Communication

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J. Am. Chem. Soc., **2004**, 126 (35), 10804-10805• DOI: 10.1021/ja0400281 • Publication Date (Web): 17 August 2004

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COMMUNICATIONS

Published on Web 08/17/2004

Coacervate Formation from Natural Glycolipid: One Acetyl Group on the Headgroup Triggers Coacervate-to-Vesicle Transition

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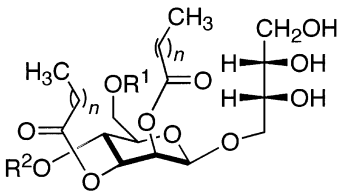
Coacervation was the most important event in the appearance of life on the early Earth.[1] It is becoming widely accepted that coacervates are the origin of all life. In his classic book,[1] Oparin mentioned that all present-day cells are generated from the organization and ordering of coacervates. All cells are, morever, surrounded by a highly ordered bilayer membrane that is the most fundamental structure for life.[2] However, the process by which the ordered bilayer membrane is generated from coacervates is still a scientific mystery. To solve this mystery, we should focus our attention on the structure−property interaction of coacervates. Generally, coacervates, including "simple coacervates"[3a] and "complex coacervates",[3b] are prepared from complicated multicomponent systems such as surfactants with salt/cosolvent or two oppositely charged polyelectrolytes.[3c] This makes their structural characterization difficult.

Recently, coacervate formation from a single organic compound, a "synthetic" gemini surfactant, was confirmed for the first time using a cryo-high-rosolution scanning electron microscope (cryo-HRSEM) by Menger and co-workers.[4a] They also proposed that the surfactant phase of sponge morphology,[4a−c] which has been often called "L_3 phase",[5a] "plumber's nightmare",[5b] or "blue I phase",[5c] should be redefined as coacervates.[4b] However, there is no report on the formation of a coacervate from a single "natural" compound which exists in nature; therefore, little is known about the mechanism of coacervates evolving into ordered bilayer membranes.

In this communication, we describe for the first time simple coacervate formation from a single "natural" glycolipid biosurfactant, manosyl-erythritol lipid-A (MEL-A), together with vesicle formation from its derivative, MEL-B. We also demonstrate that only a slight decrease in spontaneous curvature resulting from the absence of the 4′-*O*-acetyl group induces a drastic morphological change in the self-assembled structure from coacervates to vesicles, ordered bilayer membranes.

Figure 1 shows the structure of MEL-A, 4-*O*-(4′,6′-di-*O*-acetyl-2′,3′-di-*O*-alkanoyl-*β*-D-mannopyranosyl)-D-erythritol, and MEL-B, 4-*O*-(6′-*O*-acetyl-2′,3′-di-*O*-alkanoyl-*β*-D-mannopyranosyl)-D-erythritol.[6a] Both MELs have nearly the same fatty acid composition; C8 (18%), C10 (71%), and C12 (11%). MELs are one of the most promising biosurfactants known. They are abundantly produced by yeast strains of *Pseudozyma antarctica* from vegetable oils,[6b] or can be prepared by only organic synthesis.[7] We have reported that MELs have great potential for antitumor compounds,[6b] protein affinity ligands,[6c] and gene carriers.[6d]

The mixture of MELs was prepared as reported previously.[6c] Each MEL was dissolved in acetone, and the stock solution was moved into a test tube. After the solvent was removed by a rotary evaporator, distilled water was added in the test tube. The colloidal dispersion of MEL-A or MEL-B was obtained by vortexing the test tube for 1 min at room temperature (25 °C) and then was



MEL-A: R^1=R^2=Ac
MEL-B: R^1=Ac, R^2=H

(n = 6-10)

Figure 1. Structure of mannosyl-erythritol lipids (MELs).

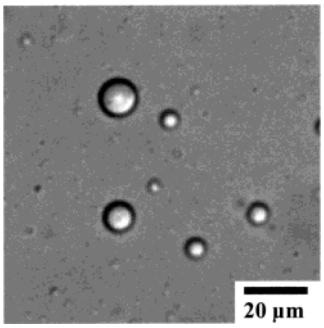


Figure 2. DIC image of a colloidal dispersion formed from MEL-A.

observed with the use of a microscope (E600, Nikon, Japan) with a differential interference contrast (DIC) attachment.

Figure 2 shows the DIC image of a colloidal dispersion formed from MEL-A (2 mM). When MEL-A was dispersed in water, immiscible oily droplets with a low viscosity were immediately separated from the equilibrium water. The figure shows spherical droplets with a diameter of 1−20 μm, which well resemble coacervate droplets formed from zwitterionic Gemini surfactants.[4a,b]

On the other hand, MEL-B, which has a hydroxyl group at the C-4′ position instead of an acetyl group, spontaneously formed giant vesicles with diameters similar to those of the droplets. Then, these two colloidal dispersions were confirmed using a confocal laser scanning microscope (CLSM, LSM5 PASCAL, Zeiss, Germany) to evaluate the effect of the acetyl group on their self-assembled structures.

Panels A and B of Figure 3 show CLSM images of colloidal dispersions (2 mM) formed from MEL-A and MEL-B, respectively. The lower two graphs show the fluorescence intensity distribution following the lines indicated in the upper images. From these observations, the droplet obtained with MEL-A was found to have an inner structure different from that of the giant vesicle with MEL-B. This clearly indicates that only one functional group on the sugar headgroup decided the direction of the molecular self-assembly of MEL.

10.1021/ja0400281 CCC: $27.50

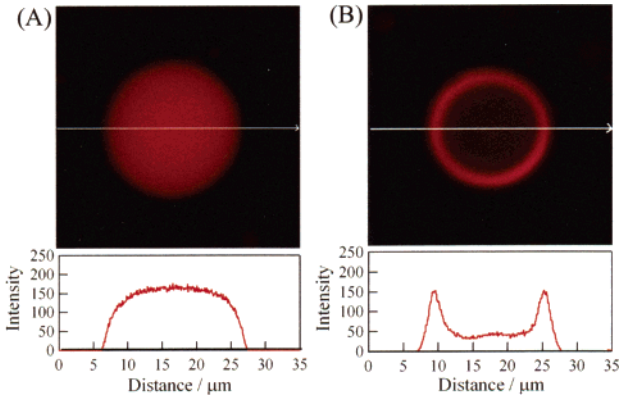


Figure 3. Fluorescence intensity distributions of colloidal dispersions formed from MEL-A and MEL-B. (A) MEL-A. (B) MEL-B.

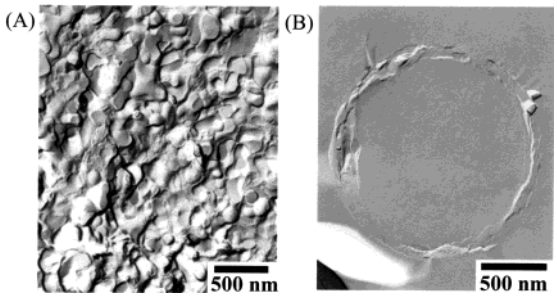


Figure 4. Freeze-fracture transmission electron micrographs of colloidal dispersions formed from MEL-A and MEL-B. (A) MEL-A. (B) MEL-B.

The structures of the colloidal dispersions were further determined using a freeze-fracture transmission electron microscope (FF-TEM, JEM-1010, JEOL, Japan).

Panels A and B of Figure 4 show typical freeze-fracture electron micrographs of droplet and vesicle (1 mM) formed from MEL-A and MEL-B, respectively. Figure 4 A clearly exhibits the typical coacervate morphology of a sponge phase composed of randomly connected 3D network of the bilayers,[3a,5b,8] whereas Figure 4 B gives the giant vesicle morphology which corresponds well to Figure 3 B. In Figure 4 A, the spherical and ellipsoidal objects with a planelike appearance are the water domains because the fracture through the water phase occurred perpendicular to the curved bilayers. In many parts of the micrograph, we can follow the bilayer over a range of several micrometers, indicating the bilayer is indeed continuous. Where the fracture occurred along two bilayers, a bilayer having both a negative and a positive spontaneous curvature can be seen. It is truly remarkable that coacervates of sponge morphology were easily obtained from the single yeast glycolipid, MEL-A, even though it possessed different alkyl chain lengths (C8 to C12). To our knowledge, this is the first observation of coacervates forming from a single "natural" compound.

On the basis of the detailed theoretical and structural study of surfactant systems,[4a−d] Menger et al. recently demonstrated that the surfactant coacervate phase is almost equal to the L_3 phase with regard to its solution properties. A L_3 phase is a well-defined surfactant phase, which appears when the spontaneous curvature is slightly negative.[9] The spontaneous curvature (H_0) is a useful parameter to characterize the lipid assembled structures. It is given by $H_0 = 1/R_0$, where H_0 is the curvature of one monolayer which forms a bilayer, and R_0 is the spontaneous radius of curvature.[10] When the spontaneous curvature is nearly zero, the lipids self-assemble into the lamella phase (L_α). The spontaneous curvature of MEL-B assemblies is thus nearly zero since vesicles are obtained by the dispersion of the L_α phase. The L_α phase of MEL-B seems to be stabilized by the hydrogen-bonding network between the headgroups resulting from the C-4′ hydroxyl group because L_α phase is less dynamic than L_3 phase.[11]

On the other hand, the presence of an acetyl group on the mannose moiety is likely to induce a slightly negative spontaneous curvature of the glycolipid assemblies: this should lead to the formation of the L_3 phase. The spontaneous curvature is also critical, when membrane fusion takes place between vesicles. Generally, lipids with a negative curvature promote the fusion, while those with a positive curvature give a reverse effect.[12] In our previous study, phospholipid vesicles containing MEL-A promoted membrane fusion that drastically increased gene transfection efficiency, whereas those containing MEL-B showed no effect.[6d] This finding provides supporting evidence of the preferential coacervate formation from MEL-A.

The L_3 phase obtained from MEL-A would be a potential host for water-soluble proteins and DNA using the interior water channels of the phase, because the L_3 phase resembles the bicontinuous cubic phase. Although the cubic phase acts as a better solvent for membrane protein bacteriorhodopsin crystallization,[13] it is a stiff liquid crystal, making it difficult to handle in high-throughput screening systems.[14] In contrast, the L_3 phase is fluid, giving it the potential to overcome this problem.

In summary, this is the first observation of the coacervate formation from a single component of a "natural" glycolipid biosurfactant. We also found that only a slight decrease in the spontaneous curvature resulting from the absence of the 4′-*O*-acetyl group induced a drastic morphological change in the 3D self-assembled structure from coacervates (L_3 phase) to ordered vesicles (L_α phase). Our results are particularly interesting from the perspective of "chemical evolution" because they might give a better explanation of the transition process from the coacervate to the vesicle system.

Supporting Information Available: Experimental details for preparation of MELs and microscopic studies. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Oparin, A. I. *The Origin of Life*; Dover Publications: New York, 1953
(2) Hanczyc, M. M.; Fujikawa, S. M.; Szostak, J. W. *Science* **2003**, *302*, 618−622.
(3) (a) Zapf, A.; Hornfeck, U.; Platz, G.; Hoffmann, H. *Langmuir* **2001**, *17*, 6113−6118. (b) Kaibara, K.; Okazaki, T.; Bohidar, H. B.; Dubin, P. L. *Biomacromolecules* **2000**, *1*, 100−107. (c) Schmitt, C.; Sanchez, C.; Desobry-Banon, S.; Hardy, J. *Crit. Rev. Food Sci. Nutr.* **1998**, *38*, 689−753.
(4) (a) Peresypkin, A. V.; Menger, F. M. *Org. Lett.* **1999**, *1*, 1347−1350. (b) Menger, F. M.; Peresypkin, A. V.; Caran, K. L.; Apkarian, R. P. *Langmuir* **2000**, *16*, 9113−9116. (c) Menger, F. M.; Peresypkin, A. V. *J. Am. Chem. Soc.* **2001**, *123*, 5614−5615. (d) Menger, F. M.; Sykes, B. M. *Langmuir* **1998**, *14*, 4131−4137.
(5) (a) Balinov, B.; Olsson, U.; Söderman, O. *J. Phys. Chem.* **1991**, *95*, 5931−5936. (b) Strey, R.; Jahn, W.; Porte, G.; Bassereau, P. *Langmuir* **1990**, 6, 1635−1639. (c) Laughlin, R. G. In *Micelles, Microemulsions, and Monolayers*; Shah, D. O., Ed.; Marcel Dekker: New York, 1998; pp 73−99.
(6) (a) Kitamoto, D.; Ghosh, S.; Nakatani, Y.; Ourisson, G. *Chem Commun.* **2000**, 861−862. (b) Kitamoto, D.; Isoda, H. Nakahara, T. *J. Biosci. Bioeng.* **2002**, *94*, 187−201. (c) Im, J.-H.; Yanagishita, H.; Ikegami, T.; Kitamoto, D. *J. Biomed. Mater. Res.* **2003**, *65*, 379−385. (d) Inoh, Y.; Kitamoto, D.; Hirashima, N.; Nakanishi, M. *J. Controlled Release* **2004**, *94*, 423−431.
(7) Crich, D.; de la Mora, M. A.; Cruz, R. *Tetrahedron* **2002**, *58*, 35−44.
(8) Hoffmann, H.; Thunig, C.; Munkert, U. *Langmuir* **1992**, *8*, 2629−2638.
(9) Anderson, D.; Wennerström, H.; Olsson, U. *J. Phys. Chem.* **1989**, *93*, 4243.
(10) Aota-Nakano, Y.; Li, S. J.; Yamazaki, M. *Biochim. Biophys. Acta* **1999**, *1461*, 96−102.
(11) Nilsson, P.-G.; Lindman, B. *J. Phys. Chem.* **1984**, *88*, 4764−4769.
(12) Marrink, S. J.; Mark, A. E.*J. Am. Chem. Soc.* **2003**, *125*, 11144−11145.
(13) Landau, E. M.; Rosenbuch, J. P.; *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14532.
(14) Ridell, A.; Ekelund, K.; Evertsson, H.; Engström, S. *Colloids Surf. A* **2003**, *228*, 17−24.

JA0400281