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Enzyme Mediated Extracellular Synthesis of CdS Nanoparticles by the Fungus, *Fusarium oxysporum*

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Developing reliable protocols for the synthesis of nanometer scale semiconductor particles is a problem of great importance,[1,2] resulting in researchers seeking inspiration from biological systems where biominerals are routinely synthesized.[3,4] Both prokaryotic (bacteria)[5,6] and eukaryotic organisms such as yeast[7] have been found to produce semiconductor nanoparticles within the cell wall of the microorganisms. While enzymatic processes in sulfate reducing bacteria are relatively well understood[8] and identified in the formation of biofilms of sphalerite (ZnS)[5] and CdS,[6] the intracellular synthesis of CdS in yeast[7,8] occurs by a process involving sequestering of the Cd^{2+} ions by glutathione-related peptides and a consequent production of CdS within the yeast cells.[9] We report here our discovery that eukaryotic organisms such as fungi may be used to synthesize CdS and some other metal sulfide nanoparticles *extracellularly* by a purely enzymatic process. The use of specific enzymes such as reductases secreted by fungi opens up the exciting possibility of designing a rational biosynthesis strategy for nanomaterials of different chemical compositions.

Microorganisms, particularly prokaryotic bacteria, are often exposed to extreme environmental conditions, forcing them to resort to specific defense mechanisms to quell such stresses, including the toxicity of foreign metal ions or metals. The toxicity of metal ions is reduced or eliminated by changing the redox state of the metal ions and/or precipitation of the metals intracellularly,[10] thus forming the basis of many important applications of microorganisms such as bioleaching, bioremediation, microbial corrosion, as well as the synthesis of nanoparticles.[7,11] In a radical departure from the predominantly bacteria-based methods for the synthesis of inorganic nanoparticles, we have investigated the use of eukaryotic organisms such as fungi in nanomaterials synthesis and have demonstrated that the reaction of metal ions with *Verticillium* results in the intracellular synthesis of metal nanoparticles such as Au[12] and Ag.[13] Recognizing that such biotransformation-based protocols would better serve potential applications if nanoparticle synthesis could be accomplished extracellularly, we have recently identified the fungus *Fusarium oxysporum* for the extracellular synthesis of gold nanoparticles.[14] In this communication, we report our surprising discovery that this fungus may also be used to synthesize technologically important semiconductor CdS nanoparticles *extracellularly* by a purely enzymatic process. More specifically, *Fusarium oxysporum*, when exposed to aqueous Cd^{2+} and SO_4^{2-} ions, leads to the formation of extremely stable CdS nanoparticles in solution. To the best of our knowledge, this is the first report on the extracellular secretion of sulfate reductases by any microorganism including fungi and harnessing it in the synthesis of stable metal sulfide nanoparticles in aqueous solution.

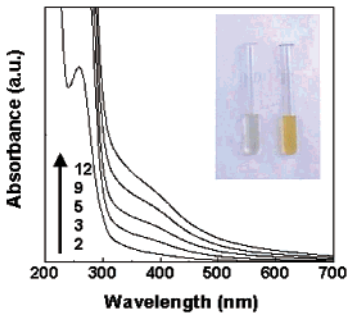

Figure 1. UV−vis spectra recorded from the aqueous 10^{-3} M $CdSO_4$ solution as a function of time (in days) of addition of the fungal biomass. The inset shows test tubes containing $CdSO_4$ solution before (test tube on the left) and after reaction with the fungal biomass for 12 days (test tube on the right).

The inset of Figure 1 shows test tubes of the $CdSO_4$ reaction solution at the beginning (time $t = 0$, test tube on the left) and after 12 days of reaction (test tube on the right) with the *Fusarium oxysporum* biomass. The bright yellow color of the solution after reaction indicates the presence of CdS nanoparticles in solution (experimental details in Supporting Information, S1). UV−vis spectra (Figure 1) recorded from the *Fusarium oxysporum*−$CdSO_4$ solution reaction medium at different time intervals (in days) exhibit the appearance of a weak absorption edge, which progressively increases in intensity as the reaction progresses. The presence of the absorption edge at ca. 450 nm is characteristic of CdS particles in the quantum size regime.[15] The colloidal solution of CdS nanoparticles was extremely stable in time with no evidence for aggregation even after 1 month of storage. The presence of an absorption at ca. 280 nm (Figure 1) attests to the presence of proteins in the reaction medium.[16] We believe the long-term stability of the CdS nanoparticle solution is due to the presence of the proteins in the nanoparticle solution that bind to the surface of the nanoparticles and prevent aggregation.

A bright field transmission electron micrograph (TEM) obtained from a drop-coated film of CdS nanoparticles formed by reaction of $CdSO_4$ with *Fusarium oxysporum* for 12 days is shown in Figure 2A. Well-dispersed, individual CdS particles in the size range 5−20 nm are clearly observed in this image. Figure 2B shows the X-ray diffraction (XRD) pattern recorded from a CdS nanoparticle film deposited on a Si(111) substrate. Prominent Bragg reflections corresponding to hexagonal CdS have been identified in the figure.[17,18]

The presence of proteins in solution (absorption at 280 nm, Figure 1) suggested a possible sulfate reducing enzyme-based process for

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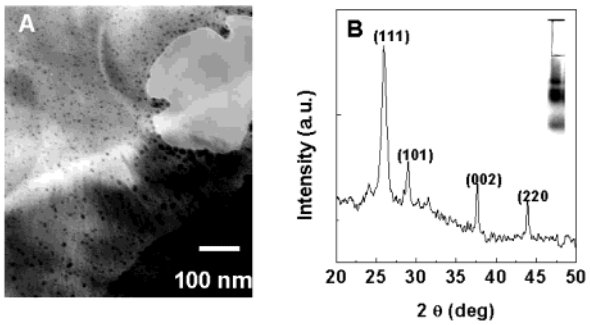

Figure 2. (A) Bright field TEM picture of CdS nanoparticles formed by reaction of $CdSO_4$ with the fungal biomass for 12 days. (B) XRD pattern recorded from the CdS nanoparticle film deposited on a Si(111) wafer. The inset shows the native gel electrophoresis of aqueous protein extract obtained from *Fusarium oxysporum* mycelia; 10% (w/v) polyacrylamide slab gel, pH 4.3.

the synthesis of CdS nanoparticles starting from Cd^{2+} and SO_4^{2-} ions. Accordingly, control experiments were performed wherein the *Fusarium oxysporum* biomass was reacted with (i) a 10^{-3} M aqueous solution of $CdNO_3$ and (ii) an aqueous solution containing a mixture of the salts $CdCl_2$ and Na_2SO_4 (1:1 Cd^{2+}:SO_4^{2-} concentration in solution = 10^{-3} M). CdS nanoparticles were observed to form in the latter case after just 4 days of reaction, while in the former case, there was no evidence of nanoparticle formation even after 21 days of reaction. To rule out the possibility of entrapment of the Cd^{2+} ions in the fungal mass followed by reaction with sulfide ions and then release of the nanoparticles into solution, another control experiment was performed wherein the fungal biomass was immersed in sterile water for 12 days, the reaction mixture was filtered, and the filtrate was reacted with 10^{-3} M $CdSO_4$ solution. It was observed that CdS nanoparticles formed readily within 6 days of reaction and resulted in a bright yellow solution similar to that shown in the inset of Figure 1. From the above experiments, it is clear that *Fusarium oxysporum* releases reductase enzymes into solution that are responsible for the formation of CdS nanoparticles from Cd^{2+} and SO_4^{2-} ions. While such processes are known in sulfate reducing bacteria (which are strictly anaerobic),[5,6] to the best of our knowledge, this is the first report on the secretion of sulfate reducing enzymes by a fungus.

The aqueous solution exposed to the fungal biomass for 12 days was analyzed for its protein content. The inset of Figure 2B shows the polyacrylamide gel electrophoresis (PAGE) results of the aqueous extract carried out at pH 4.3 (S1). The electrophoresis measurements indicate the presence of at least four different protein bands. Addition of an aliquot of the aqueous protein extract after dialysis to remove small molecular weight compounds (using a dialysis bag of 3 K molecular weight cutoff) to an aqueous solution of $CdSO_4$ did not result in the formation of CdS nanoparticles. However, addition of ATP and NADH to the protein dialysate restores the CdS nanoparticle formation ability of the protein solution either by direct reaction with $CdSO_4$ solution or by an aqueous solution consisting of a mixture of $CdCl_2$ and Na_2SO_4. The slow reduction of sulfate salts in comparison with sulfite salts indicates that the rate-limiting sulfate activation step is responsible for this slow rate of conversion.

The formation of extracellular CdS nanoparticles by enzymatic reduction of sulfate ions by *Fusarium oxysporum* was extended to the formation of PbS, ZnS, and MoS_2 nanoparticles starting with appropriate sulfate-containing salts. Preliminary investigations indicate that it is indeed possible to realize such chemical compositions by fungi-based extracellular biotransformations. Another important potential benefit of the process described herein is the fact that the semiconductor nanoparticles, which are quite stable in solution, are synthesized extracellularly, and this is thus a very important advantage over other biosynthetic methods where the nanoparticles are entrapped within the cell matrix.[5−9,11−13] The extracellular synthesis of semiconductor nanoparticles makes it possible to harness and immobilize/deposit such nanoparticles onto desired solid surfaces for different practical purposes with ease.

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Supporting Information Available: Experimental details of the biosynthesis of CdS nanoparticles using *Fusarium oxysporum* (S1) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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