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Stimuli-Responsive Supramolecular Nanocapsules from Amphiphilic Calixarene Assembly

Myongsoo Lee,* Sun-Ja Lee, and Li-Hong Jiang

Center for Supramolecular Nano-Assembly and Department of Chemistry, Yonsei University, Seoul 120-749, Korea

Received July 8, 2004; E-mail: mslee@yonsei.ac.kr

Controlled self-assembly of specific amphiphilic molecules is a challenging topic of research in the fields of chemical biology and materials science because it provides the spontaneous generation of a well-defined, discrete aggregate structure from molecular components under thermodynamic equilibrium.[1] For example, amphiphilic molecules consisting of hydrophilic and liphophilic block segments have been proved to be promising scaffolds for nanometer-sized aggregate structures that can be able to effectively encapsulate hydrophobic guest molecules in aqueous solution.[2] Increasing chain length of hydrophilic and hydrophobic segments and introducing a rigid segment into a self-assembling system have been reported to enhance aggregation stability.[3,4] In addition to stability, another important issue regarding the preparation of these self-assembling systems is their capability to respond to external stimuli such as pH and temperature.[5] To obtain precisely controlled and well-defined aggregates that are able to respond to external stimuli such as pH, however, the more elaborate design of corresponding building blocks is required, since the information determining their specific assembly should be encoded in their molecular architecture. Accordingly, we synthesized amphiphilic tetramers based on a calixarene building block consisting of four liphophilic decyl chains at the lower rim and four dibranched hydrophilic chains connected to amine at the upper rim that can endow aggregates with enhanced stability and a pH-responsive character.

In this communication, we report significant size and structural changes of the aggregates in aqueous solution, from large vesicles to small spherical micelles, with only small variation in the hydrophilic chain length or pH in amphiphilic tetramers based on a calixarene building block (Figure 1). The amphiphilic calixarenes having four decyl chains and eight oligo(ethylene oxide) moieties on the opposite sides were obtained in a multiple synthesis from commercially available starting materials. The resulting amphiphilic calixarenes **3a**−**3c** were characterized by ^{1}H and ^{13}C NMR spectroscopy, elemental analysis, and MALDI-TOF mass spectroscopy and shown to be in full agreement with the structures presented.

The aggregation behavior of the amphiphilic tetramers based on a calixarene building block in aqueous solutions was investigated by using dynamic light scattering (DLS), field-emission scanning electron microscopy (FE-SEM), and transmission electron microscopy (TEM). DLS experiments were performed with the solutions (1×10^{-5} g/mL) of **3a**−**3c** over a scattering angular range of 30−145° at 25 °C. All of the molecules showed an aggregation behavior with a narrow size distribution, indicating well-equilibrated structures (Figure 1). The average hydrodynamic radii (R_H) of **3a** and **3b** were observed to be approximately 100 nm (polydispersity = 0.12) and 18 nm (polydispersity = 0.15), respectively, indicating that the amphiphilic molecule with longer hydrophilic chains assembles into much smaller aggregates. However, the measured diameters of the aggregates in both molecules exceeded the

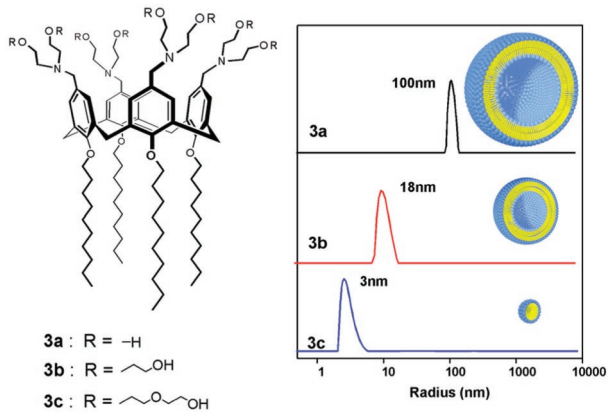

Figure 1. Molecular structure and DLS results at the scattering angle of 90° of the aggregates formed by **3a**−**3c** in aqueous solution.

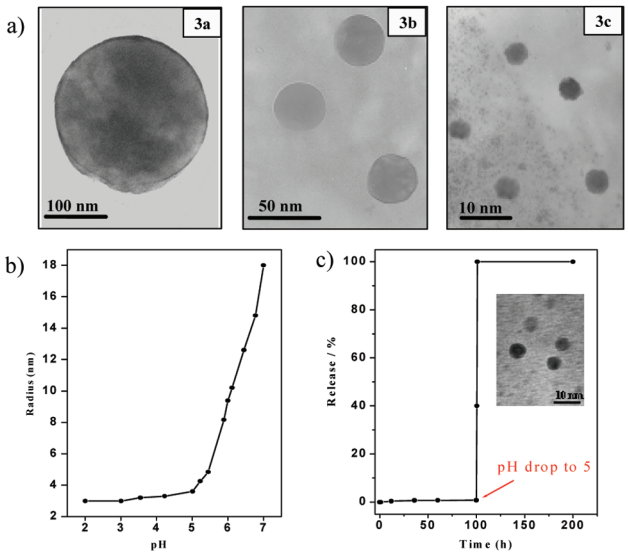

Figure 2. (a) TEM images of **3a**−**3c** (5 mg/ml) in H_2O. (b) Hydrodynamic radius of aggregates of **3b** (0.5 mM) as a function pH at 25 °C. (c) Release percent of calcein from the vesicle of **3b** as a function of time.

corresponding extended molecular length (approximately 2 nm), suggesting that these aggregates are rather vesicular entities than simple micelles. Further evidence for the formation of the vesicles in **3a** and **3b** was provided by TEM experiments. As shown in Figure 2a, the images revealed that there is obvious contrast between the periphery and center in the sphere, characteristic of the projection images of hollow spheres. The formation of spherical aggregates of these molecules was also confirmed by FE-SEM experiments. The micrographs showed spherical aggregates that are approximately 200 and 35 nm in diameter for **3a** and **3b**, respectively, and are thus consistent with the results obtained from DLS and TEM experiments.

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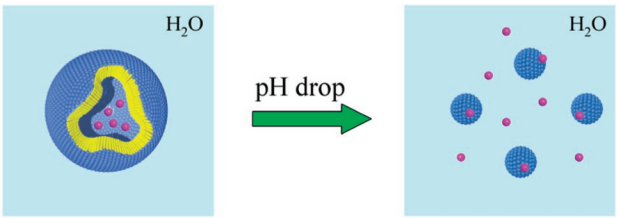

Figure 3. Schematic representation of a vesicle-to-micelle transition of **3b** with a pH and subsequent release of encapsulated calcein.

In contrast, **3c** based on a long oligo(ethylene oxide) chain assembles into spherical micellar aggregates. DLS measurements of **3c** showed that the R_H is approximately 3 nm with a narrow size distribution (polydispersity = 0.16). TEM micrograph showed spherical aggregates that are roughly 5−7 nm in diameter and are thus in accord with the DLS results. These dimensions in diameter of the aggregates correspond to approximately twice the extended molecular length, implying that the aggregates of **3c** are micellar in nature. These results demonstrate that aggregate size can be regulated by systematic variation in the hydrophilic chain length of the molecule. The variation of aggregate size can be rationalized by considering the effective cross-sectional area of a hydrophilic headgroup.[6] The effective area of a hydrophilic headgroup increases as a function of chain length. Thus, the interface between the hydrophilic and hydrophobic domains changes from a more flat to a curved interface, causing a large interfacial area and resulting in the reduction of the aggregate size.

More importantly, the vesicular structure appeared to transform into a micellar structure with the decrease of pH. The effect of pH on the aggregation behavior was investigated with **3b** by DLS measurements. As shown in Figure 2b, the small drop of pH induces a dramatic decrease of the aggregate size, from 36 nm in diameter at pH 7 to 6 nm at pH 5, while maintaining a narrow size distribution. This unique aggregation behavior seems to be attributed to a transition from a vesicular structure to a micellar structure. Therefore, one can envision that the vesicles formed by **3b** encapsulate hydrophilic guest molecules within their interior at neutral condition and release the guest molecules in response to a decrease in pH. With this in mind, calcein as a hydrophilic fluorescent guest was encapsulated at a sufficiently high, self-quenching concentration, and free calcein was removed by filtration over a Sephadex column. Release of calcein from the inside of a vesicle was accompanied by an increase in fluorescence emission as the free calcein in solution was dequenched.[7,8] As shown in Figure 2c, no leakage of entrapped calcein was observed over periods of 100 hr, indicating that the vesicles are highly stable toward leakage. However, exposure of the calcein-loaded vesicles to pH 5 resulted in a rapid and complete release of encapsulated calcein. This result can be explained by considering a pH-triggered vesicular-micellar phase transition. The decrease of pH results in the quarternization of the amine groups. This increases the surface area of the hydrophilic headgroup[9] and triggers the collapse of the vesicles into a micellar structure with concomitant release of the encapsulated calcein (Figure 3). This is further supported by TEM images measured at pH 5 that revealed small spherical aggregates with dimensions of 5−7 nm in diameter, indicative of transformation into a micellar structure with a pH drop (Figure 2c, inset).

The results described here demonstrate that the amphiphilic calixarene molecules with a small hydrophilic part assemble into well-defined and tunable vesicles that decrease significantly in diameter with increasing hydrophilic chain length. Further increasing the chain length induced the collapse of the vesicles into spherical micelles. Remarkably, the vesicles were also observed to transform into small globular micelles at lower pH, which can be used to trigger the release of the encapsulated hydrophilic guest molecules. This stimuli-responsive nanocapsule might have potential application for selective drug delivery in tissues of a lower pH value such as infected tissues and tumor tissues.[10,11] Further work on this line is in progress.

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Supporting Information Available: Detailed synthetic procedures, characterization, emission spectra, DLS data, and SEM images (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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