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Evaluate the potential environmental toxicity of quantum dots on ciliated protozoa by microcalorimetry

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ABSTRACT

In the present study, we evaluated the toxic effects of mercaptoacetic acid (MAA)-capped CdSe QDs and CdSe/ZnS QDs to particle-ingesting model ciliated protozoa *Tetrahymena thermophila* BF_5 (*T. thermophila* BF_5) by using a TAM air isothermal microcalorimeter. These results suggested that both MAA-CdSe QDs and MAA-CdSe/ZnS QDs were indeed acutely toxic for *T. thermophila* BF_5 growth in a dose-dependent manner, and the toxicities of both MAA-CdSe QDs and MAA-CdSe/ZnS QDs increased dramatically after UV irradiation due to the liberation of more toxic Cd^{2+}, which indicated that the toxicity of MAA-CdSe/ZnS QDs was less than that of MAA-CdSe QDs. Furthermore, the toxicity of different ligands-capped CdSe/ZnS QDs on *T. thermophila* BF_5 was also investigated. The uptake of MAA-CdSe/ZnS QDs and adenosine 5′-monophosphate (AMP)-CdSe/ZnS QDs by cells and the morphological change during the process of *T. thermophila* BF_5 growth incubated with these QDs were further studied by fluorescence inverted microscopy.

1. Introduction

The fluorescent quantum dots (QDs) have attracted great attention in biological imaging and biomedical applications areas in the past two decades [1–4]. In comparison with organic dyes and fluorescent proteins, QDs have high quantum yield of fluorescence, broad excitation spectrum and narrow/symmetric emission spectrum. In addition, QDs exhibit high photobleaching threshold and excellent photostability [5–8]. Owing to the tremendous focus on the biological imaging and biomedical applying of QDs, there has been increasing interest in the estimation of the toxicity of QDs [9–13]. The existing knowledge of the toxicity of QDs is very incomplete compared with some drugs or other chemical agents and the toxicity of QDs may depend on the particle sizes, surface ligands, charges, release of toxic Cd^{2+} ions, generation of reactive oxygen species and so forth [14–20]. Recently, the potential *in vivo* ecotoxicity of QDs has attracted wide attention and been widely investigated [21–24]. Gagné et al. have examined the toxic potential of CdTe QDs to the freshwater mussel *Elliptio complanata* by tracking changes in immune parameters. The results indicated that CdTe QDs were immunotoxic to freshwater mussels and resulted in the oxidative stress in gills and DNA damage in both organs [21]. Ke et al. found that the water-soluble CdSe/ZnS QDs have a high affinity for the *Chlamydomonas* sp. algae and the adsorption of QDs could hinder the photosynthetic activity of algae by reducing CO_2 depletion for QDs over 100 ppm and declining O_2 production for small dosages of QDs [22]. Lee and co-worker have employed freshwater macroinvertebrate, *Daphnia magna*, to evaluate the toxicity of CdSe/ZnS QDs in relation to surface coatings and light conditions systematically [23]. Moreover, Jackson et al. have reported on bioavailability, toxicity and bioaccumulation of CdSe/ZnS QDs to the amphipod *Leptocheirus plumulosus*. The results indicated that QDs were accumulated to a greater extent than the dissolved ion and QDs ingested with algae resulted in toxicity [24]. However, still no specific mechanism for the ecotoxicity of QDs was discussed detailed. Considering increasing application of QDs in various areas, their eco-toxic potentials deserve further investigation.

The ciliated protozoa *Tetrahymena thermophila* BF_5 (*T. thermophila* BF_5), belonging to the class of oligohymenophorea, subclass Hymenostomia, order Hymenostomatida, suborder trahymenina [25], is widely distributed in different natural environments. The ciliated is an eukaryotic unicellular environmental microorganism and has been widely used as a useful model

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organism for environmental toxicological research for decades [26–30], because *T. thermophila* BF_5 can grow well in axenic culture and the growth of *T. thermophila* can indicate the status of the aquatic environment. Compared to some simplex chemical tests, it can reveal the toxicology influence of some drugs and nanoparticles truly.

Microcalorimetry has attracted great attention in ecotoxicology research, because it is a universal, integral, non-invasive, non-destructive and highly sensitive approach for many biological investigations and rapid bioassay procedures in recent years [31–35]. Microcalorimetry is an effective approach to provide lots of kinetic and thermodynamic information of the interaction between microorganism and toxic substances by using the automatic calorimeter and the specific experimental method [36–39]. It can provide helpful evidences to evaluate the interaction mechanism. With the expansion of the application range and the increase of the detection accuracy, the experimental results become much more influential and persuasive.

It has been proved that the bioenergetics investigations become much more important in the evaluation of some properties of toxic substances in environmental toxicology, and the direct microcalorimetric method is quite suitable for toxicological tests by analyzing the metabolic activities processes and the kinetic parameters. Microcalorimetry can provide more growth kinetic information of ciliated protozoa which are very important and helpful for the understanding of biological processes of ciliated protozoa and evaluating the cytotoxicity of QDs on organisms in biomedical fields.

In the present study, the growth thermogenic curves of *T. thermophila* BF_5 under the influence of different concentrations of mercaptoacetic acid (MAA)-capped CdSe QDs and MAA-CdSe/ZnS QDs were measured by a TAM air Isothermal Calorimeter. Then, the growth thermogenic curves of *T. thermophila* BF_5 under the same concentrations of MAA-CdSe QDs and MAA-CdSe/ZnS QDs after UV irradiation were all measured by a TAM air Isothermal Calorimeter. To our knowledge, this is the first comprehensive evaluation of the *in vivo* ecotoxicity of MAA-CdSe QDs and MAA-CdSe/ZnS QDs. The growth thermogenic curves of *T. thermophila* BF_5 under these QDs were obtained and some growth thermogenic parameters were also calculated. The ecotoxicity of these QDs were analyzed from both the qualitative and the quantitative aspects. Furthermore, the toxicities of the same concentrations of CdSe/ZnS QDs with different ligands (bovine serum albumin, human serum albumin, NH_2-PEG-6000, nucleotide) on *T. thermophila* BF_5 growth were also investigated. The results showed that the toxicity of MAA-CdSe/ZnS QDs was the strongest and that of adenosine 5′-monophosphate (AMP)-CdSe/ZnS QDs was the weakest among them, no matter these QDs were pretreated under UV irradiation or not. In addition, the fluorescence inverted microscopy was used to record the morphology of *T. thermophila* BF_5 cells affected by MAA-CdSe/ZnS QDs and AMP-CdSe/ZnS QDs, so that there is confirmation of the general results obtained by microcalorimetry.

2. Experimental

2.1. Materials

Selenium (Se), stearic acid, hexadecylamine (HDA), octadecene (ODE), dioctylamine (DOA), tributylphosphine (TBP) and tri-n-octylphosphine oxide (TOPO) were purchased from Aldrich (Milwaukee, WI, USA). Sulfur (S), zinc acetate ($Zn(Ac)_2$), cadmium oxide (CdO) and MAA were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Bovine serum albumin (BSA), human serum albumin (HSA), NH_2-PEG-6000 and AMP were all purchased from Sigma (St. Louis, MO, USA). All other reagents were of analytical-reagent grade and used as received from Sigma (St. Louis, MO, USA). Ultrapure water with a resistivity of 18.2 MΩ cm was produced by passing through a RiOs 8 unit followed by a Millipore-Q Academic purification set (Millipore, Bedford, MA, USA).

T. thermophila BF_5 (mononuclear) was provided by the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, PR China. The culture medium was a solution containing 1.5% peptone, 0.1% glucose and 0.5% yeast extract (pH 7.2–7.4). It was sterilized in high-pressure steam at 120 °C for 30 min.

2.2. Instruments

T. thermophila BF_5 cells were cultivated in constant temperature incubator at 28 °C. A TAM air Isothermal Calorimeter (Thermometric AB, Sweden) was used to determine the metabolic power-time curves of *T. thermophila* BF_5 cells. This microcalorimeter is an eight-channel twin instrument and thermostated at the range of 5–60 °C, with a limit of detectability of 2 μW. The biomass was calculated on the haemacytometer (homemade, volume was 100 μL) with microscope imaging system (XSP-18B, Jiangnan, China). UV–vis absorption spectrophotometer (TU-1900, Beijing Puxi Analytic Instrument Ltd., China) and fluorescence spectrometer (LS-55, Perkin Elmer, USA) were used to characterize the properties of QDs. Fluorescent inverted microscope (DMIRB, Leica, USA) was used to observe and photograph the *T. thermophila* BF_5.

2.3. Preparation of hydrophobic CdSe and CdSe/ZnS QDs

The hydrophobic CdSe and CdSe/ZnS QDs were prepared according to the method described previously [3,11]. In a typical synthesis, 0.0254 g CdO and 0.2280 g stearic acid were put into a three-neck flask. The mixture was heated to 150 °C under argon flow until it became a clear solution and was then allowed to cool to room temperature. 3.88 g TOPO and 3.88 g HDA were added to this solution and the resulting mixture was heated to 310 °C under argon flow. At this temperature, the Se precursor (0.158 g Se solution in 0.476 g TBP and 3.362 g DOA) in glove box was injected swiftly into the hot reaction mixture. The cold injection solution brought the reaction temperature down to 280 °C for the growth of CdSe core QDs. The reaction was stopped about 3 min at 280 °C after the injection and then heat was immediately removed. When the mixture cooled to 60 °C, the chloroform and acetone were added to precipitate the QDs. The QDs were collected by centrifugation at 10,000 rpm for 5 min by washing with methanol for three times. The purified CdSe core QDs were dried in a vacuum oven and then the vacuum-dried CdSe core QDs were redispersed in hexane.

For the growth of ZnS shell, the reaction solution of CdSe core QDs cooled down to 200 °C. Typical synthesis of CdSe/ZnS core/shell QDs was performed by thermal cycling. S (0.0192 g S in 4 mL ODE) and Zn (0.0556 g $Zn(Ac)_2$ in 4 mL ODE, 1.94 g HDA and 1.94 g TOPO) precursors (10 mL total) were added successively to the reaction flask with CdSe core QDs, the injection was finished within 20 min. After that, the temperature cooled down to 90 °C for 1 h to allow the growth of ZnS shell. When the synthesis was ended, the solution cooled down to room temperature. The precipitate was collected by centrifugation at 10,000 rpm for 5 min by washing with acetone for three times. The purified CdSe/ZnS core/shell QDs were dried in a vacuum oven and then redispersed in hexane.

2.4. Preparation of MAA-CdSe QDs and MAA-CdSe/ZnS QDs

MAA-capped QDs were synthesized according to the scheme described [3,11]. The hydrophobic CdSe or CdSe/ZnS QDs were precipitated with acetone and then redispersed in chloroform. MAA was added to chloroform/QDs solution until it became obscure. Then, the QDs were centrifuged out at 10,000 rpm for 5 min.