Formation of cage-like hollow spherical silica *via* a mesoporous structure by calcination of lysozyme–silica hybrid particles†

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Calcination of lysozyme–silica hybrid hollow particles gives novel cage-like hollow spherical silicas with differently patterned through-holes on their shell structure.

The controlled formation of inorganic materials with well designed shapes and patterns at the micron- and nano-size level is extremely important in materials science. In particular, the preparation of hollow inorganic capsules with a defined structure has received increasing attention, because of their potential applications, such as the encapsulation of various substances, as controlled release systems for drug delivery and the manufacture of advanced materials.[1] The key to using hollow inorganic shells in these applications is the design of the interior space as a storage space or a reaction chamber and the shell structure containing paths through which desired molecules can be loaded or released.[2] Many researchers have found sophisticated methods for creating inorganic hollow structures with sponge-like interiors, mesoporous shell structures, micro-spheres with surface perforations or self-organized porous spherical particles.[3] However, to the best of our knowledge, a hollow spherical structure, with a defined interior space and a shell provided with through-holes of >100 nm diameter, sufficiently large to allow the passage of biomacromolecules, is essentially unknown.[4] Very recently, silica hollow spheres with nano-macroholes like diatomaceous earth have been synthesized using a water/oil/water emulsion system, although the formation mechanism of the macroholes is still unclear.[5] The generation of capsule shapes with complex microscopic structures like cell walls remains the ultimate challenge to workers in the field.

Herein we report the morphologies of novel cage-like hollow spherical silica (CHS) with a through-hole (50–250 nm diameter) structure obtained by the calcination of lysozyme–silica hybrid hollow particles (L-SHHs). L-SHHs were synthesized by the combination of sonochemical treatment and the silica precipitation activity of lysozyme. Lysozyme molecules were found to be uniformly dispersed within the silica matrix.[6] One can envision the removal of such encapsulated lysozyme as a way to control the morphology of the silica structure. The removal of organic templates by calcination from organic–inorganic hybrid materials is a general technique to transfer the diverse structure of organic molecular to inorganic products.[7] Several examples of templated formation of inorganic materials have employed biopolymers such as DNA, peptide fibrils and polysaccharides, whose characteristic shapes have been faithfully transferred to inorganic materials.[8] However, our synthesis route to CHS is somewhat different from the transcription method using a bioorganic polymer just as a template, because the through-hole sizes of the CHS synthesized in this study were ten times larger than the molecular size of lysozyme. This difference is an interesting hint towards understanding the formation process of CHS. Based on the observation of the morphologies at each calcination temperature, we will also discuss the formation mechanism of through-holes whose sizes are larger than that of a lysozyme molecule.

Our synthesis of CHS consists of two simple steps.‡ First, L-SHHs were prepared by reacting TEOS with 2 mg ml^{-1} lysozyme solution *via* a sonochemical treatment, as previously reported.[6] The obtained L-SHHs with a particle diameter of 0.5–15 μm were subsequently calcined at 700 °C for 2 h in air, resulting in the formation of CHS. Fig. 1 shows scanning electron microscope (SEM) and transmission electron microscope (TEM) images of the CHS. The hollow spherical structure of L-SHHs was preserved even after calcination, and the characteristic through-holes which are similar to those in an archean radiolarian shell[9] were formed in the silica shell structures. The typical CHS structure has a silica shell with thickness of ~100 nm and through-holes with a diameter of 50–250 nm. The particle size distribution of typical CHS appeared not to differ from that of L-SHHs (0.5–15 μm), even though slight shrinkage might occur during calcination. The through-holes of CHS shell structures were individually patterned at random even in the same sample. The morphological variety of the through-hole structure was observed in relation to the number, size, and shape of through-holes and the connectivity of adjacent through-holes. It appears that the through-holes pattern is very sensitive to the structural nature of the L-SHHs. CHS is mechanically fragile so that fragmentation or collapse of particles as seen in Fig. 1C easily occurred. Additionally, the broad range distribution of shell thickness in L-SHHs should be mentioned. In the case where the shell thickness is above 100 nm, the alternative shape of CHS shown in Fig. 2 was also observed. Instead of a thin silica shell layer and complete through-holes, some CHS have a bilayer shell structure with complicated voids between silica shell layers (Fig. 2A and C), and others have a slightly thicker silica shell with incomplete through-holes (Fig. 2B and D). It appears that one of the essential factors

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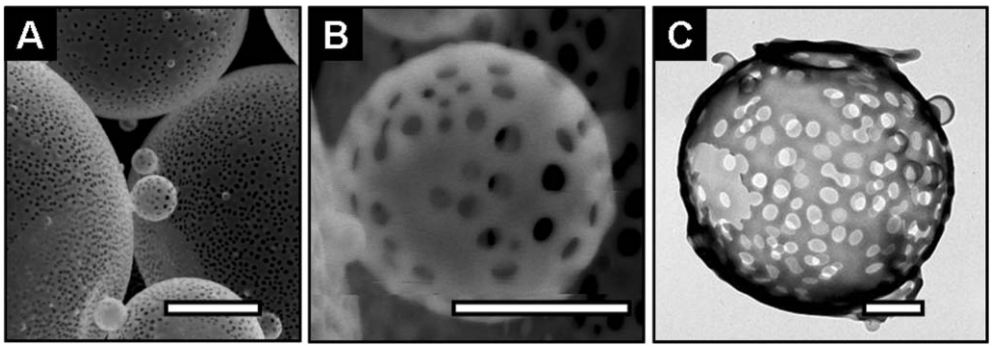

Fig. 1 SEM (A), (B) and TEM (C) images of CHS obtained by calcining L-SHHs (2 mg ml^{-1} lysozyme) at 700 °C for 2 h. Scale bars: (A) 3 μm, (B) and (C) 500 nm.

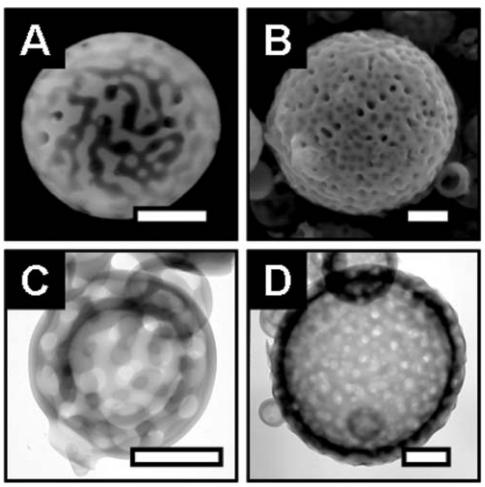

Fig. 2 SEM and TEM images of CHS with different shell thickness. The CHS structures were also observed in the same sample prepared by calcining L-SHHs (2 mg ml^{-1} lysozyme) at 700 °C for 2 h. All scale bars: 500 nm.

operating to form a complete CHS shell structure is the shell thickness of the L-SHHs.

The composition of the L-SHHs is also likely to be another key factor that controls the variable nature of the patterns of CHS shell structures. To investigate this, we examined how the initial lysozyme concentration used to prepare L-SHHs influences the morphology of the CHS formed after calcination. When the L-SHHs were prepared with a 1 mg ml^{-1} lysozyme solution, calcination resulted in a CHS with a similar shape to those made from 2 mg ml^{-1}, while those prepared from a 10 mg ml^{-1} solution exhibit a different morphology. As Fig. 3A reveals, the SEM and TEM images of calcined L-SHHs made from a 10 mg ml^{-1} solution show a significantly rough shell surface with incomplete through-holes and serpentine wormlike structures. Under the high magnification of the TEM, it was apparent that mesopores with diameters of several tens of nanometers were formed even on the inside of the wormlike structures (indicated by an arrow in Fig. 3B). Thermal gravimetric analysis (TGA) clearly showed that the different surface morphologies of the calcined L-SHHs are linked to the composition ratios of lysozyme to silica (Fig. 4). Total weight losses between 30 and 700 °C of materials made from 2 mg ml^{-1} and 10 mg ml^{-1} solutions were *ca.* 55 and 63%,

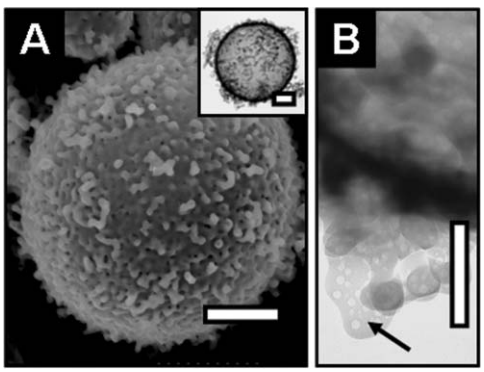

Fig. 3 SEM (A) and TEM (Inset) and (B) images of CHS obtained by calcining L-SHHs (10 mg ml^{-1} lysozyme) at 700 °C for 2 h. Scale bars: (A) and (Inset) 1 μm, (B) 300 nm.

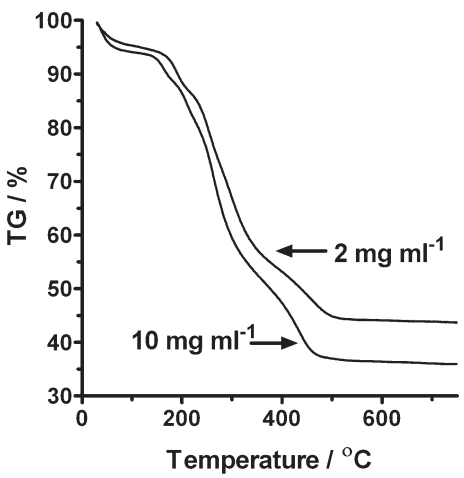

Fig. 4 TGA analysis of L-SHHs obtained with different lysozyme concentrations.

respectively. The relatively high lysozyme content of L-SHHs which were synthesized with 10 mg ml^{-1} lysozyme solution probably represents the increase of lysozyme ratio within the near surface area of L-SHHs. This difference would cause the formation of a rough surface and serpentine wormlike structures.

Having established the role of the properties of the L-SHHs in the calcination process, we went on to investigate the formation mechanism of CHSs. One aspect of this important topic is the

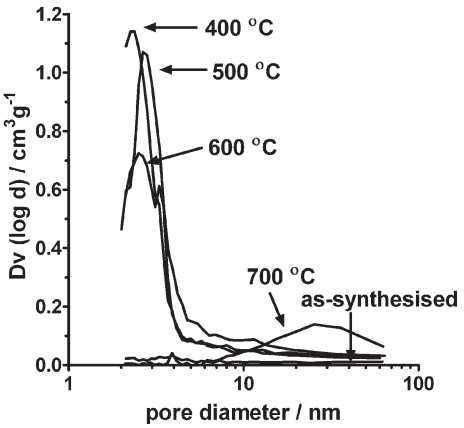

Fig. 5 Pore size distributions calculated from nitrogen adsorption measurements of as-synthesized L-SHHs and L-SHHs calcined at different temperatures.

curious question of how the large through-holes diameter (50–250 nm) of the CHS is created from the small lysozyme molecule (3.0 nm × 3.0 nm × 4.5 nm) encapsulated within the silica matrix of L-SHHs during calcination. To resolve this question, we performed nitrogen adsorption–desorption experiments with samples obtained from the calcination of L-SHHs (prepared with 2 mg ml^{-1} lysozyme solution) at different temperatures.

Fig. 5 shows the pore size distribution calculated by the BJH method using the adsorption branch. As-synthesized L-SHHs were found to have no mesopore structure. When calcination was performed at 400 °C, pores with diameter of about 2 nm or less were observed. It is worth noting that pores with a diameter of about 3 nm, which is consistent with the size of the lysozyme molecule, appeared after calcination at 500 °C. Increasing the calcination temperature from 500 to 600 °C decreases the size of the pore distribution peak. The BET surface areas also decreased with an increase in the calcination temperature from 400 to 600 °C (621, 420 and 338 m^2 g^{-1}, respectively). These mesopores disappeared after calcination at 700 °C (the temperature used in the preparation of the CHSs), while larger pores appeared in the range of several tens of nanometers or more, which probably corresponds to creation of the through-holes observed in CHS.

Returning to the TGA results, Fig. 4 shows that the removal of lysozyme from L-SHHs finishes at about 500 °C. Thus, the mesopores of around 3 nm diameter are probably derived from the removal of lysozyme molecules, that is, lysozyme molecules act as a template at this temperature. This suggestion is also supported by SEM and TEM observation of powder samples calcined at 500 °C, showing that porous shell structures without through-holes were formed. The nitrogen sorption isotherms of L-SHHs calcined at 500 °C exhibit a diagnostic type IV isotherm with very little hysteresis consistent with these TEM observations. These results also prove that the lysozyme molecules are dispersed one by one within L-SHHs without aggregation. The extinction of the 3 nm diameter mesopores and the appearance of through-holes on increasing the calcination temperature from 600 to 700 °C imply the reconstruction of the silica network after the removal of the lysozyme. Bicontinuous structures observed among the CHS by SEM suggest that these morphologies are indicative of the restructuring from mesoporous structures to a cage-like structure with through-holes (Fig. 2A). In order to explain the formation mechanism of CHS in more detail, further work needs to be performed on the synthesis method using more precisely-controlled, well-defined CHSs. Nevertheless, it can be concluded that the formation process of CHS during calcination is comprised of two steps: the removal of lysozyme resulting in the formation of mesopores by the templating effect of lysozyme (first step) and the subsequent restructuring of the silica network leading to a morphological change to the CHS (second step).

We have demonstrated that a novel cage-like hollow spherical silica (CHS) was synthesized by the calcination of lysozyme–silica hybrid hollow particles (L-SHHs). The shell thickness and the composition ratio of L-SHHs were key factors in the resulting CHS structure. Based on the formation of cage-like hollow spherical particles with a through-hole structure, we suggest not only that lysozyme molecules operate as a sacrificial organic template during the calcination of L-SHHs, but also that reconstruction of the silica structure occurs after removal of the lysozyme molecules. The through-holes and interior space offered by the CHS structures have the potential to be developed for applications such as controlled release systems. The results of our findings reported herein suggest the possibility of developing a practical synthesis of cage-like particles and other shaped nanomaterials using biopolymer–inorganic hybrid structures as the primary material.

Notes and references

‡ A typical synthesis of CHS was performed as follows. A 1 ml volume of TEOS was added to 9 ml of lysozyme solution (final concentration of lysozyme: 2 mg ml^{-1}, 0.05 M glycine buffer, pH 9). Immediately the mixture was sonicated for 15 min at RT. The resultant solutions were dispensed onto a polystyrene plate and dried at 60 °C for 24 h, after which a white powder was obtained (L-SHHs). The L-SHHs were then calcined in air at 700 °C for 2 h. SEM observations of CHS and other silica samples were carried out using a Hitachi S-800 instrument operated at 10 kV. The nitrogen adsorption/desorption experiments were carried out using a NOVA 3000 series instrument (Quantachrome Instruments) after drying the sample powder at 200 °C for 2 h (except for as-synthesized L-SHHs: 60 °C for 24 h). The TGA data were recorded on a TG/DTA 300 instrument (SEIKO) at 10 °C min^{-1} in an air atmosphere.

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