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LETTER

Aggregates of cholic acid and benzylamine as templates for the formation of hollow silica spheres

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Introduction

Silica is an abundant and important biomineral in one-celled diatoms through to higher animals and plants. Due to its amorphous structure, silica morphogenesis principally depends upon the suitable templates [1–6]. It is well known that the sufficient interaction between the aggregates and negatively charged silica precursors, such as tetraethyl orthosilicate (TEOS), is indispensable for effective transcription [7–9]. Thus, silica transcription via TEOS polycondensation was limited to molecules possessing positive charges or hydrogen bond sites, like amino groups, until people discovered the utilization of amine additives to promote the silica transcription even in the absence of cationic groups or hydrogen bond sites on the aggregates [10–14]. The self-assembly morphologies of poly(L-lysine) vesicles [10] and organogel fibers [11] have been successfully transcribed into their silica analogs with the help of benzylamine. As a result, a great amount of organic molecules, including anionic and neutral molecules, can be considered as templates for silica transcription.

Bile acids and their salts are biosynthesized in liver. Bile salts are natural surfactants, which act as solubilizer and emulsifier for cholesterol, lipids, and fat-soluble vitamins in the intestine. The solubilization and association behaviors of bile salts in aqueous environment are distinct from those of ordinary aliphatic amphiphilic compounds, which have received much attention from the scientific community including us [15–17].

Here we report the self-assembly structure of cholic acid (CA), one of the bile acids in human bodies, with benzylamine in aqueous solution and the transcription of such aggregates into silica analogs. The influence of solution conditions on aggregation behaviors of the CA–amine intermixture and the role played by benzylamine in the transcription process have been discussed. This method will not only reveal the solution behaviors of certain natural molecules but also offer an easy way for the fabrication of silica materials with peculiar structures.

Experiments

Cholic acid (CA) was recrystallized twice from ethanol before use. Benzylamine was purified by vacuum distillation with zinc powder. The other agents were used as received. At ambient condition, CA is hardly soluble in water (15 °C: 0.028 g/100 g (H_2O)) [18]. However, the powder of CA crystal disappeared gradually in the presence of exceeding benzylamine, leading to a limpid solution. Silica transcription was performed by the addition of TEOS to the solution of CA–benzylamine intermixture at room temperature. 10 mL of TEOS was added dropwise to the solution containing CA (1.0 g, 2.4 mmol) and benzylamine (6.0 g, 56 mmol) in 200 mL of distilled water. The molar ratio of CA and the amine was 1/23 and the original pH value was 10.9 before the addition of TEOS. White suspended solids were obtained after 30 min of

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stirring and then kept under static condition for 24 h before collection. The product was washed thoroughly with water and ethanol and dried at 80 °C.

Scanning electron microscopy (SEM) measurements were taken on a Hitachi S-4500 microscope. Dried samples were sputter-coated with Au. The accelerating voltage used was 5–15 kV. Transmission electron microscopy (TEM) measurements were performed with a Hitachi H-7100 microscope operating at an accelerating voltage of 100 kV. The samples were placed on carbon-coated copper grids (400 mesh). Dynamic light scattering (DLS) experiments were carried out on a laser light scattering spectrometer (BI-200SM) equipped with a digital correlator (BI-9000AT). The samples were prepared by filtering about 1 mL of the aggregate solution with a 0.45 μm Millipore filter into a clean scintillation vial and then measured at 25 °C. Phase contrast microscopy (PCM, Olympus LX70 inverted fluorescence microscope) was used to observe the morphologies of the aggregates in aqueous solution.

Results and discussion

SEM image of the product (Fig. 1a) shows the presence of a large amount of spheres with diameters ranging from about 200 nm to 1 μm. A possible explanation for the wide size distribution might be the complex association behaviors of cholate in aqueous solution.

The typical TEM image of the product (Fig. 1b) reveals the hollow nature of the silica spheres with outer diameters close to the size observed under SEM. Bigger spheres possess larger interior empty space, whereas the shell thicknesses are nearly the same (ca. 60 nm). Without any further chemical or physical treatment, hollow spheres were obtained as synthesized, which could keep their structures even after calcination (observed with TEM).

The relatively smooth surface of the silica spheres has provided a reasonable mechanism that the silica growth is initiated on the surface of templates [10]. To confirm the presence of aggregates in solution prior to silica transcription, DLS experiments were carried out. The results show that there were aggregates with particle size ranging from 200 to 800 nm (Fig. 2). The value is in good agreement with the size of the silica spheres mentioned above, proving that the formation of the silica spheres was templated by organic aggregates presented in solution.

Whereas DLS experiments do not provide any information about the shape of the CA-benzylamine aggregates, PCM images allow us to directly visualize the aggregates responsible for the formation of the inorganic shell. PCM image taken of the solution of CA and benzylamine clearly shows that the aggregates possessing perfect spherical shape (Fig. 3), in accordance with that of the silica particles obtained after transcription, confirming that these aggregates are in fact the templates for the formation of the silica hollow spheres.

Additional evidence for the template process was obtained from FT-IR measurements (KBr pellet) of the products. The absorptions at 1558 and 1396 cm^{-1} and the disappearance of the characteristic stretching frequency of COOH group (1720 cm^{-1}) were ascribed to the formation of COO^{-} of negative CA [19]. The peaks around 1800 cm^{-1} could be attributed to benzylamine. The absorption bands at 1097, 805, and 465 cm^{-1} were due to Si–O–Si group and 943 cm^{-1} due to Si–OH [20]. It has confirmed the formation of silica structures and the existence of cholate molecules in the spheres.

Test experiments were carried out to prove that both CA and benzylamine were required for the formation of these hollow silica spheres. Reaction in the absence of CA resulted in the exclusive formation of granular silica. While in the test without benzylamine, as CA could hardly dissolve in water, soluble salts like sodium cholate was used instead, but no silica formation was observed even after long time. These results strongly indicate that both CA and benzylamine need to be present in order to obtain hollow spherical silica.

The use of organic vesicles as templates for silica deposition asks for non-trivial approaches since the template structure is sensitive to the solution conditions

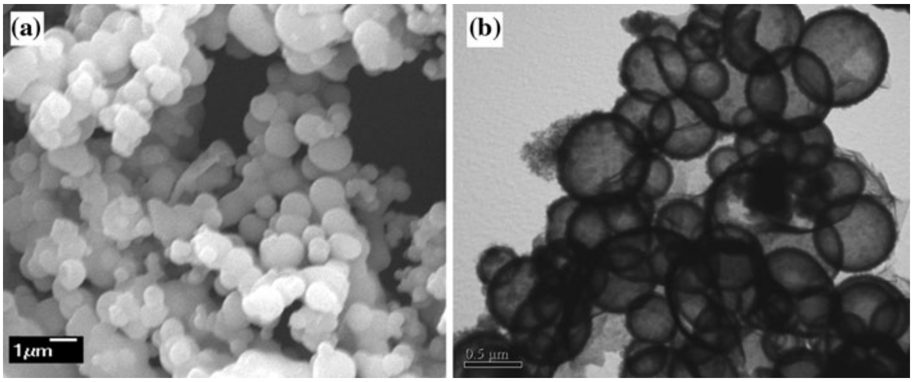

Fig. 1 SEM image (**a**) and TEM image (**b**) of as-synthesized products

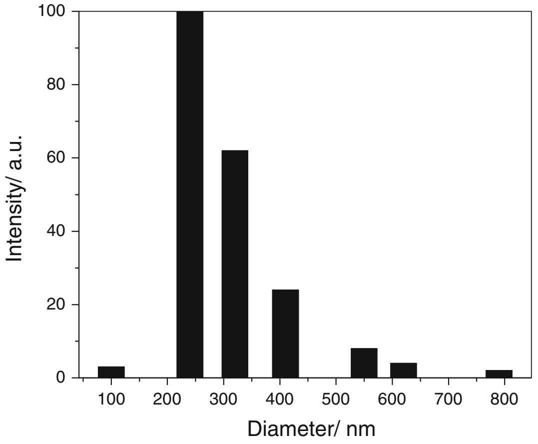

Fig. 2 Size distribution of the aggregates of CA and benzylamine in aqueous solution

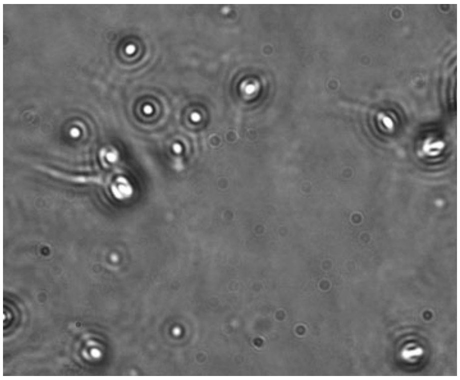

Fig. 3 PCM image of the spherical aggregates presented in the solution of CA and benzylamine (×400)

including organic solvents, pH values, and total concentrations. The classical Stöber approach is unfit due to the use of ethanol [21], which can lead to the break up of the vesicle structure and the formation of solid silica particles. When the transcription was performed in relatively strong basic conditions, such as pH 12 by increasing the content of benzylamine (molar ratio of CA and benzylamine 1/47), narrowly dispersed, solid spheres were obtained (Fig. 4). The vesicle structures of aggregates might be destroyed by exceeding amine. As decreasing the content of amine to the molar ratio of CA/benzylamine 1/12, the pH value was under 10, resulted in the formation of a small amount of granular silica. This can be explained that the basicity of the solution was not high enough to catalyze TEOS polycondensation.

As diluting the total concentration of the CA-benzylamine solution, variation of the final structures of the silica products was observed, indicating a micelle-to-vesicle transition of the organic aggregates. Solid spheres were formed at high concentration (100 mL of water totally), while hollow shells in wide size distribution were obtained by diluting (300 mL of water totally). There is no obvious change from the synthesized products to the dilution one. As reported, there is a mixed micellar phase limit for multicomponent micelle systems containing bile salt [22, 23]. At concentrations below the critical point, the transition of micelle to vesicle occurs and unilamellar vesicles form.

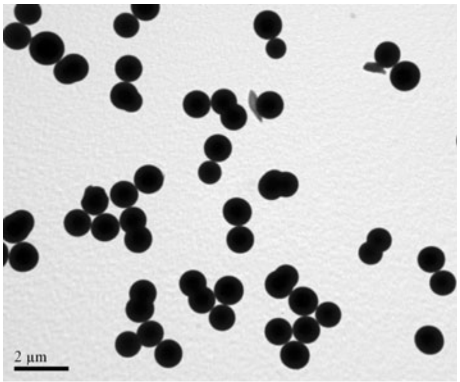

Fig. 4 TEM image of the sample prepared at pH 12.0 with molar ratio of CA and benzylamine 1/47

It was the hydrogen bonds between benzylamine and silica precursor that acted as recognition interaction in the transcription at the interface. Tests using *n*-butylamine (Fig. 5a) or diethylamine (Fig. 5b) as basic additives have both resulted in a mixture of granular silica and a small number of spherical shells. These inferior transcriptions can be ascribed to the poor abilities of the bases to offer hydrogen bonding sites. Duo to the molecular structure, triethylamine cannot act as a hydrogen bond donor and only granular silica was obtained when triethylamine was used.

Conclusions

The natural origin of CA makes this method friendly to environment and commercially available reagents may contribute to the ease of preparation as well as low cost of products. The simplicity of the experimental procedure, which involves only one-step aggregation and growth procedure, adds to the attractiveness of this system. Benzylamine has acted as a co-structure-directing agent in this anionic template method, which takes part in aggregation process and modulates the self-assembly structure of the aggregates, catalyzes TEOS sol–gel polycondensation, and promotes silica transcription as a connecting bridge of the organic templates and inorganic precursors. Although the investigation on the assembly model of CA is still in process, the results presented here will be helpful in deep insight into the aggregation behaviors of bile acids in

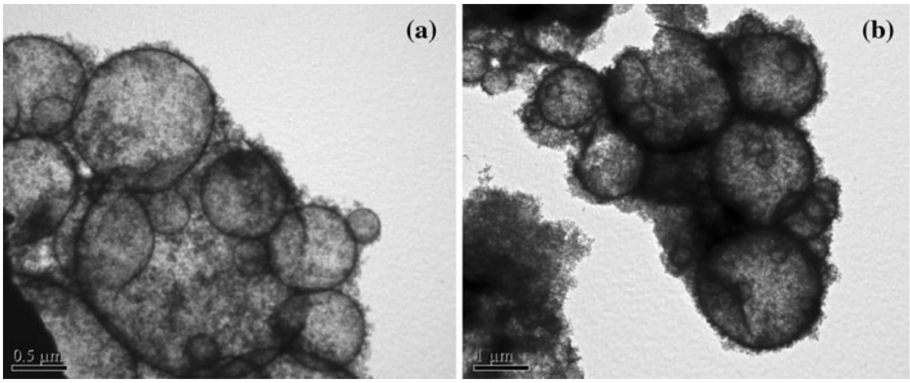

Fig. 5 TEM images of the samples prepared using *n*-butylamine (**a**) and diethylamine (**b**) as additives

aqueous solution, as well as in fabricating variety of inorganic materials with interesting new shapes and properties.

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