Synthesis of protein–silica hybrid hollow particles through the combination of protein catalysts and sonochemical treatment

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Hollow spherical particles with protein–silica hybrid shell structures have been synthesized through a combination of the catalytic activity of the protein and sonochemical treatment; the morphologies of the particles were controlled by varying the protein concentration.

The preparation of organic–inorganic hybrid materials with well-defined morphologies has received increasing attention. In particular, hollow spherical particles have received attention because of the large number of their potential applications (as sorbents and in catalysis, chromatography, drug delivery, *etc.*).[1] So far, a number of papers have described the synthesis of hollow spherical silica-based particles based on W/O emulsion methods with copolymers as templates[2] or surfactants for stabilization of the organic phase.[3] However, such conventional chemical synthesis has to rely on extreme reaction conditions of pH, temperature and pressure. In contrast, the biomineral formation of silica structures with hierarchical morphologies is known to occur in diatoms and sponges even under ambient conditions.[4] Furthermore the complexity of their highly controlled silicon architectures is greater than that of artificial materials. Recent studies of silica formation in diatoms have suggested that polypeptides and their derivatives catalyze the biomineralization of silica and regulate its morphology.[5] Although the use of biopolymers for catalyzing silica formation *in vitro* would seem to allow a path to synthesis of differently patterned silica structures under ambient conditions,[6] synthesized peptides cannot be obtained readily, and thus studies of such systems and their industrial applications are restricted. In this paper, we report a novel catalytic activity of commercially available lysozyme for polysiloxane formation from tetraethoxy-orthosilicate (TEOS). The control of the morphologies of the protein–silica hybrid structures produced by a sonochemical method is also described. The effects of ultrasound, for example thermal decomposition and removal of surface contamination, have been studied with regard to inorganic hollow materials.[7] However, to the best of our knowledge, this is the first report describing the use of a combination of a protein solution and sonication treatment for the formation of hollow spherical particles with a protein–silica hybrid shell structure.

In a typical synthesis of protein–silica hybrid particles, 9 ml of an aqueous solution of lysozyme was prepared by dissolving lyophilized lysozyme powder derived from egg white into 0.05 M glycine buffer at given concentrations. The lysozyme solution was brought to pH 9 by the addition of 5 N NaOH, after which 1 ml of TEOS was added. Immediately, the mixture was stirred or sonicated for 15 min. The resultant solutions were dispensed onto a polystyrene plate and dried at 60 °C for 24 h, after which a white powder was obtained. Fig. 1 shows the scanning electron microscopy (SEM) images of sample powders obtained by stirring and sonication. When a lysozyme–TEOS mixture was stirred for the sol–gel reaction of TEOS, granular particles without any hollow structure were mainly observed (Fig. 1A and B). The granular spherical particles have a diameter of 250–1000 nm. Interestingly, the granular shape observed is quite similar to the silica nanospheres produced by coacervate formation using polyamine/phosphate mixtures as templates.[8] No spherical particles were observed without lysozyme in the reacting solution, suggesting that the biomimetic patterning of silica can be catalyzed by lysozyme. When sonication treatment was applied to the lysozyme–TEOS mixture, hollow spherical particles were observed as shown in Fig. 1C and D. SEM images reveal their particle diameter to be between 500 nm and 15 μm. Moreover, it is clear that the shell of hollow spherical silica has a well-defined surface and flexibility, evident in curled fragments which can be observed in the inside of the hollow spherical particles in Fig. 1D. The shell thickness of the hollow spheres, as estimated by SEM images of fractured particles, appears to be approximately 100 nm or more.

For both reaction routes (stirring and sonication), elemental analysis using an energy-dispersive X-ray (EDX) analyzer (which can detect the peak of silicon atoms and that of sulfur atoms derived from lysozyme) indicates that the obtained particles are composed of lysozyme and siloxane polymer, that is they are lysozyme–silica hybrid particles. Lysozyme molecules are probably

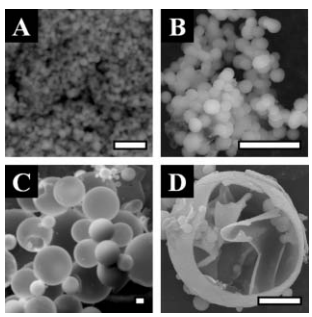

Fig. 1 SEM images of lysozyme–silica hybrid particles. (A) and (B): granular particles obtained by treating the lysozyme–TEOS mixture without sonication. (C) and (D): Hollow spherical particles obtained by sonication treatment. Scale bar: 3 μm.

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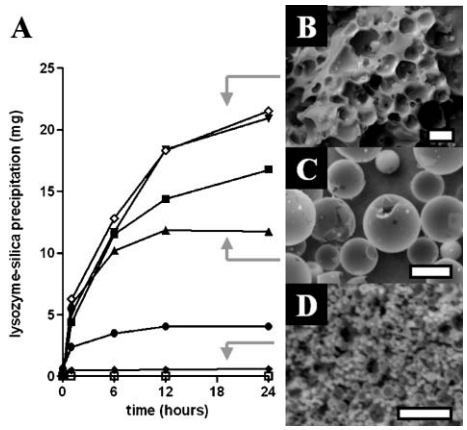

Fig. 2 The influences of lysozyme concentration on the formation of hollow spherical lysozyme–silica hybrid particles. (A) Lysozyme–silica precipitation from 1 ml of a lysozyme–TEOS mixture using lysozyme solutions of various concentrations (0 (empty squares), 0.5 (filled rhombus), 2.0 (filled circles), 5.0 (filled triangles), 10.0 (filled squares), 20.0 (filled inverted triangles), 40.0 mg ml^{-1} (filled rhombus)). The *x*-axis shows the time that a mixture was left under static conditions at room temperature after sonication. The weight of precipitations were measured after the precipitation of the lysozyme–silica hybrid was dried at 60 °C for 24 h (precipitations had been washed with 50% ethanol repeatedly in order to remove free lysozyme and TEOS). (B)–(D) SEM images obtained with lysozyme concentrations of 40, 5.0, 0.5 mg ml^{-1}, respectively. Scale bar: 10 μm.

held within the silica matrix by hydrogen bonding and electrostatic interaction between the hydroxyl residues and cationic groups of lysozyme molecules and the silanol residues of the silica matrix. This result of EDX analysis explains why the flexibility of these hollow spherical particles differs from other silica-based hollow spherical particles.

On the basis of the EDX analysis results, a structural change in the hollow spherical silica was expected with varying lysozyme concentration. We performed the reaction of the lysozyme–TEOS mixture with different lysozyme concentrations and the amount of lysozyme–silica hybrid precipitation measured (Fig. 2A). In the absence of lysozyme, no precipitation was observed. Below 5.0 mg ml^{-1} lysozyme, the rate of lysozyme–silica precipitation increases with increasing lysozyme concentration. This suggests that the reason that the amount of lysozyme–silica precipitation depends on lysozyme concentration, is that once lysozyme is included in the silica structure, such entrapped lysozyme probably loses its catalytic activity for silica formation. In addition to the increasing amounts of precipitation, an increase in average particle size of the hollow spherical silica was also observed (Fig. 2C and D). However the particle size distribution of the hollow spherical silica was not changed with increases in lysozyme concentration between 5.0 mg ml^{-1} and 40 mg ml^{-1}. Furthermore, it is clear from Fig. 2A that the increase in lysozyme–silica precipitation in the 20 and 40 mg ml^{-1} samples is very similar. This would suggest that TEOS works as a limiting factor in the lysozyme–silica precipitation. It is interesting to note that the sponge-like silica structures which can be seen in Fig. 2B start to appear at concentrations of lysozyme of 20 mg ml^{-1}.

The morphological changes observed in relation to changes in lysozyme concentration are associated with the adsorption of lysozyme onto the surface of the TEOS emulsion and its catalytic activity. We propose a mechanism for the formation of lysozyme–silica hybrid hollow particles as shown in Fig. 3. Firstly, lysozyme adsorbs to the TEOS emulsion (which is generated by sonication), through interactions between the lysozyme surface and the TEOS emulsion surface. This adsorption mechanism is supported by a similar property of lysozyme known as the foaming effect.[9] Subsequently inner lysozyme–silica shell structures are produced on the surface of TEOS emulsion through the catalytic activity of lysozyme. This step seems to start with a smaller TEOS emulsion, which causes the average particle size to be gradually changed along with lysozyme concentration below 5.0 mg ml^{-1}. Secondly, the lysozyme–silica shell structures grow by trapping TEOS which leaks through gaps in the lysozyme–silica shell on the outer surface. The excess of free lysozyme molecules and/or other lysozyme–TEOS aggregations cause lysozyme–silica hybrid hollow particles to combine with each other during their formation, as observed with the 40 mg ml^{-1} lysozyme concentration (Fig. 2B). When TEOS molecules both inside and outside of the silica shells have run out or when all lysozyme molecules are buried in shell structures, the hydrolysis and polymerization catalysed by lysozyme is finished.

As described above, the process of lysozyme–silica hybrid formation is comprised of several steps. The different interaction between TEOS (silica) and lysozyme seems to be present at each step. It was reported by Stone *et al.*[10] that -OH groups and cationic charges of peptides have important effects for the hydrolysis and polymerization process, meaning that hydrogen bonding and electrostatic interaction between lysozyme molecules and TEOS (silica) play a key role. Additionally, the foaming effect of lysozyme is also an important consideration for the formation of lysozyme–silica hybrid hollow particles. In order to determine the

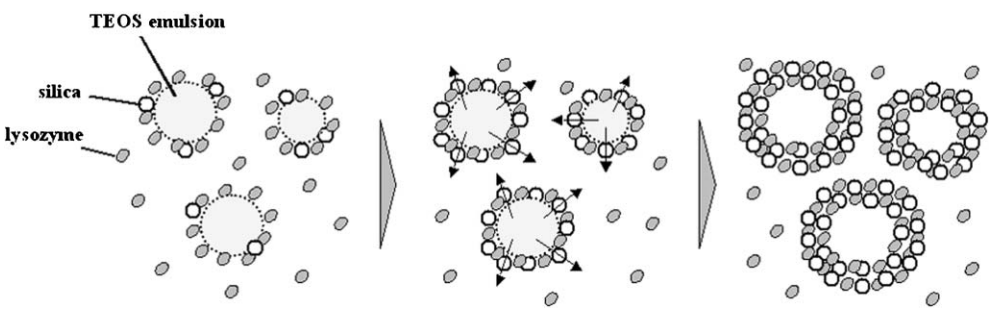

Fig. 3 The proposed model of the mechanism for the formation of lysozyme–silica hybrid hollow spherical particles through sonication treatment. TEOS emulsion (dotted circles), lysozyme (gray ellipsoids), induced silica (white spheres).

mechanism of formation of hybrid hollow particles in detail, we are currently investigating the influence of protein surface structures on the catalytic ability needed for silica formation and the morphologies of the obtained particles.

In conclusion, we have demonstrated the formation of hollow spherical particles with a lysozyme–siloxane hybrid shell structure produced through the combination of sonochemical treatment and the catalytic activity of lysozyme. The morphologies of the particles can be controlled by altering reaction conditions (stirring or sonication) or by changing lysozyme concentrations. Furthermore, our method needs no process to remove the template, which is inevitable in other protocols used to obtain hollow silica particles.[11] We believe that our method for the formation of inorganic–organic hybrids induced by biopolymers as described here sheds light on the controlled formation of silica structures and goes some way to the creation of advanced materials.

Notes and references

1 J. P. Photos, L. Bacakova, B. Discher, S. F. Bates and E. D. Discher, *J. Controlled Release*, 2003, **90**, 323; J. Ding and G. Lie, *J. Phy. Chem. B*, 1998, **102**, 6107; J. Du and Y. Chen, *Macromolecules*, 2004, **37**, 5710; F. Caruso, *Chem. Eur. J.*, 2000, **6**, 413; R. K. Sharma, S. Das and A. Maitra, *J. Colloid Interface Sci.*, 2005, **284**, 358; J. F. Chen, H-M. Ding, J-X. Wang and L. Shao, *Biomaterials*, 2004, **25**, 723.

2 J. Wang, Y. Xia, W. Wang, R. Mokaya and M. Poliakoff, *Chem. Commun.*, 2005, 210; Q. Sun, P. C. M. M. Magusin, B. Mezari, P. Panine, R. A. van Santen and N. A. J. M. Sommerdijk, *J. Mater. Chem.*, 2005, **15**, 256; Q. Sun, P. J. Kooyman, J. G. Grossmann, P. H. H. Bomans, P. M. Frederik, P. C. M. M. Magusin, T. P. M. Beelen, R. A. van Santen and N. A. J. M. Sommerdijk, *Adv. Mater.*, 2003, **15**, 1097.

3 W. Li, X. Sha, W. Dong and Z. Wang, *Chem. Commun.*, 2002, 2434; J-H. Park, C. Oh, S-I. Shin, S-K. Moon and S-G. Oh, *J. Colloid Interface Sci.*, 2003, **266**, 107.

4 Y. Zhou, K. Shimizu, J. N. Cha, G. D. Stucky and D. E. Morse, *Angew. Chem., Int. Ed.*, 1999, **38**, 779.

5 N. Kroger, R. Deutzmann and M. Sumper, *Science*, 1999, **286**, 1129; N. Kroger, G. Lehmann, R. Rachel and M. Sumper, *Eur. J. Biochem.*, 1997, **250**, 99; J. N. Cha, K. Shimizu, Y. Zhou, S. C. Christiansen, B. F. Chmelka, G. D. Stucky and D. E. Morse, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 361.

6 J. N. Cha, G. D. Stucky, D. E. Morse and T. J. Deming, *Nature*, 2000, **403**, 289.

7 N. A. Dhas and K. S. Suslick, *J. Am. Chem. Soc.*, 2005, **127**, 2368.

8 M. Sumper, S. Lorenz and E. Brunner, *Angew. Chem., Int. Ed.*, 2003, **42**, 5192.

9 R. Marchal, D. Chaboche, R. Douillard and P. Jeandet, *J. Agric. Food Chem.*, 2002, **50**, 1420.

10 F. Rodriguez, D. D. Glawe, R. R. Naik, K. P. Hallinan and M. O. Stone, *Biomacromolecules*, 2004, **5**, 261.

11 M. Jafelicci, Jr., M. R. Davolos, F. J. dos Santos and S. J. de Andrade, *J. Non-Cryst. Solids*, 1999, **247**, 98; F. Caruso, R. A. Caruso and H. Mohwald, *Science*, 1998, **282**, 1111.