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Biosynthesis of cadmium sulfide nanoparticles by photosynthetic bacteria *Rhodopseudomonas palustris*

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ABSTRACT

A simple route for the synthesis of cadmium sulfide nanoparticles by photosynthetic bacteria *Rhodopseudomonas palustris* has been demonstrated in this work. The cadmium sulfate solution incubated with *R. palustris* biomass changed to a yellow color from 48 h onward, indicating the formation of cadmium sulfide nanoparticles. The purified solution yielded the maximum absorbance peak at 425 nm due to CdS particles in the quantum size regime. Also, X-ray analysis of the purified nanoparticles confirmed the formation of cadmium sulfide. Transmission electron microscopic analysis of the samples showed a uniform distribution of nanoparticles, having an average size of 8.01 ± 0.25 nm, and its corresponding electron diffraction pattern confirmed the face-centered cubic (fcc) crystalline structure of cadmium sulfide. Furthermore, it was observed that the cysteine desulfhydrase producing S^{2-} in the *R. palustris* was located in cytoplasm, and the content of cysteine desulfhydrase depending on the growth phase of cells was responsible for the formation of CdS nanocrystal, while protein secreted by the *R. palustris* stabilized the cadmium sulfide nanoparticles. In addition, *R. palustris* was able to efficiently transport CdS nanoparticles out of the cell.

1. Introduction

The optoelectronic and physicochemical properties of nonmaterials are size and shape dependent. So the synthesis of cadmium sulfide nanoparticles of different sizes and shapes is of great importance for their applications in optical devices, electronics and biotechnologies [1,2]. Living organisms have the endogenous ability to exquisitely regulate synthesis of inorganic materials such as amorphous silica (diatoms), magnetite (magnetotactic bacteria), gypsum, and calcium carbonate layers (S-layer bacteria) and minerals such as calcite into functional superstructures [3]. Because of this ability to precisely direct the shape and crystallinity of a developing inorganic material, there is great interest in exploiting living organisms such as bacteria and fungi for inorganic materials synthesis.

An earlier study found that *Clostridium thermoaceticum* could precipitate CdS at the cell surface as well as in the medium from $CdCl_2$ in the presence of cysteine hydrochloride in the growth medium. Most probably, cysteine acts as the source of sulfide [4]. *Klebsiella pneumoniae* exposed to Cd^{2+} ions in the growth medium were found to form 20–200 nm CdS on the cell surface [5]. Intracellular CdS nanocrystals, composed of a wurtzite crystal phase, are formed when *Escherichia coli* is incubated with cadmium chloride and sodium sulfide. Nanocrystal formation varies dramatically depending on the growth phase of the cells and increases about 20-fold in *E. coli* grown in the stationary phase as compared with that grown in the late logarithmic phase [6].

It has long been recognized that among the eukaryotes, yeasts are explored mostly in the biosynthesis of the semiconductor nanoparticles. Exposure of *Candida glabrata* to Cd^{2+} ions leads to the intracellular formation of CdS quantum dots [7]. Kowshik et al. have shown that CdS quantum dots synthesized intracellularly in *Schizosaccharomyces pombe* yeast cells exhibit ideal diode characteristics. Biogenic CdS nanoparticles in the size range 1–1.5 nm have been used in the fabrication of a heterojunction with poly (p-phenylenevinylene) [8]. Even more exciting is the finding that the exposure of *F. oxysporum* to the aqueous $CdSO_4$ solution yields CdS quantum dots extracellularly [9]. The particles are reasonably monodisperse and range in size from 5 to 20 nm. Reaction of the fungal biomass with the aqueous $CdNO_3$ solution for an extended period of time does not yield CdS nanoparticles, indicating the possibility of the release of a sulfate reductase enzyme into the solution. However, the intracellular biosynthesis of CdS nanoparticles and then effluence from cells is still scarce.

Phototrophic bacteria are ubiquitous in fresh and marine water, soil, wastewater, and activated sludge. They are metabolically

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the most versatile among all procaryotes: anaerobically photoautotrophic and photoheterotrophic in the light and aerobically chemoheterotrophic in the dark, so they can use a broad range of organic compounds as carbon and energy sources [10]. In this study, phototrophic bacteria *Rhodopseudomonas palustris*, a typical purple non-sulfur bacterium, have been chosen to synthesize CdS nanocrystals at room temperature through a single step process.

2. Materials and methods

2.1. Materials

Photosynthetic bacteria *R. palustris* were cultured in the medium containing purvate, yeast extract, NaCl, NH_4Cl and K_2HPO_4 at pH 7 and 30 °C. Cells were harvested at a various time points by centrifugation (4000 × *g*) at 4 °C for 10 min. After a minimum of 42 h of growth, the cells were no longer dividing, and the optical density (OD) at 650 nm was approximately 2.0. The culture was considered to be in stationary phase. After about 36 h of growth, the culture was close to but did not yet reach saturation, and the $OD_{650\,nm}$ was around 1.6. These cells were considered to be in late logarithmic phase. Mid-logarithmic phase cells were harvested after 24 h of growth when the cells were dividing exponentially, and the $OD_{600\,nm}$ was about 0.8. The collected bacteria were washed five times with distilled water to obtain about 1 g wet weight of cells and then resuspended in phosphate-buffered saline (PBS) (10 mM phosphate (pH 7.2), 0.8% NaCl) containing 1 mM $CdSO_4$. The solutions were incubated on an orbital shaker at 30 °C for 72 h and agitated at 150 rpm. At the same time, the possible synthesis of cadmium sulfide nanoparticles by the growth media without the cells of bacteria present was also investigated.

2.2. Characterization

The solution was centrifuged at 4000 × *g* for 20 min after CdS nanoparticles were synthesized. The biomass pellet was discarded and the medium without cells was centrifuged at 15,000 × *g* at 4 °C for 60 min. The supernatant was discarded, and the pellet with the CdS-containing particles was washed in deionized water three times. The purified CdS nanoparticles were redispersed in water by ultrasonication and were kept at room temperature (30 °C) for two months. The UV–vis spectra of the solution were recorded on a Shimadzu spectrophotometer (model UV-2101PC) from 200 to 800 nm. Deionized water was used as the blank. The sample obtained from stationary phase stationary for transmission electron microscopy (TEM), high-resolution transmission electron microscopy (HRTEM) and selected area electron diffraction (SAED) were prepared by drop coating onto a carbon-coated copper grid. TEM was performed on a Hitachi H-600 instrument operated at an accelerating voltage of 120 kV while HRTEM and SAED were performed on a Hitachi H-2010 instrument operated at a lattice image resolution of 0.14 nm. The purified CdS nanoparticles were used for powder X-ray diffraction (XRD) analysis. The spectra were recorded on a Rigaku Dmax-γA automatic instrument. The diffracted intensities were recorded from 10° to 70° 2θ angles. For FTIR, the purified CdS nanoparticles diluted with potassium bromide (KBr) at a ratio of 1:100 and the spectra was recorded with a Shimadzu FTIR-8300 Spectrum in the range 4000–400 cm^{-1}.

2.3. Cysteine desulfhydrase assay

Cysteine desulfhydrase activity of the cell was measured using a colorimetric assay adapted from Chu et al. [11]. Total protein was measured by the method of Chen et al. [12].

Zymogram was performed to detect cysteine desulfhydrase activity as previously described [13,14]. Unboiled enzyme samples were applied to a non-denaturing protein gel (10% Tris–glycine gel). After electrophoresis, the gel was washed twice for 10 min in 50 ml of Tris–Cl (0.05 mol/l, pH 7.4). The gel was then incubated at 37 °C for 4–8 h in the following solution: 0.05 mol/l Tris–Cl (pH 7.4), 0.01 mol/l $MgCl_2$, 0.01 mol/l L-cysteine, 5×10^{-4} mol/l $Pb(NO_3)_2$, 5×10^{-3} mol/l DTT and 4×10^{-4} mol/l PLP. H_2S formed during the enzymatic reaction precipitated as insoluble PbS. Cysteine desulfhydrase activity was therefore detected by precipitated PbS.

2.4. Subcellular fractionation

Subcellular fractionation was obtained by the method of Kumar and Upretil [15]. In the stationary phase, the cells grown in presence of 1 mM cadmium were harvested by centrifugation (15,000 × *g*, 25 min, 4 °C). The supernatant were extracellular liquid. The pellet was washed twice with 0.03 mol/l Tris buffer containing 2.5×10^{-3} mol/l EDTA, pH 8.0, and was resuspended in the same buffer. To prepare spheroplasts, lysozyme was added to a final concentration of 200 mg/ml and cells were incubated for 30 min at 25 °C. All subsequent steps were carried out at 0–4 °C. Spheroplasts were collected by centrifugation at 15,000 × *g* for 15 min and were resuspended in 0.03 mol/l Tris buffer containing 3×10^{-3} mol/l EDTA, pH 8.1. The obtained supernatant was the periplasmic fluid consisting of a peptidoglycan layer. Then the spheroplasts were then disrupted by four 15 s bursts with the vibronic ultrasonic processor and were centrifuged at 2000 × *g* for 10 min to remove debris and unbroken cells. The resulting supernatant consisting of membrane and cytoplasm fractions was centrifuged at 50,000 × *g* for 60 min. The pellet consisting of both outer and inner membrane envelopes, now being termed crude membrane fraction, was washed twice and was resuspended in a suitable buffer system for enzyme assays. The final supernatant was termed the cytoplasm fraction. For the blank, deionized water was substituted for subcellular fractionation.

2.5. Different forms of cadmium separated by different centrifugation speed

Nanocrystal formation was initiated by adding $CdSO_4$ (1 mM) to a cell sample (about 1 g wet weight) suspended in phosphate-buffered saline (PBS) (10 mM phosphate (pH 7.2), 0.8% NaCl). The solutions were incubated on an orbital shaker at 30 °C and agitated at 150 rpm. Samples were taken at predefined time intervals (0, 12, 24, 36, 42 and 48 h). The sample was centrifuged at 4000 × *g* for 20 min. The biomass pellet (P_1) was collected and the medium without cells was centrifuged at 15,000 × *g* at 4 °C for 60 min. The supernatant (S_1) was collected, and the pellet (P_2) with the CdS-containing particles was washed in deionized water three times. Each experiment was repeated three times. The contents of cadmium in different forms of P_1, S_1 and P_2 were determined using Shimadzu AA-6300 atomic absorption spectrophotometer in an air–acetylene flame at 228.8 nm wavelength [16].

2.6. Sucrose gradient centrifugation

Sucrose gradient centrifugation was operated by the method of Kessi et al. [17]. Polyallomer tubes (20 ml; Beckman L8-8, America) were filled with four layers of sucrose as follows: 3.5 ml of 2.5 M sucrose, 5.0 ml of 2.0 M sucrose, 3.0 ml of 1.5 M sucrose, and 3.0 ml of 0.1 M sucrose. A 2-ml culture sample was overlaid, and centrifugation was performed at 20 °C and 50,000 × *g* for 2 h. Samples from cultures containing 1 mM $CdSO_4$ were concentrated twice before centrifugation. Fractions (0.5 ml) were collected from the tubes manually from the top by using a precision pipette.