



Biosynthesis of CdS nanoparticles: A fluorescent sensor for sulfate-reducing bacteria detection



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ABSTRACT

CdS nanoparticles were synthesized with an environmentally friendly method by taking advantage of the characteristic metabolic process of sulfate-reducing bacteria (SRB), and used as fluorescence labels for SRB detection. The presence of CdS nanoparticles was observed within and immediately surrounded bacterial cells, indicating CdS nanoparticles were synthesized both intracellularly and extracellularly. Moreover, fluorescent properties of microbial synthesized CdS nanoparticles were evaluated for SRB detection, and a linear relationship between fluorescence intensity and the logarithm of bacterial concentration was obtained in the range of from 1.0×10^2 to 1.0×10^7 cfu mL⁻¹. The proposed SRB detection method avoided the use of biological bio-recognition elements which are easy to lose their specific recognizing abilities, and the bacterial detection time was greatly shortened compared with the widely used MPN method which would take up to 15 days to accomplish the detection process.

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1. Introduction

CdS is an important II–VI group semiconductor with a wide band gap energy of 2.42 eV at room temperature, it has been extensively studied for its prominent optical and optoelectronic properties. Band gap energy of CdS nanoparticles can be obviously changed by varying their morphologies and particle sizes [1], so CdS nanoparticles with varied optical and optoelectronic properties have been synthesized by tuning their morphologies, and particle sizes, and CdS nanoparticles hold great application potential in displays [2], photodiodes [3], electronics [4,5], solar cells [6] and sensors [7,8].

Various approaches were developed for synthesis of CdS nanoparticles, including hydrothermal or solvothermal methods, sol–gel techniques, template methods, microwave approaches, and chemical vapor deposition techniques [9–11]. In most cases, these methods require high energy consumption to initiate the reaction, and the toxicity and potential negative biological effects of the nanoparticles cannot be ignored when they were used *in vivo*. Thus, it is necessary to develop non-toxic, biocompatible and eco-friendly methods for the synthesis of CdS nanoparticles. A promising approach to achieve this objective is to exploit and use natural sources like biological systems, since biosynthesis of

nanoparticles have been regarded cost-effective, sustainable and environmentally friendly. In the past several years, nontoxic metallic nanoparticles including Au, Ag, Pt, FeS, CdS, ZnS, PbS, Fe₃O₄, UO₂, and Co₃O₄ nanoparticles have been produced by plants, algae, fungi, bacteria, and viruses [12–14].

Sulfate-reducing bacteria (SRB) are a large group of anaerobic microorganisms that obtain their growth energy by reducing sulfate to sulfide [15], they are widely distributed in anoxic environments, such as oilfield waters and offshore sediments. Since one of the metabolic products, sulfide, is corrosive, toxic and reactive [16,17], SRB has been known as one of the key microorganisms in microbiological induced corrosion (MIC), and the presence of SRB can lead to environmental and industrial problems [18,19]. On the other hand, this pathogen was very useful in waste water treatment, numerous reports have reported the possibility of using SRB for sulfate and heavy metals removal [20,21]. Hence, selective and sensitive detection of SRB is essential for corrosion analysis and environmental monitoring.

In SRB cells, the anaerobic reduction of sulfate was initially activated by an ATP sulphurylase, the resulting adenosine-phosphosulphate (APS) complex was then reduced to sulfite with ferredoxin or NADH, and finally sulfide was formed by reduction of sulfite with the help of assimilatory or dissimilatory sulfite reductase [15]. Thus, the bacteria could provide the sulfide source when they are employed in synthesis of CdS nanoparticles.

In this paper, we developed a novel method for microbial synthesis of CdS nanoparticles by SRB. The synthesized CdS

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nanoparticles were characterized with TEM, SAED, and fluorescent spectrum. Then, CdS nanoparticles were used as a powerful fluorescent label for SRB detection, and the fluorescence intensity of CdS nanoparticles formed in SRB culture solution exhibited a linear response with the logarithm of bacterial concentration in the range of 1.0×10^2 – 1.0×10^7 cfu mL⁻¹. The proposed SRB detection method could avoid the use of biological recognition elements, which are expensive and easy to lose specific recognizing abilities. In addition, compared with the widely used MPN method which would take up to 15 days to accomplish the detection process, the proposed method based on microbial synthesized CdS nanoparticles could shorten the detection time greatly.

2. Experimental

2.1. Chemicals

All chemicals were analytical grade and purchased from Sino-pharm Chemical Reagent Co., Ltd. Magnesium sulfate, ammonium chloride, sodium sulfate, dipotassium hydrogen phosphate, calcium chloride, sodium hydroxide, sodium lactate and yeast extract were used to prepare the modified Postgate's culture medium. Milli-Q water (Millipore, USA) was used throughout.

2.2. Bacterial cultivation

Seed SRB bacteria, *Desulforibrio caledoniensis*, were isolated from marine mud collected from the Bohai Sea, China. The bacterial cultivation and enumeration methods have been reported in our previous work [22,23]. Bacterial cells were harvested through centrifugation (8000 rpm, 5 min) and then rinsed twice with 0.2 M PBS (pH 7.4). The bacteria, *pseudomonas aeruginosa*, *escherichia coli*, *staphylococcus aureus*, and *vibrio alginolyticus* were used as control microorganisms.

2.3. Microbial synthesis of fluorescent CdS nanoparticles

Firstly, SRB cells were centrifuged (8000 rpm, 5 min) and rinsed with PBS thrice. Then, bacterial cells were dispersed in 2 mL modified Postgate's culture containing 200 μ M Cd²⁺ and cultivated anaerobically for 2 days at 30 °C. After cultivation, the culture solution was centrifuged at 12,000 rpm for 5 min, and the supernatant was discarded. A mixture of SRB cells and CdS nanoparticles was obtained by washing the insoluble residues with

PBS thrice.

The obtained mixture was dispersed in 1 mL PBS (50 mM, pH 7.4) containing 0.3% dodecyl sodium sulfate (DSS) and 10 μ g mL⁻¹ proteinase K, and the mixture solution was incubated overnight at 40 °C. DSS and proteinase K were used to digest SRB cells. After incubation, purified CdS nanoparticles were obtained by centrifuging the reaction solution at 12000 rpm for 5 min and washing with 50 mM PBS.

2.4. SRB detection with fluorescent CdS nanoparticles

A series of SRB suspensions with concentrations from 10⁰ to 10⁸ cfu mL⁻¹ were prepared by serial dilution and washed by centrifugation, and then bacterial cells were cultivated in modified Postgate's culture containing 200 μ M Cd²⁺. Mixtures of SRB cells and CdS nanoparticles were obtained as described in Section 2.3 and dispersed in PBS (50 mM, pH 7.4) for fluorescence measurement.

Fluorescence spectra were recorded on a Hitachi F-4500 fluorescence spectrophotometer with an excitation wavelength at 440 nm. Fluorescence emission spectra range was recorded from 500 nm to 675 nm. Excitation and emission slits were both set as 5 nm. Each experiment was performed in triplicate, and the means of the results were presented with the standard deviations.

3. Results and discussion

3.1. Characterization of microbial synthesized CdS nanoparticles

Microbial synthesis of CdS nanoparticles was highly related with the sulfide metabolic process in SRB cells. In order to better understand the CdS formation process, the mixtures of SRB cells and CdS nanoparticles were characterized by TEM. As shown in Fig. 1A and B, SRB cellular structures were maintained integrally after a series of pretreatments, and CdS nanoparticles were uniformly distributed around bacterial cells in the region with a diameter of about 1 μ m. These results indicated that CdS synthesis process was extracellularly and highly related to SRB metabolic process.

However, these results could not definitely show whether the synthesis process was intracellularly related. In order to classify this issue, ultrathin sections of samples were prepared for TEM observation by cutting the resin embedded mixture of SRB cells and CdS nanoparticles. The results were displayed in Fig. 2A. In

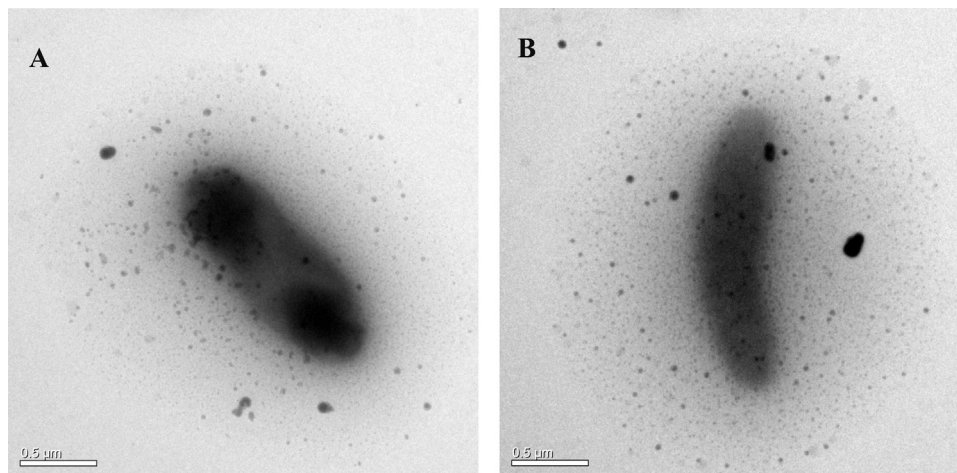


Fig. 1. TEM images of the mixture of SRB cells and CdS nanoparticles (A) and (B). The sample was acquired by cultivation SRB of 1.0×10^7 cfu mL⁻¹ in Cd²⁺ containing Postgate's culture for 2 days.

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