



The interactions between CdSe quantum dots and yeast *Saccharomyces cerevisiae*: Adhesion of quantum dots to the cell surface and the protection effect of ZnS shell



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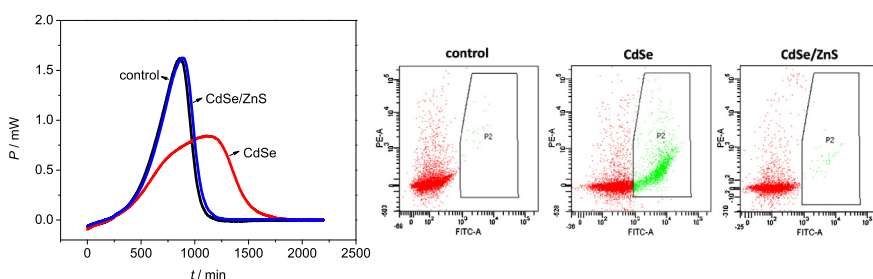
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HIGHLIGHTS

- Detailed comparison of the biological effect between CdSe QDs and CdSe/ZnS QDs on yeast *Saccharomyces cerevisiae*.
- Carboxyl-QDs adhere to the surface of *S. cerevisiae*.
- Mechanism of toxicity induced by heavy metal ion was investigated.
- Epitaxial coating of ZnS shell efficiently reduces the toxicity of Cd-containing QDs.

GRAPHICAL ABSTRACT

The biological effects of CdSe and CdSe/ZnS QDs (ZnS: protection shell) on yeast *Saccharomyces cerevisiae* were investigated via microcalorimetric, spectroscopic and microscopic methods, clearly demonstrating a toxic order CdSe > CdSe/ZnS QDs.



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ABSTRACT

The interactions between quantum dots (QDs) and biological systems have attracted increasing attention due to concerns on possible toxicity of the nanoscale materials. The biological effects of CdSe QDs and CdSe/ZnS QDs with nearly identical hydrodynamic size on *Saccharomyces cerevisiae* were investigated via microcalorimetric, spectroscopic and microscopic methods, demonstrating a toxic order CdSe > CdSe/ZnS QDs. CdSe QDs damaged yeast cell wall and reduced the mitochondrial membrane potential. Noteworthy, adhesion of QDs to the yeast cell surface renders this work a good example of interaction site at cell surface, and the epitaxial coating of ZnS could greatly reduce the toxicity of Cd-containing QDs. These results will contribute to the safety evaluation of quantum dots, and provide valuable information for design of nanomaterials.

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1. Introduction

Quantum dots (QDs), namely semiconductor nanocrystals, have gained considerable attention owing to their unique size-dependent optical and electronic properties that make them attractive

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for a wide range of applications, such as biomedical imaging, DNA detection, drug delivery, solar energy generation, and electronics industries (Murray et al., 2000; Zhang et al., 2005; Portney and Ozkan, 2006; Kamat, 2008; Delehanty et al., 2009; Shirasaki et al., 2012). Nevertheless, the possible threats to human health and environment have also attracted increasing attention as the production and applications of QDs increase rapidly while standard evaluation of the safety is lack to some extent (Holbrook et al., 2008; Teow et al., 2011; Tsoi et al., 2012). Hence, clear elucidation of the relationship between biological effects and the unique properties of nanomaterials, e.g. size, shape, surface coatings, etc. for better design and syntheses with good biocompatibility has become a hot topic (Zrazhevskiy et al., 2010; Rivera-Gil et al., 2012). The diverse types of QDs bring certain difficulties for toxicological evaluation because each individual type of QDs owns its unique physicochemical properties, which will dominate its interactions with biological systems. Consequently, it is necessary to continuously study the biological effects of QDs on different organisms and the possible mechanisms.

Previous studies indicated that the toxicity of QDs comes from the leakage of heavy metal ions (Derfus et al., 2004; Lewinski et al., 2008), the enhancement of reactive oxygen species (ROS) level (Slaveykova et al., 2009; Mahto et al., 2010), and other causes (Chang et al., 2006; Hardman, 2006). For heavy metal-containing QDs, such as cadmium selenide (CdSe) and cadmium telluride (CdTe), both of which bear excellent optical properties, e.g. really high quantum yields over 60%, the release of toxic metal ions causes cellular toxicity. Several studies have evidenced the inconspicuous toxicity of QDs in several cellular models (Voura et al., 2004; Chaves et al., 2008; Zhang et al., 2010). Pace et al. (2010) showed that QDs with thiol stabilizer induced toxicity on *Daphnia magna* caused by releasing Cd²⁺. Contradictory results obtained from Priester et al. (2009) demonstrated that QDs themselves were more toxic to planktonic *Pseudomonas aeruginosa* PG201 than cadmium ions, suggesting that the release of Cd²⁺ is not the exclusive factor. In addition, other studies pointed out that QDs themselves could also induce cellular damage besides the leakage of heavy metal ions (Male et al., 2008; Liu et al., 2011; Aye et al., 2012). Moreover, physicochemical properties of QDs such as chemical composition (Cho et al., 2007), size (Duan and Nie, 2007), dosage (Ryman-Rasmussen et al., 2007), surface charge (Geys et al., 2008) and surface coating (Nabiev et al., 2007) led to toxicity. Furthermore, the stability of QDs was a vital factor to their toxicity. Mahendra et al. (2008) found that QDs were potentially safe materials at near-neutral pH but exerted toxicity under acidic or alkaline conditions. This was proven to be the weathering effect in extreme condition that destabilized QDs followed by release of the cadmium and selenite ions rapidly.

In our previous studies, we have found CdTe QDs were toxic due to the released cadmium ions (Li et al., 2010), while sizes and surface coatings of QDs could influence their biological effects (Li et al., 2011; Han et al., 2012; Lai et al., 2012a,b; Xu et al., 2013). It is widely accepted that epitaxial growth of another material with wider band gap can make the lattice more stable as well as reduce the toxicity (Bao et al., 2011; Chen et al., 2012). Aaron et al. studied the interactions between CdSe QDs and immune cells, suggesting that their particle geometry significantly affected their interactions with the plasma membrane, uptake into cells, and localization within intracellular vesicles (Aaron et al., 2011). Gosso et al. used neurosecretory mouse chromaffin cells of the adrenal gland for testing the effect of CdSe/ZnS QDs on Ca²⁺ channels functionality and Ca²⁺-dependent neurosecretion, showing that exposure to CdSe/ZnS QDs impaired Ca²⁺-influx and severely interfered with the functionality of the exocytotic machinery, thus compromised the overall catecholamine supply from chromaffin cells (Gosso et al., 2011). However, detailed comparison of the biological effects

between CdSe QDs and CdSe/ZnS QDs is thus far less documented. In fact, the results from the comparisons will give valuable information for safe use of CdSe QDs in future. To address this issue, herein, by using *Saccharomyces cerevisiae* (*S. cerevisiae*) as a model eukaryotic organism, we attempt to evaluate and compare the toxicity of CdSe and CdSe/ZnS QDs, both of which are orange-emitting with similar core sizes as well as hydrodynamic sizes, and coated with amphiphilic polymer octylamine-modified polyacrylic acid (OPA) to become water-soluble. The biological effects of QDs on the metabolism of *S. cerevisiae* were determined by microcalorimetry, spectroscopy, transmission electron microscopy (TEM), confocal laser scanning microscopy and flow cytometry. The mechanism of CdSe QDs toxicity towards *S. cerevisiae* was investigated via spectroscopy, microcalorimetry, inductively coupled plasma atomic emission spectroscopy (ICP-AES) so as to determine the main threats that brought by the applications of QDs.

2. Materials and methods

2.1. Reagents

Cadmium oxide (99.99%), selenium powder (99.99%, about 100 mesh), trioctylphosphine oxide (TOPO, 90%), hexadecylamine (HDA, 90%), zinc acetate (99.99%), 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide (JC-1), hexamethyldisilathiane (90%) and CdCl₂ (99.99%) were obtained from Sigma-Aldrich. Trioctylphosphine (TOP, 90%) was purchased from Alfa Aesar. Poly (acrylic acid) (PAA, Mw ~1800) and n-Octylamine (99%) were obtained from Aladdin. All reagents were used without further purification. Deionized (DI) distilled water was prepared from a Milli-Q-RO₄ water purification system (Millipore).

2.2. Preparation and characterization of water-soluble CdSe QDs

The preparation of highly luminescent CdSe and CdSe/ZnS nanocrystals were synthesized as previously reported (Qu and Peng, 2002; Xie et al., 2005). Experimental details for preparation and characterization of CdSe and CdSe/ZnS QDs are described in the supporting information. The water-soluble QDs were coated with amphiphilic polymer octylamine-modified polyacrylic acid (OPA), which was synthesized according to Zhou's method (Zhou et al., 2007). QDs concentrations were determined using the extinction coefficients according to a literature (Yu et al., 2003). The quantum yield (QY) values were determined by the following equation:

$$QY_{\text{sample}} = (F_{\text{sample}}/F_{\text{ref}}) \times (A_{\text{ref}}/A_{\text{sample}}) \times (n_{\text{sample}}^2/n_{\text{ref}}^2) \times QY_{\text{ref}}$$

where F , A , and n are the measured fluorescence (area under the emission peak), absorbance at the excitation wavelength and refractive index of the solvent respectively. PL spectra were spectrally corrected and quantum yields were determined relative to Rhodamine 6G (QY = 94%) (Grabolle et al., 2009).

2.3. Microorganism culture

Yeast *S. cerevisiae* (BY4742) was provided by the microbial genetics laboratory, Wuhan University, PR China. The yeast extract peptone dextrose (YPED) medium was consisted of yeast extract (1%), peptone (1%) and glucose (2%) at natural pH, which was sterilized under high-pressure steam at 120 °C for 30 min.

A single colony of yeast *S. cerevisiae* grown on YPED agar plates was inoculated in fresh medium and grown in a shaking incubator at 30 °C for 12 h. Subsequently, 1% inoculated yeast and QDs were incubated together at the beginning.

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