Bio-Nanoreactors

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Urease as a Nanoreactor for Growing Crystalline ZnO Nanoshells at Room Temperature**

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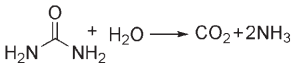
The room-temperature synthesis of oxide semiconductor materials is of prime interest for the industry, as mild manufacturing conditions will ensure low energy consumption, economical production, less manpower, and compatibility with other fabrication processes, all important factors in technological applications.[1] Oxide semiconductor nanoparticles have been synthesized at low temperature in basic aqueous media because this basic condition could catalyze condensation and dehydration of the hydroxide intermediates.[2–5] In the past, biological templates, such as DNA, peptides, and proteins, have been used to grow various metals[6] because of their potential to control pH at the molecular level.[7] However, this local pH value is sensitively changed by conformation changes occurring in these template molecules. As a consequence, it is difficult to control the reaction parameters to obtain those desired for the synthetic procedure.

Recently, enzymes were recognized as a new class of smart nanomaterials in the areas of regenerative medicine, drug delivery, and diagnostics.[8] In the work presented herein, we applied the enzyme urease as a nanoreactor for the room-temperature synthesis of ZnO nanoshells. An advantage of this biomolecular template is that the local pH around the enzyme particle can be controlled by the hydrolysis of urea (Scheme 1). Mainly, the production of ammonia by the



Scheme 1. Hydrolysis of urea catalyzed by urease.

enzyme finely tunes the local pH at the template surface such that it is adequate for the growth of oxide semiconductor (Figure 1). Urease also presents another advantage in having high affinity for metal ions, so that the particle will intercalate semiconductor precursors very efficiently.[9] Thus, these

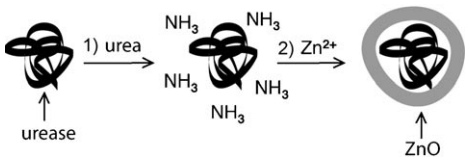


Figure 1. Synthesis of ZnO nanoshells using the enzyme urease as a catalytic template. Step 1: ammonia, generated by the hydrolysis of urea, adjusts the pH value around the enzyme. Step 2: after addition of Zn^{2+}, the growth of ZnO nanoshells is catalyzed by the local pH value at the enzyme/solution interface.

precursors are preconcentrated at the enzyme where the chemical environment is adjusted at high pH, suitable for the subsequent semiconductor growth process.

To demonstrate the proof of principle of this biomimetic room-temperature growth of oxide semiconductor materials, we mineralized ZnO as nanoshells around the enzyme nanoparticles. ZnO is a model oxide semiconductor as a wide-bandgap material, and various shapes, such as nanorings, nanobelts, nanohelices, nanowires, and nanoparticles, of ZnO were grown to yield unique electrical, optical, and catalytic properties.[10–17] Herein, we report the production of crystalline ZnO nanoshells around an enzyme core at room temperature by coupling the reaction catalyzed at the enzyme surface with a common procedure for the synthesis of oxide semiconductor materials.

When urease molecules were incubated with urea and zinc nitrate hexahydrate, ammonia was produced on the enzyme (see the Supporting Information) and ZnO shells were grown around the urease core (Figure 2a). From this TEM image, the average outer diameter of the nanoshell was 18 nm with a fairly narrow distribution (Figure 2b). A selected-area electron diffraction (SAED) pattern for the resulting nanoshells (inset in Figure 2a) shows the (102), (104), and (203) faces of ZnO. The high-resolution transmission electron microscopy (HRTEM) image in Figure 2c reveals the lattice fringe of the (102) face of ZnO, and this observation indicates that the ZnO nanoshell is highly crystalline. In this image, two ZnO nanoshells were aggregated; however, each particle maintained its core–shell structure without collapsing. This structure consists of an electro-transparent core of the protein surrounded by an electro-dense shell of ZnO. The outcome demonstrates the feasibility of the proposed methodology for the room-temperature synthesis of zinc oxide.

Based upon our hypothesis, ammonia produced by the enzyme core catalyzes the mineralization of the crystalline ZnO nanoshells. Therefore, if the enzyme is denatured, the growth of ZnO nanoshells should be inhibited, as urease can no longer exert its catalytic function and produce ammonia (see the Supporting Information). We demonstrated this

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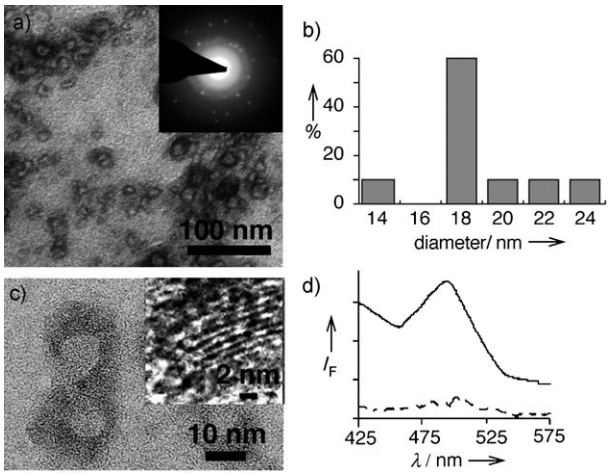

Figure 2. a) TEM image of enzyme–ZnO core–shell nanoparticles. Inset: SAED pattern of ZnO nanoshells. b) Size distribution of the outer diameter of ZnO nanoshells. c) HRTEM image of enzyme–ZnO core–shell nanoparticles. Inset: HRTEM image of ZnO nanoshell. d) Fluorescence spectra of ZnO nanoshell in (c) (——) and denatured enzyme particles in the presence of ammonia and Zn ions in Figure 3a (-----). The excitation wavelength was 390 nm.

control experiment to justify our hypothesis (Figure 3). When the enzyme was unfolded by heating the protein solution at 100 °C and then cooling to room temperature, these enzyme

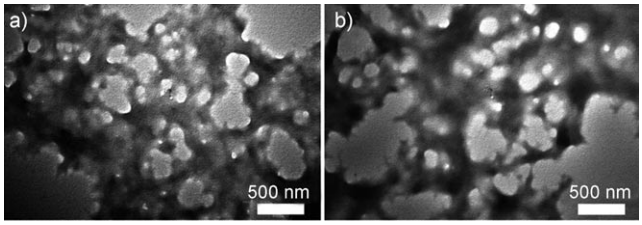

Figure 3. TEM images of a) denatured enzyme in the presence of ammonia and b) denatured enzyme in the presence of ammonia after incubation with Zn ions.

molecules were heavily aggregated (Figure 3a) and the addition of the zinc precursor did not produce ZnO, even in the presence of an external source of ammonia (Figure 3b). The absence of ZnO particles in Figure 3b was also confirmed by their fluorescence spectrum.

The ZnO nanoshells in Figure 2c showed the characteristic ZnO peak in the visible region that resulted from the presence of oxygen defects in the crystal and organic capping agents (solid line, Figure 2d).[18–20] However, the denatured enzymes in the presence of ammonia and Zn ions (Figure 3b) lack this peak centered at 547 nm, as shown by the dotted line in Figure 2d. These results support the notion that the native conformation of the proteins plays an important role in the production of ZnO nanoshells. The control experiments demonstrate the catalytic role of urease through the generation of ammonia at its surface for the synthesis of oxide semiconductor materials.

Urease has an isoelectric point of 4.9.[21] Hence, the protein has an overall negative charge at pH 9, and zinc precursors electrostatically interact with the protein. Previously reported data suggest that Zn ions are hydrolyzed to produce zinc hydroxide in basic media, and therefore it is reasonable that zinc ions are also hydrolyzed at the enzyme surface to yield zinc hydroxide intermediates.[22] The reaction requires some additional energy, such as heat, to further stimulate the dehydration of the hydroxide intermediates and yield ZnO.[3,23] Alternatively, the exothermic decomposition of an organometallic zinc precursor has been proposed as a suitable source of energy in a room-temperature synthetic process.[24]

In the case of enzymes, it has been well studied that proteins are surrounded by a layer of highly ordered and tightly bound water molecules; when certain ions bind protein surfaces, this water solvation layer is disrupted and the entropy of the system increases. This process is usually termed the "salting out" effect.[25] We hypothesize that this concomitant increase in entropy could be converted into sufficient energy for the subsequent growth of ZnO. The fact that ZnO is not produced when the reaction takes place in the presence of the unfolded enzyme supports this idea, as wrongly folded proteins have a less-developed solvation shell.[26]

In conclusion, we have demonstrated the room-temperature synthesis of ZnO nanoshell structures with urease as a catalytic template. The enzyme preconcentrates the precursors and catalyzes their conversion into ZnO by finely controlling the pH at its surface. The crystallization takes place under mild biological conditions, therefore allowing its easy implementation in the room-temperature synthesis of other oxide semiconducting materials, such as ZrO_2, SnO_2, Ga_2O_3, WO_3, IrO_2, TiO_2, and NiO, which can be grown by pH-sensitive sol–gel methods. The dimension of the ZnO nanoshells is determined by the size of the urease core, although there are catalytically active isozymes of urease available in various sizes,[27] and the selection of these isozymes will enable the production of nanoshells in different sizes. Notably, this catalytic biotemplating strategy for room-temperature synthesis could also have a significant impact on reducing energy consumption in the manufacturing process, as it will decrease the production costs, size of the facility (such as cooling systems), and manpower required if these syntheses can be conducted at low temperature.

Experimental Section

A suitable amount of urea solution was added to a solution of urease (1 mg mL^{-1}) in sodium nitrate (0.1 M). The final concentration of urea was 10 mM. After 2 min, zinc nitrate hexahydrate was added to a final concentration of 0.1 mM. The solution was left at room temperature overnight. Alternatively, the catalytic activity of the enzyme was mimicked by adding an NH_3/HNO_3 solution (pH 9) instead of the urea solution. When required, the enzyme was unfolded by heating at 100 °C for 15 min. Samples for TEM imaging were centrifuged for 30 min to concentrate the ZnO nanostructures. After removing most of the supernatant, a TEM grid was immersed in the resulting ZnO-enriched solution. The size distribution of the ZnO nanoshells was determined by measuring 30 samples in a series of TEM images. An Au substrate was used as a standard to assign the lattice indices for

ZnO in SAED. After 2 h the grids were washed with deionized water and the excess liquid was removed with the aid of a filter paper. Photoluminescence spectra were obtained with a Fluorolog fluorometer.

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