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A sustainable biogenic route to synthesize quantum dots with tunable fluorescence properties for live cell imaging

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ABSTRACT

Quantum dots (QDs), owning to their unique optical and electronic properties, are increasingly used for bio-imaging/biosensing in medical and energy fields. Biosynthesis of QDs offers several advantages over the conventional chemical synthesis route, such as low cost, environmental-benignity, and no need for further modification of the products. However, information about the regulation and mechanism of synthesis of QDs with great fluorescence properties is still limited. In this work, we prepared cadmium-selenium (CdSe) QDs in vivo using *Candida utilis* WSH02-08. We regulated the fluorescence properties of the bio-QDs through different precursor concentrations, and found that the bio-QDs with high fluorescence intensity and long photostable lifetime were favoured at an elevated Cd content over Se. The synthesis of the high-quality bio-QDs was attributed to the less formation of Se(0) and the increased formation of CdSe and CdS. Furthermore, such synthesized QDs were directly used in live-cell imaging without further modification. These findings are expected to enable a further design of biosynthesized QDs with great fluorescence properties and expand our understanding of the regulation mechanism of nanoparticles fabrication in microorganisms.

1. Introduction

Quantum dots (QDs) are attractive luminescent materials with broad applications in bioimaging [1], biosensing [2–4], or manufacture of light-emitting diodes [5]. They exhibit superior fluorescence properties over the conventional organic dyes in terms of fluorescence intensity, stability and tenability [6,7]. However, the conventional chemical route for QDs synthesis usually involves toxic reagents, intensive energy input and extreme reaction conditions [8,9]. Moreover, such chemical-synthesized QDs are not suitable for direct biological applications because of their poor solubility and biocompatibility. Thus, complicated surface modifications are usually needed [10,11].

Biosynthesis of QDs with microorganisms such as bacteria and fungi offers a promising alternative to overcome these limitations [12,13]. To date, an increasing number of in vivo biosynthesized QDs (bio-QDs) with excellent hydrophilicity and inherent biocompatibility by various types of microorganisms have been reported [14–17]. However, it is still challenging to produce high-quality luminescent nanoparticles for practical bioimaging/biomedical applications. Among the fluorescence properties of bio-QDs, several aspects are of particular interests for practical bioimaging/biomedical applications: fluorescence intensity and photostablility [18]. High photostablility means a slow photobleaching and a long photostable lifetime, which is an important feature of QDs required for bioimaging and biomonitoring applications [19,20].

To enhance the production of QDs in *Saccharomyces cerevisiae*, a strategy that glutathione (GSH)-based Se (IV) reduction was coupled with intracellular Cd(II) detoxification in time and space has been proposed by Pang et al. [21]. They successfully prepared CdSe QDs with a relatively high fluorescence intensity using genetically engineered *S. cerevisiae* cells [15]. Adding strong chemical reducer sodium boronhydride stimulated the extracellular synthesis of CdTe QDs by *S. cerevisiae* [22]. Effective regulation of fluorescence properties will facilitate the applications of QDs. Changing dosage and dosing time of precursors is a very important strategy to the tunable synthesis of nanomaterials in a chemical routine. Correlating the synthesis parameters with the composition and function of products could undoubtedly enable a better understanding of

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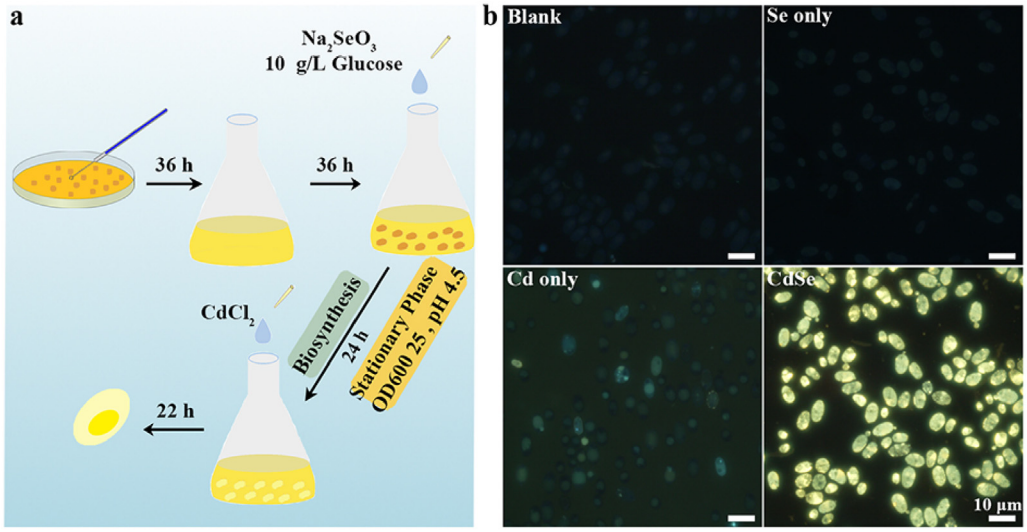

Fig. 1. Biosynthesis of QDs by *C. utilis* WSH02-08. a, Diagram of bio-QDs synthesis process by *C. utilis* WSH02-08. b, Fluorescence images in negative control groups (Blank, 1 mM Na_2SeO_3 only, 6 mM $CdCl_2$ only) and synthesized group (treated with 1 mM Na_2SeO_3 and 6 mM $CdCl_2$).

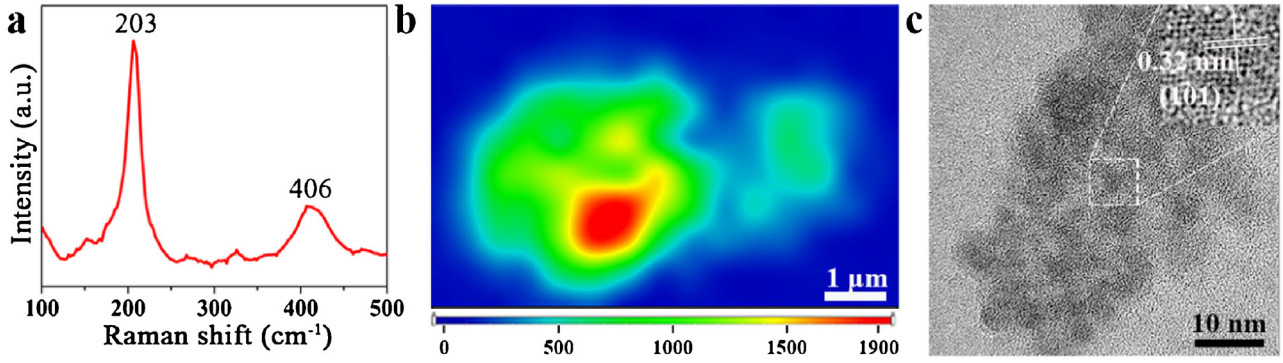

Fig. 2. Characteristics of the bio-QDs synthesized by *C. utilis* WSH02-08 with 1 mM Na_2SeO_3 and 6 mM $CdCl_2$. a, *In situ* micro-Raman spectrum of the cells (excited at 532 nm) shows CdSe characteristic peaks at 203 cm^{-1} and 406 cm^{-1}. b, Raman mapping of cells at 203 cm^{-1} (the characteristic peaks of CdSe) reveals the location of CdSe inside cells. c, High-resolution TEM image of the purified QDs, insets show the nanocrystals with lattice planes of 0.32 nm spacing.

the synthesis mechanism. Inspired by this strategy in chemical synthesis, we adopted the methodology of changing precursor concentration into the biosynthesis of QDs.

According to previous reports about bio-assembled QDs, GSH is found to play critical roles in Se reduction and Cd chelation procedures [14,15,17]. Thus, *Candida utilities* [23], the organism that is able to produce GSH at an industrial scale could be a model microorganism to fabricate QDs. In this work, we applied *C. utilis* WSH02-08 [24], to synthesize cadmium-selenium (CdSe) QDs, and evaluated the fluorescence intensity and photostability of the in vivo synthesized CdSe bio-QDs in WSH02-08. The feasibility of these bio-QDs for live cell imaging was investigated. Furthermore, we developed a simple and facile approach to tune the fluorescence properties of CdSe bio-QDs synthesized by *C. utilis* WSH02-08 through adjusting the precursor compositions.

2. Materials and methods

2.1. Culture

C. utilis WSH02-08 strain was obtained from Southern Yangtze University, China [23]. The medium for pre-culture contained (per liter): 30 g glucose, 14.7 g sodium citrate dehydrate, 10 g ammonium sulfate, 6 g yeast extract, 4.5 g potassium dihydrogen phosphate, 0.75 g magnesium sulfate. The culture was inoculated in flask at pH 5.5 and 30 °C and shaken at 200 rpm.

2.2. Biosynthesis of CdSe QDs in cells

C. utilis WSH02-08 strain was cultured into the medium and grown for 36 h. The activated strain was tenfold diluted with fresh medium and inoculated in a 250 mL erlenmeyer flask for 36 h and grown to its stationary phase. The biomass was controlled by measuring the optical density at 600 nm (OD 600) and pH was set at 4.5 before the Se and Cd treatment. Then, sodium selenite and glucose (10 g/L) were added, after 24 h incubation, the cadmium chloride was added and the mixture was placed in the incubator for 22 h. The biosynthesis process were conducted at 30 °C under 200 rpm sharking. The WSH02-08 cells were collected by centrifugation (6000*g*, 4 °C, 5 min) and washed two times with 10 mM Tris-Cl (pH = 7.6).

2.3. Fluorescence microscopic observation

An aliquot of the fluorescent strain was loaded on a glass slide. Images were taken by a wide field fluorescent microscope (BX-51, Olympus Co., Japan) under 120 W mercury lamp (X-Cite 120 Q) irradiation. The lamp was equipped with a wideband MWU2 filter (Ex 330–385 nm) and a water immersion objective (100×). Images were recorded under the same irradiation intensity by the