Bilayer Vesicle Formation in Ethanol from Calix[4]arene Derivative with Two Guanidinium Groups

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(Received July 20, 2005; CL-050941)

Stable and regular bilayer vesicles can be formed in ethanol by 5,11,17,23-tetra-*tert*-butyl-25,27-bis(2-guanidinoethoxy)-26,28-dihydroxycalix[4]arene hydrochloride (BGC). Hydrophobic interactions between aromatic cavity and *t*-butyl are main self-organization driving force. This is the first example of the formation of bilayer vesicles in ethanol from calixarene without long alkyl chains.

Because of the potential role of vesicles in biology, chemsitry, and material science, many synthetic vesicles have been investigated.[1,2] Intermolecular non-covalent interactions, such as hydrophobic interaction, hydrogen bond, electrostatic attraction, and metal coordination, are responsible for the formation of vesicles.[2] For those typical amphiphiles with long alkyl chains, the hydrophobic interaction among the alkyl chains is the main driving force to form such high-order aggregates. Therefore, the corresponding vesicles were mostly found in aqueous solution.[1] In fact, studies on aggregate morphology in non-aqueous media can extend the function of vesicles as microreactor, drug delivery carrier, and synthetic template for materials.[3] Vesicle formation in aprotic organic solvents, such as reversed bilayer vesicles from peptide–lipids and fluorocarbon bilayer vesicles from fluorocarbon amphiphiles, has been reported.[4] However, research on vesicles in protic organic media is few.[5] Calixarenes have been widely studied as self-assembling materials in the past decade, the bilayer vesicles in water–tetrahydrofuran from calix[4]-resorearene with four long chains has been reported.[6] Herein, we show that the non-typical amphiphile 5,11,17,23-tetra-*tert*-butyl-25,27-bis(2-guanidinoethoxy)-26,28-dihydroxycalix[4]arene hydrochloride (BGC) without any long alkyl chains can self-organize to form bilayer vesicles not only in water–ethanol media but also in pure ethanol. To our knowledge, it is the first example of bilayer vesicle formed in ethanol from calixarene without long alkyl chains.

BGC was synthesized in our laboratory.[7] The BGC dispersions ($1.0\,\mathrm{mmol{\cdot}L^{-1}}$) were prepared at ca. 25 °C by sonicating 5 mL solvent containing 0.005 mmol powdery BGC for 10 min. The solvents are water, water–ethanol mixtures of different ratios, and pure ethanol, respectively. Transmission electron microscopy (TEM) (JEOL Model JEM-200CX) was adopted to study the dispersion. The TEM samples were prepared by placing one drop of the dispersion on the Cu grid coated with a conductive polymer film followed by drying in air. Due to the poor solubility of calixarene in water, its suspension in water is quite opaque and unstable. No regular aggregate of BGC molecules but small particles can be found by TEM. While in ethanol–water (1:4 v/v), a white suspension was obtained, and precipitates were found in 3 h. From corresponding TEM images, one can find that many tiny particles are scattering among the larger vesicular entities (diameter around 100 nm). When increasing the ratio of ethanol in the mixed media, the tiny particles decrease evidently with the dispersion getting more and more translucency, and a clear dispersion can be obtained in pure ethanol finally. The dispersion in ethanol stands for more than a month and there is no change for its appearance and properties. The TEM images recorded for the sample from pure ethanol solution reveal the regular vesicular morphologies with diameters of 200–500 nm (Figure 1). The result infers that BGC molecules are more dispersable in ethanol and ethanol seems to facilitate BGC molecules to self-organize into stable regular aggregates.

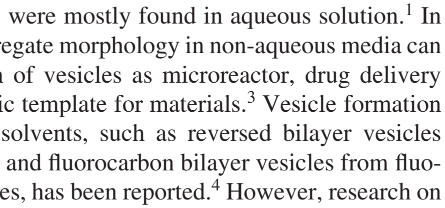

Chart 1. The chemical structure of BGC.

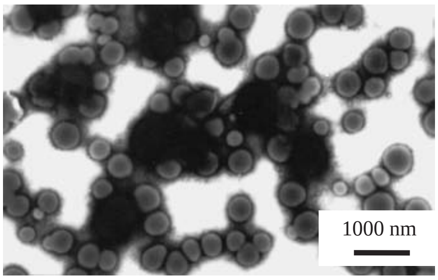

Figure 1. Transmission electron micrographs of BGC dispersion in pure ethanol. Samples were post-stained with 2% uranyl acetate aqueous solution.

The BGC bilayer vesicles in ethanol had been further studied by atomic force microscopy (AFM), small-angle X-ray diffraction (SAXRD, Rigaku Model D/Max-RA), and differential scanning calorimetry (DSC). From typical AFM images of the cast film formed by spreading BGC dispersion in ethanol on mica, one can find that all the particles are of round edges and slightly convex top surface. The diameters of particles varied from 200 to 500 nm, consisting with the TEM images. The average height of the particles was approximately 6 nm. According to the rough estimation by MM+ calculation, the length of single BGC molecule in the optimized conformation is about 1.36–1.38 nm. The thickness of the bright particles on mica is approximately four times of BGC monolayer, which infers the formation of BGC bilayer vesicles.

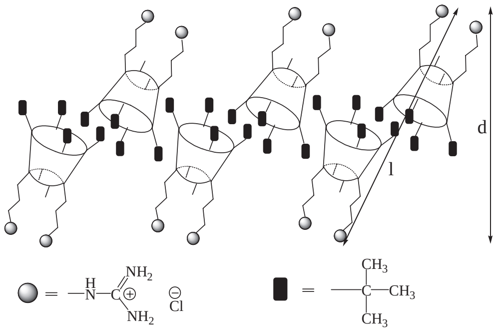

Figure 2. Proposed packing model of the formation of bilayer from BGC dispersed in ethanol.

The cast films for XRD study was prepared by dropping the BGC ethanol solution onto glass plate followed by desiccation in desiccator. The XRD pattern of the cast film exhibits periodic peaks indicating the existence of an ordered layer structure. In addition, the long spacing (D value) of the aggregates is 2.42 nm. This data is quite close to the length of two BGC molecules (2.7–2.8 nm). On the other hand, DSC study of BGC dispersion in ethanol found an endothermic peak at ca. 40 °C, indicating a gel-to-liquid crystal phase transition process of bilayer structure.

Ripmeester has reported the self-inclusion crystal structure of *p-tert*-butylcalix[4]arene,[8] in which the hydrophobic cavity of one calixarene molecule can be occupied by *t*-butyl groups from a guest calixarene molecule situated in the adjacent layer to form bilayer. Moreover, Zerbetto has demonstrated further through calculation that such self-inclusion can be formed via CH–π interaction and van der Waals interaction.[9] Our previous study has demonstrated that the protons from the bridging methylenes in BGC molecules give a pair of doublets at 4.19 and 3.33 ppm in ^{1}H NMR spectrum and the corresponding coupling constant is around 12 Hz.[7] The result indicates BGC molecules adopt a *cone* conformation. On the other hand, geometry optimization by MM+ shows the *cone* conformation is the most stable one. Therefore, the analogous structure may be adopted in the structure of BGC bilayer vesicles. The polarity of ethanol makes the hydrophobic upper rims of BGC molecules come close and interact with each other via intermolecular hydrophobic interaction. Meanwhile, as polar head group, the guanidinium ion can form hydrogen bonds and dipole–dipole interaction with ethanol, which makes the BGC lower rims to have stronger affinity to ethanol. Moreover, the suitable polarity of ethanol makes BGC molecules more dispersible in ethanol than in water. All these make the solvophilic/solvophobic balance of BGC molecules more fit to self-assembly to form bilayer vesicle in ethanol. A speculative packing model of BGC bilayer vesicles in ethanol is given in Figure 2. Similar to the bilayer in crystal structure, the BGC bilayer, with guanidinium groups stretching outside to contact ethanol, is formed via multiple CH–π interaction and van der Waals interaction between *t*-butyl groups and aromatic cavities. BGC molecules are packed in a tilting form to appease the demand for more effective host–guest interaction. As a result, the thickness of the bilayer (d) become slightly shorter than the twice of the BGC molecular length (l).

In conclusion, we have shown that bilayer vesicles are constructed in ethanol by calix[4]arene derivative without long alkyl chains via hydrophobic interactions. This work offer such possibility that non-typical amphiphiles can be used to study bilayer assembly in wider solvent system, even in protic and polar organic media. Moreover, it may broaden the application of calixarenes in material synthesis, ion transportion, and molecular recognition.

We especially thank Ms. Anneliese Heilig, Max-Planck Institute of Colloids and Interfaces, Germany, for AFM observation. This work was supported by National Natural Science Foundation of P. R. China (No. 20372032).

References

1 a) T. Kunitake, *Angew. Chem., Int. Ed. Engl.*, **31**, 709 (1992). b) N. Kimizuka, *Curr. Opin. Chem. Biol.*, **7**, 702 (2003). c) J. A. Zasadzinski, E. Kisak, and C. Evans, *Curr. Opin. Colloid Interface Sci.*, **6**, 85 (2001).

2 a) T. Kawasaki, M. Tokuhiro, N. Kimizuka, and T. Kunitake, *J. Am. Chem. Soc.*, **123**, 6792 (2001). b) F. Ilhan, T. H. Galow, M. Gray, G. Clavier, and V. M. Rotello, *J. Am. Chem. Soc.*, **122**, 5895 (2000). c) H. Yin, Z. Zhou, J. Huang, R. Zheng, and Y. Zhang, *Angew. Chem., Int. Ed. Engl.*, **42**, 2188 (2003). d) X. Luo, S. Wu, and Y. Liang, *Chem. Commun.*, **2002**, 492.

3 a) J. H. Fendler, *Chem. Rev.*, **87**, 877 (1987). b) M. V. Kitaeya, N. S. Melik-Nubarov, F. M. Menger, and A. A. Yaroslavov, *Langmuir*, **20**, 6796 (2004). c) A. Fischer, A. Franco, and T. Oberholzer, *ChemBioChem*, **3**, 409 (2002). d) Y. Jin, L. Bi, Y. Shao, and S. Dong, *Chem.—Eur. J.*, **10**, 3225 (2004).

4 a) N. Yamada, K. Ariga, M. Naito, K. Matsubara, and E. Koyama, *J. Am. Chem. Soc.*, **120**, 12192 (1998). b) Y. Ishikawa, H. Kuwahara, and T. Kunitake, *J. Am. Chem. Soc.*, **111**, 8530 (1989). c) H. Kuwahara, M. Hamada, and T. Kunitake, *J. Am. Chem. Soc.*, **115**, 3002 (1993).

5 a) N. Kimizuka, T. Wakiyama, H. Miyauchi, T. Yoshimi, M. Tokuhiro, and T. Kunitake, *J. Am. Chem. Soc.*, **118**, 5808 (1996). b) J.-B. Huang, B.-Y. Zhu, G.-X. Zhao, and Z.-Y. Zhang, *Langmuir*, **13**, 5759 (1997).

6 Y. Tanaka, M. Miyachi, and Y. Kobuke, *Angew. Chem., Int. Ed. Engl.*, **38**, 504 (1999).

7 F. Liu, G.-Y. Lu, W.-J. He, J. Hu, Y.-H. Mei, and L.-G. Zhu, *Synthesis*, **2001**, 607.

8 E. B. Brouwer, K. A. Udachin, G. D. Enright, J. A. Ripmeester, K. J. Ooms, and P. A. Halchuk, *Chem. Commun.*, **2001**, 565.

9 S. León, D. A. Leigh, and F. Zerbetto, *Chem.—Eur. J.*, **8**, 4854 (2002).