Nanoassembly of biocompatible microcapsules for urease encapsulation and their use as biomimetic reactors†

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Biocompatible polypeptide capsules with high enzyme loading and activity prepared by templating mesoporous silica spheres were used as biomimetic reactors for performing $CaCO_3$ synthesis exclusively inside the capsule interior *via* urease-catalyzed urea hydrolysis.

Biomineralization is a process that natural living organisms use to produce inorganic solids from bioorganic molecules. Compared to the usual techniques of preparative solid-state chemistry, this process usually occurs at room temperature and under mild conditions.[1] To learn from nature, scientists from diverging fields, ranging from mineralogy to biochemistry and material science, have made great efforts to understand the biomineralization mechanism and mimic the process. During the last decade biomineralization has also become one of the most promising methods for the preparation of high-performance nanocomposite materials.[2,3] Depending on the structure of the organic templates and/or additives involved (*e.g.*, amphiphilic polymers,[2a,2b] enzymes,[2c] fatty acids[2d]) and the reactor type (*e.g.*, monolayer films,[3a] polyelectrolyte capsules,[3b] micelles,[3c] membranes,[3d] nanotubes[3e]), inorganic/organic hybrid materials possessing a wide range of polymorph modifications and properties can be obtained.

In a recent study, we reported an alternative, facile process for encapsulating biomacromolecules within polyelectrolyte microcapsules using mesoporous silica (MS) spheres as sacrificial templates for both enzyme immobilization and polyelectrolyte (PE) multilayer capsule formation.[4] This method yields microcapsules with high enzyme loading, retained enzyme activity, and notably enzyme in a "free" (non-immobilized) state. Herein we demonstrate that these enzyme loaded polyelectrolyte capsules can be used as microreactors for inorganic fermentative synthesis. As illustrated in Fig. 1, the MS spheres (diameters 2–4 μm) were exposed to urease (M_w ~ 480,000) solution, resulting in immobilization of urease on the MS spheres. Following several washing cycles to remove loosely adsorbed enzyme, the urease-loaded MS particles were alternately coated with three bilayers of poly(L-lysine) (PLL)/poly(L-glutamic acid) (PGA) *via* the layer-by-layer (LbL) self-assembly technique. After removing the porous silica template cores by exposure to a hydrofluoric acid (HF)/ammonium fluoride (NH_4F) buffer (pH 5) for 5 min, urease encapsulated hollow capsules were obtained. The presence of urease inside the polyelectrolyte (PE) capsule stimulated the catalytic decomposition of urea to form carbonate anions, which then reacted with metal cations from the surrounding solution to precipitate calcium carbonate exclusively in the capsule interior.

The MS spheres used for urease encapsulation, as shown in Fig. 2a, have a bimodal pore structure (small pores of 2–3 nm and large pores of 10–40 nm), a surface area of 630 $m^2\ g^{-1}$ and a pore volume of 1.72 mL g^{-1}.[4,5] A high magnification TEM image (Fig. 2a, inset) shows the high porosity of these spheres. Compared to MS materials with only smaller pores, the MS particles used in this study show faster immobilization rates and higher enzyme loadings, especially for large enzymes.[5] As the main driving force for enzyme adsorption onto negatively charged MS spheres is electrostatic interaction, an enzyme with a positive charge favors the adsorption process. Adsorption studies showed that adsorbing in 2.0 mg/mL urease (isoelectric point: pH 6) in pH 5.5 2-(*N*-morpholino)ethane sulfonic acid (MES) buffer solution yielded adsorbed amounts of ~ 67 mg urease g^{-1} MS spheres (or 6.7 wt%) within 72 h. The three bilayer PLL/PGA was then deposited on the enzyme loaded MS spheres in the same buffer solution. The pH value for PE coating (pH 5.5) was chosen to be similar to the urease adsorption conditions and also to the silica dissolution solution (2 M HF/8 M NH_4F, pH 5) in order to reduce possible enzyme loss during the PE coating and silica dissolution process (~ 20%). The final enzyme amount encapsulated in the $(PLL/PGA)_3$ microcapsules was calculated to be ~ 25 mg/mL capsule volume, assuming a density of MS spheres of 0.46 g cm^{-3} and no change in size of capsules during core dissolution. The high

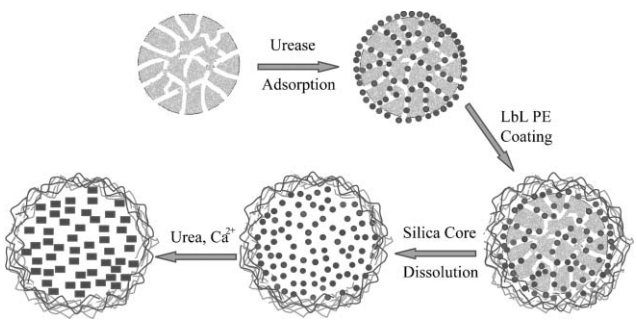

Fig. 1 Schematic representation of the procedure for encapsulating urease in polypeptide microcapsules using MS spheres as templates and their use as biomimetic reactors to form $CaCO_3$ within the capsules.

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† Electronic supplementary information (ESI) available: Details of experimental, SEM images of urease-loaded $(PLL/PGA)_3$ capsules after carbonate precipitation for 24 h and $CaCO_3$ prepared without PE capsules, XRD patterns of $CaCO_3$ prepared in different concentrations of urease. See DOI: 10.1039/b601490a

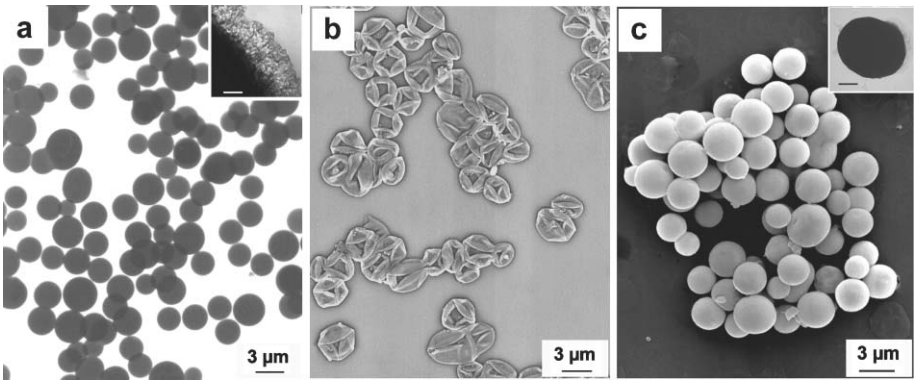

Fig. 2 Low (a) and high magnification (a, inset, scale bar 100 nm) TEM images of MS spheres. SEM images of urease-loaded $(PLL/PGA)_3$ capsules before (b) and after (c) carbonate precipitation in 0.5 M urea and 1 M $CaCl_2$ solution for 20 min. Inset of image c: TEM image of urease-loaded $(PLL/PGA)_3$ capsules after carbonate precipitation for 20 min. Scale bar: 1 μm.

enzyme loading is noteworthy as urease is a large enzyme (diameter ~ 13 nm[6]) and difficult to load into pre-formed PE hollow capsules. The activity of the encapsulated urease, as measured by a pH-sensitive dye,[7] is ~ 49% of the activity of free urease in solution. Despite the high enzyme loading, it is worth noting that the activity of the encapsulated urease is much higher than, for example, urease immobilized in halloysite nanotube (~ 17%)[3e] and urease post-infiltrated in poly(allylamine hydrochloride)/poly(styrene sulfonate) capsules (~ 13%),[8] which is mainly due to the free state of the enzyme in the polypeptide capsule provided by this method.[4]

Urease-containing polypeptide capsules were then injected into a solution of 0.5 M urea and 1 M $CaCl_2$, which had been degassed by nitrogen bubbling. In the presence of urease, urea decomposes to form CO_3^{2-} inside the polyelectrolyte capsules. The carbonate anions then react with metal cations from the surrounding solution to precipitate calcium carbonate. It is observed that after adding urease-containing PE capsules for *ca.* 10 min, the mixture solution begins to turn turbid. A white solid is seen to slowly precipitate from the solution after *ca.* 20 min reaction. Fig. 2b and 2c show typical SEM images of urease-loaded $(PLL/PGA)_3$ multilayer capsules before (b) and after carbonate precipitation in 0.5 M urea and 1 M $CaCl_2$ solution for 20 min (c). It can be seen that urease-containing PLL/PGA capsules totally collapse upon drying due to the removal of silica core. Folds and creases are clearly seen on their surface. The diameter of the microcapsules (approximately 2–4 μm) is similar to the initial diameter of the MS sphere template (Fig. 2a). After reaction in 0.5 M urea and 1 M $CaCl_2$ solution for 20 min, bulky, spherical particles with smooth surfaces are formed (Fig. 2c). The particles are well separated with diameters ranging from 2–4 μm, which is the same size as the PE capsule. TEM (Fig. 2c, inset) and SEM images of broken spheres confirmed that the particles are totally filled after 20 min reaction. It has been shown that the concentration of urea and calcium does not significantly influence the reaction rate, which mostly depends on the concentration of urease.[9] Thus the fast and uniform $CaCO_3$ precipitation process observed here is mainly due to the high enzyme loading and notable enzymatic activity within the PE capsules. It should also be noted that no $CaCO_3$ forms outside the capsules during the reaction, indicating that the PE shell can effectively prevent the leakage of the enzyme and restrict the $CaCO_3$ precipitation to only occur within the PE capsules. If the reaction time is prolonged to 24 h, the particles can slowly swell to a diameter of 15–25 μm but with broken PE shells. However, even without the protection of the capsule shell, the crystal still only grows from the particle surface as no $CaCO_3$ was observed in the solution.

Wide-angle X-ray diffraction (XRD) analysis of the resultant capsules after $CaCO_3$ synthesis (Fig. 3) revealed that the particles are mixed phases of calcite and vaterite. As calcite is the more stable polymorph of $CaCO_3$, the stable presence of vaterite phase in the final materials may be due to the high concentration of urease in the PE capsule, as high enzyme concentrations in the reaction system are found to favor and to some extent stabilize the vaterite phase.[9] This is further confirmed by our XRD analysis of calcium carbonate precipitates obtained in mixed solutions of 0.5 M urea and 1 M $CaCl_2$ containing 0.3 or 30 mg mL^{-1} free

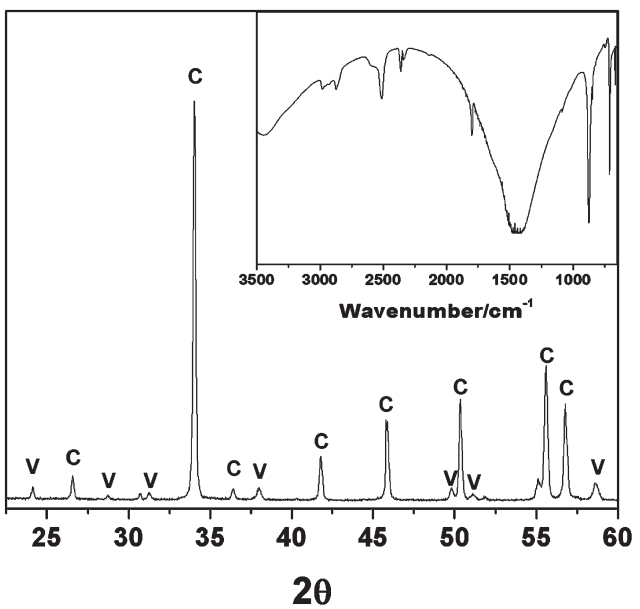

Fig. 3 X-ray diffraction pattern and FTIR spectrum (inset) of calcium carbonate precipitates obtained after reaction in 0.5 M urea and 1 M $CaCl_2$ solution for 24 h. Samples were separated from the reaction solution and washed with water by centrifugation and dried overnight under vacuum. Peaks coded as C and V, respectively, are characteristic of calcite and vaterite.

urease. The former product shows only a calcite phase but the latter shows a mixture of calcite and vaterite. This result gives further evidence of the high urease loading within the PE capsules. The FTIR spectrum (Fig. 3, inset) further confirms the presence of these two crystal phases of $CaCO_3$ (peaks at 875, 710 cm^{-1} for calcite and 730 cm^{-1} for vaterite[10]). The weak intensity seen between 2800 and 3000 cm^{-1} could be attributed to the C–H stretching vibration of urease and polypeptides remaining in the sample.

In summary, we have described an effective and novel biomimetic approach for carrying out inorganic synthesis exclusively inside biocompatible polypeptide capsules, based on the fermentative formation of the precipitative agent (CO_3^{2-}) in the capsule interior by urease-catalyzed urea hydrolysis. The final $CaCO_3$ precipitate has mixed phases of calcite and vaterite and the particle size can be controlled by the reaction time. The current method provides a promising strategy for studying crystal engineering and fundamental aspects of biomineralization processes, due to the immobilization capacity of MS materials for various materials and the broad range of MS materials available with tunable size, morphology and porosity.

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