3-(8*Z*,11*Z*-pentadecadienyl)-phenol and 29% of 3-(8*Z*,11*Z*,14-pentadecatrienyl)phenol.[6] The unique feature of cardanol is that it contains 1) lipid chains with varying degree of allylic *cis* double bonds, 2) alkyl chains with odd numbers of carbon atoms, 3) a reactive phenolic group in the *meta* position for further functionalization, and 4) saturated/unsaturated versions of hydrocarbon chains. Hence one can envision an amphiphilic building block from cardanol, which show a stimuli-responsive behavior that could be utilized to reconfigure them into functional architectures. Herein, we report vesicle formation and vesicular adhesion from a biobased surfactant, *N*-cardanyl tauramide (NCT), obtained by the judicious combination of cardanol as the hydrophobic part and taurine (a vital aminosulfonic acid) as the hydrophilic head group. The molecular structure of NCT is shown in Figure 1c.

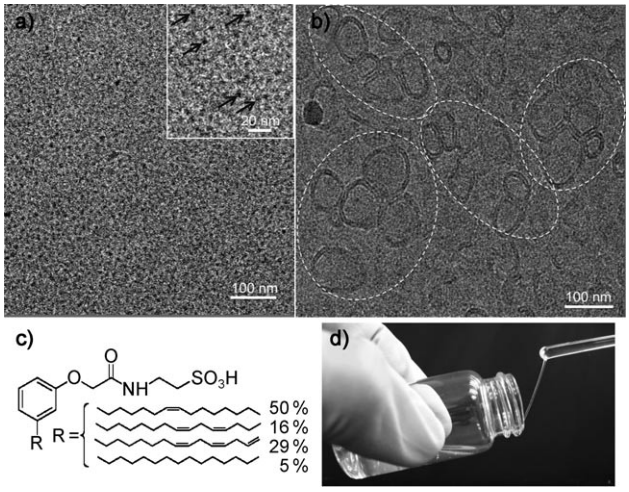

Figure 1. Cryo-TEM micrograph of a fresh 5 mM NCT solution at 25°C showing micelles as dark spots. The inset shows a high-magnification view of the micellar structures indicated by arrows. b) Viscous phase with vesicular islands marked by white circles. c) Molecular structure of NCT. d) Image of the vesicular phase showing sticky behavior.

NCT behaved as a surfactant in aqueous solution with a critical micelle concentration (CMC) of 1.2 mM at 25°C. Typically, when a 5 mM micellar solution was warmed to 55°C and annealed, the solution transformed into a viscous phase at 45°C with a restricted flow. Upon reaching room temperature, the solution resumed back to the micellar phase. Interestingly, the viscous phase appeared elastic and could be pulled into a "wet string" (Figure 1d). In order to understand this behavior, we performed a cryo-TEM analysis of the sample at 45°C in the cooling regime. The frozen-hydrated samples were observed at liquid-nitrogen temperature, suspended in a thin vitreous ice layer across the holes of the carbon support film. Cryo-TEM images of samples showed several vesicular structures that were unilamellar, 80–150 nm in size fused together (Figure 1b). For the microstructures to be observed by cryo-TEM technique, we need to rely on the phase contrast and therefore introduced an instrument-

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dependent defocus while acquiring the images.[7,8] The key observation is that, upon annealing to room temperature, the vesicles revert back to the micellar phase. When the solution was reheated, the sticky phase reappeared in the cooling regime, suggesting a monotropic-like phase transition. The viscosity of a freshly prepared micellar solution (5 mM) at room temperature was 4.621 cP whereas at 55 °C it decreased to 1.468 cP. The viscosity of the same solution at 45 °C (in the cooling regime) almost doubled to 8.506 cP compared with the fresh solution (see the Supporting Information). This observation suggests a transformation from a micellar phase to a viscous phase.

In order to understand the different phases formed, a small lump of NCT kept on a glass slide between a 150-micrometer spacer was allowed to contact with water, and the slide was mounted on a hot stage and heated to 60 °C. After a clear isotropic solution was obtained, it was cooled slowly at a rate of 1 °C per minute and observed through a phase-contrast objective of the microscope. At 50 °C, several glassy blobs were formed that were birefringent under crossed polarizers (see the Supporting Information). Interestingly, at 45 °C the blobs slowly gave rise to sticky strings progressively to form a network.

Formation of a viscous phase in surfactant solution is expected to occur when the micelles grow in one dimension, resulting in worm- or thread-like micelles[9] or vesicular structures. A viscoelastic solution shows a Weissenberg or the rod-climbing effect whereas a vesicle-rich phase would not exhibit such phenomena.[10] Analysis of different samples by cryo-TEM showed only vesicular structures and no worm-like micelles being present in the solution. If so, how then the vesicular solution formed a sticky viscous phase?

Naturally occurring polyunsaturated lipidic systems with characteristic *cis* double bonds have been shown to exist in a highly curved semicircular topology.[11] Biological phospholipids with polyunsaturated and saturated side chains exhibit energetically stable kinked and extended conformations that are characterized by a crankshaft-like topology and pack with favorable lateral van der Waals contact interactions.[12] NCT being a derivative of cardanol with varying degree of unsaturations in the form of *cis* double bonds, is expected to behave in a similar fashion. The characteristic kinks of *cis* double bonds form an angle of 30° in the molecular structure that is reflected in their effective chain length.[13,14] When the NCT micellar solution is warmed, thermal fluctuations develop in the alkyl chains triggering a homeostasis-like responsive behavior. The increased chain mobility accrues favorable conformations for a micelle-to-bilayer transformation. During reassembly, the shorter kinked and longer extended alkyl chains interlock in the bilayer arrangement and eventually undergo curvature,[15] culminating in individual vesicular structures. The stability of the assembly is complemented by the directional hydrogen bonding of the amide linkages, together with the π–π stacking of the aromatic rings (Figure 2). Such an interlocking mechanism has been reported to exist in unsymmetrical gemini surfactants with short and long alkyl chains.[16] An energetically compensated interlocking of the short and long alkyl chains provides stability of the bilayer assembly. Such lipid tail–tail coupling and inter-lipid shuffling has been found to occur in homeoviscous adaptation of biological lipid membranes.[17] In addition, similar responsive micelle–vesicle–micelle transitions have been reported in marinobactin surfactants with varied alkyl chain length.[18,19]

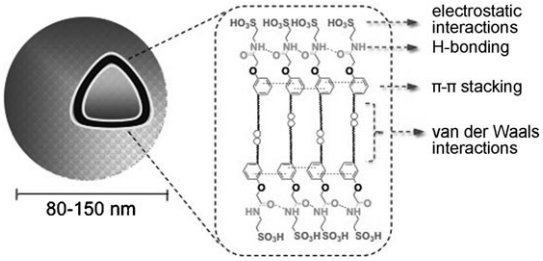

Figure 2. Non-covalent forces present in the vesicle bilayer.

By the virtue of the sulfonic acid moiety of the taurine head group, the NCT vesicles eventually coalesce, because of hydrogen-bonding-promoted intervesicular attraction, forming a viscous vesicular phase. DLS measurements of the micellar NCT solution at room temperature showed particle sizes around 6–8 nm whereas at 45 °C the size increased to approximately 110 nm, suggesting the formation of larger particles (eventually vesicles in the present case; see the Supporting Information).

The ^{1}H NMR spectra of NCT in 10 % D_2O–H_2O mixture showed a broad singlet at $\delta = 7.9$ ppm corresponding to highly strongly hydrogen-bonded amide protons characteristic of taurine amides.[20] Further evidence of the hydrogen bonding was obtained from FT-IR experiments. The FT-IR spectra of NCT were acquired in D_2O and in its non-assembled state in chloroform solution. The amide I band in chloroform was observed at 1668 cm^{-1} whereas in the associated state it shifted to 1643 cm^{-1}, suggesting an extensive hydrogen bonding of the N–H group.[21] In order to understand the temperature-induced micelle-to-vesicle formation, we recorded ^{1}H NMR spectra at different temperatures between 25 and 55 °C. With increase in temperature, the generally expected trend is that the resonance signals become sharp, when no aggregation occurs. However, for all the spectra recorded, we observed an appreciable increase in line width. The full width at half maximum of the signal at $\delta = 7.9$ ppm at 25 °C was found to be 29.67 Hz whereas at 45 °C the value increased to 33.70 Hz. While increase in temperature decreases the extent of the N–H···O=C= hydrogen bonding, in the present case it also favors the micelle-to-vesicle transformation. Hence the observed line broadening is not very high, nevertheless, it is significant and accounts for the formation of vesicular structures (see the Supporting Information).

We also followed the contribution of π–π stacking towards self-assembly by using temperature- and concentration-dependent NMR experiments. At very dilute concentration (0.05 mM, much below the CMC) and at high temperature (65 °C), the resonances for non-associated aromatic protons were found at $\delta = 7.3$, 6.96, and 6.87 ppm. Whereas, for a 5 mM solution at 45 °C, the signals of the aromatic protons shifted upfield, to $\delta = 6.99$, 6.67, and 6.54 ppm, respectively, suggest-

ing the presence of π–π stacking in the assembly (see the Supporting Information).

Since the vesicular aggregates of NCT were formed as a result of competing, as well as complementing weak non-covalent forces, any mechanical stress introduced to the system is expected to result in the destabilization of the jelly phase. However, when the jelly phase was touched with a glass rod, it could be pulled into a wet string. We speculate that the adhesion is strong enough that the one-dimensional force exerted by mechanical pulling was insufficient to disturb the intervesicular attraction. With increase in the pulling force, the wet strings reach their elastic limit and finally break. This analogy explains the unusual elastic behavior of the sticky jelly phase. The hierarchical microstructures observed are schematically represented in Figure 3. To the best of our knowledge, such an observation of mechanically alterable vesicular adhesion remains unexplored in the literature.

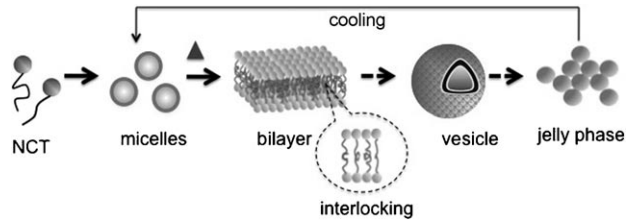

Figure 3. Temperature-induced phase transformations of NCT.

From the above discussion, one may expect that the absence of kinks would disfavor the formation of interlocking and any vesicular structures being formed. To prove this, we synthesized an all-*trans* saturated side chain derivative, which did not form such a viscous phase as expected. This might be due to the energetically costly *gauche* conformations of the all-*trans* alkyl chain on par with the kinked *cis* double bonds of cardanol. We are currently exploring the effect of saturation content on thermal responsiveness of NCT by doping with different percentages of a saturated component.

In summary, the results show an adaptive responsive behavior of unsaturated alkyl chains present in the cardanol-based amphiphile, much akin to the homeostasis of naturally occurring lipid membranes. A reversible micelle-to-vesicle transition, followed by vesicular adhesion leading to a sticky jelly phase was observed and corroborated from cryo-electron microscopic measurements and other physico-chemical techniques.

Experimental Section

Cardanol was obtained by double vacuum distillation of cashew nut shell liquid at (3 ± 4) mm Hg in which the fraction boiling at (220 ± 15) °C was collected. Bromomethylacetate was purchased from TCI America. Anhydrous Potassium carbonate and dimethylsulfoxide were procured from Acros organics, USA. All the chemicals were of analytical grade and were used as received. The molecular structure of NCT was determined by ^{1}H and ^{13}C NMR (Vega-300 Varian Inc, USA) and FT-IR (Nicolet 380 FT-IR) spectroscopic techniques. NMR experiments were done on a Vega-600 Varian spectrometer using the WATERGATE program (Varian Inc. USA). Ultrapure water (Millipore, resistivity 18.2 MΩ cm) was used to prepare aqueous solutions. Dynamic light scattering (DLS) measurements were made on a Malvern Nano*ZS* instrument (Malvern Instruments, Inc, USA). The micellar solutions were filtered through a 0.42 μm nylon-mesh filter before the measurements to avoid any scattering from stray particulates present in the solution. Viscosity measurements were made on a Ostwald viscometer of tube constant 0.0125 $cSt\,s^{-1}$ with 20 mL sample volume. The viscometer was kept immersed in a water bath and equilibrated at the desired temperature.

Cryo-electron microscopy: An aqueous solution of NCT was prepared from a 10 mM stock solution by appropriate dilution followed by vortex mixing. Thin layers of vitrified suspensions were prepared by applying a 5 μL drop of NCT solution on a 200-mesh copper grid coated with a 20 nm thick holey carbon film (purchased from Protochips, NC) without previous glow discharge. The grid was mounted on a Gatan Cp3 cryo-plunger device, blotted with filter paper for 3.5 seconds on both sides of the grid, and immediately vitrified in liquid ethane, cooled at liquid-nitrogen temperature. The chamber was maintained at the desired temperature for the microstructures to be observed. The vitrified samples were mounted in a Gatan 626 Cryo-specimen holder (Gatan, Warrendale, PA) and observed at −180 °C in a JEOL 2100 FEG cryo-electron microscope, operated at 200 kV. Images were recorded in low-dose mode (less than 1500 electrons nm^{-2}) at a typical magnification of 25 000 × using a Gatan 2k-by-2k CCD camera.

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