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Short communication

Mitochondria as target of Quantum dots toxicity

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ABSTRACT

Quantum dots (QDs) hold great promise in many biological applications, with the persistence of safety concerns about the environment and human health. The present work investigated the potential toxicity of CdTe QDs on the function of mitochondria isolated from rat livers by examining mitochondrial respiration, swelling, and lipid peroxidation. We observed that QDs can significantly affect the mitochondrial membrane properties, bioenergetics and induce mitochondrial permeability transition (MPT). These results will help us learn more about QDs toxicity at subcellular (mitochondrial) level.

1. Introduction

Quantum dots (QDs) are nanocrystals of inorganic semiconductors that demonstrate large absorption cross sections with a broad range of excitation wavelengths and narrow emission profiles with wavelengths tunable by their sizes [1–3]. Owing to their optical, electrical, chemical and biofunctional properties, QDs are widely employed in various fields, ranging from energy to medical, biomedical and bioanalytical imaging [1,4]. However, many studies have reported toxic effects of QDs at the cellular, subcellular, proteic and DNA levels [5–11]. As reported, QDs can enter cells via endocytosis and damage the plasma membrane, nucleus, further leading to cell apoptosis [6–10,12]. QDs can effectively kill *Escherichia coli* in a concentration-dependent manner [8]. At proteic level, QDs can bind to human serum albumin to form of QDs-protein complex and lead to conformational changes of proteins at both secondary and tertiary structure levels [13]. At DNA level, QDs can nick DNA due to the generation of free radicals [14].

Mitochondria, whose main function is ATP synthesis, have long been recognized as the power source of the cell [15,16]. Apart from this important function, it is now widely accepted that several mitochondrial events control the apoptosis process [15,17]. Meanwhile, the exquisite structure of mitochondria provides a number of targets for noxious agents-induced mitochondrial dysfunctions [17].

The mitochondrial permeability transition (MPT) is a key mechanism for cell apoptosis regulated by mitochondria [18,19]. MPT is described as a process of abrupt increase of mitochondrial inner membrane permeability to solutes with molecular weighting up to 1500 Da [19]. MPT is considered to be the result of opening of the mitochondrial permeability transition pore (MPTP), the molecular composition of MPTP has yet to be identified. The currently popular models point to well-known membrane components: adenine nucleotide translocator (ANT), voltage dependent anion channel (VDAC), Cyclophilin D (Cyp D). As with Pandora's box, mitochondria are full of apoptosis-related proteins, such as Cytochrome C. During the process of MPT, these harmful proteins that normally reside in mitochondria are released into the cytosol, thus to activate apoptosis [20,21]. Many factors have been shown to induce pore opening, including oxidative stress, collapse of mitochondrial membrane potential, and decrease of matrix pH [22].

So far, most conventional studies of QDs toxicity on mitochondria were performed in vitro cell-culture experiments by staining with Mito Tracker which depend on the release or entrapment of fluorescent dyes in mitochondria [6,7]. However, the evidences of a direct and immediate influence of QDs on mitochondrial inner membrane permeabilization, bioenergetics, oxidative stress, ultrastructure and permeability transition are yet to be provided. To overcome these limitations, herein we investigated the effects of CdTe QDs on the function of mitochondria isolated from rat liver,

Abbreviations: CsA, cyclosporine A; ADP, adenosine diphosphate; HP, hematoporphrin; DTT, dithiothreitol; QDs, Quantum dots; MPT, mitochondrial permeability transition; ETC, electron transport chain; MPTP, pearmeability transition pores; ANT, adenine nucleotide translocator; VDAC, voltage dependent anion channel; Cyp D, Cyclophilin D; BGA, bongkrikic acid; ROS, reactive oxygen species; AIF, apoptosis inducing factor.

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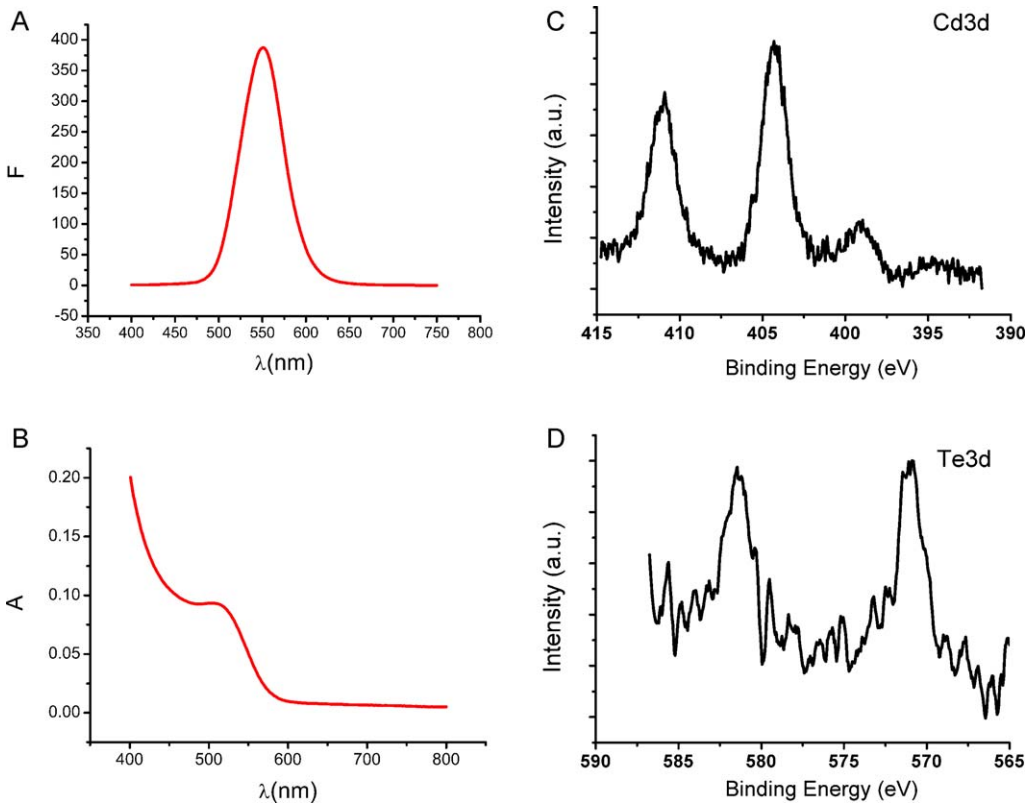

Fig. 1. Characterization of CdTe QDs. A: room temperature fluorescence spectra of CdTe QDs. B: absorption spectra of CdTe QDs. C: XPS Cd3d spectra of QDs. D: XPS Te3d spectra of QDs.

and try to define the possible mechanisms of the toxicity on liver mitochondria. We show that QDs induce MPT, increase the permeabilization of mitochondrial inner membrane to H^+ and K^+, and inhibit mitochondrial respiration. All data will help us learn more about the mechanisms underlying toxicity of QDs at subcellular (mitochondrial) level.

2. Materials and methods

2.1. Synthesis and characterization of CdTe QDs

Synthesis and characterization of CdTe QDs was performed as described in Supplementary materials (Sections 2 and 3).

2.2. Measurement of H^+ and K^+ inner membrane permeabilization

Mitochondrial inner membrane permeabilization to H^+ or K^+ was detected respectively by passive osmotic swelling of mitochondria suspended in KAc and KNO_3 media [23].

2.3. Effects of QDs on membrane lipid peroxidation and mitochondrial respiratory rate

Respiratory rate and the extent of lipid peroxidation were monitored by a Clark oxygen electrode and the Oxygraph software from Hansatech as described in Supplementary materials (Section 9).

2.4. Measurement of mitochondrial permeabilization transition

MPT was determined by QDs induced swelling of isolated mitochondria measured as a reduction in A_{520} as described in Supplementary materials (Section 11).

3. Results

3.1. Characterization of NAC-capped CdTe QDs

The optical properties of QDs were studied by Photoluminescence (PL) spectra and UV–Vis absorption spectra. The absorption spectrum in Fig. 1A displays an obvious absorption peak at 514 nm. The photoluminescence (PL) spectra for QDs were measured at room temperature using a 380 nm excitation line. The PL spectra in Fig. 1B shows a narrow band and obvious peak 551 nm. Dynamic light scattering (DLS) measurements showed that the NAC-CdTe QDs have a hydrodynamic size of 5.5 nm. ζ-Potential measurements showed that the negative surface charge of the QDs was −20.9 mV. Its surface area is 0.033 m^2/g using BET method. The compositions of the QDs were determined by X-ray photoelectron spectroscopy (XPS). The Cd3d and Te3d XPS spectrum are displayed in Fig. 1C and D.

3.2. Permeabilization of mitochondrial inner membrane to H^+ and K^+ induced by QDs

In the absence of QDs, the mitochondria undergo a little swelling in KAc media (Fig. 1A, trace a). The swelling may represent that H^+ permeates across the inner membrane due to mitochondrial respiration in normal condition. In presence of QDs (Fig. 2A, traces b–d), the permeabilizations of mitochondrial inner membrane to H^+ significantly increased as compared with untreated mitochondria, representing large proton conductance induced by QDs across the inner membrane. Mitochondrial inner membrane permeabilizations to K^+ induced by QDs were shown in Fig. 2B. The K^+ conductance across the inner mitochondrial membrane was only slightly affected by QDs at the concentration of 50 nmol/mg protein (Fig. 2B, trace b), but obviously affected by the high concentration of QDs at 1 μmol/mg protein (Fig. 2B, trace d).

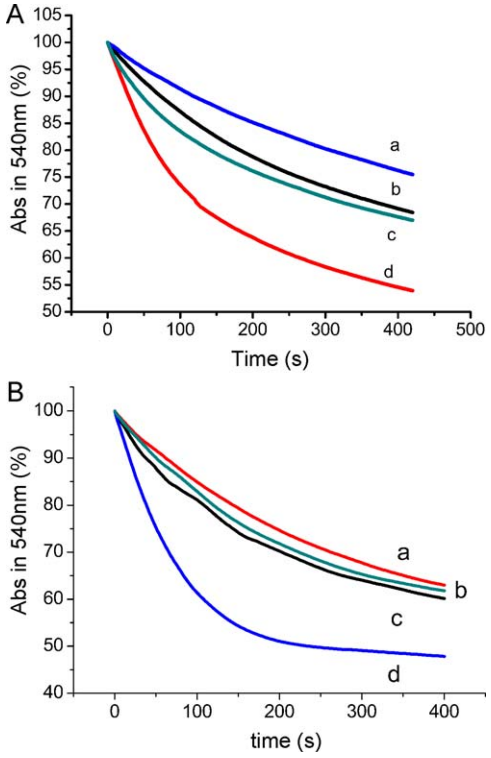

Fig. 2. Effect of QDs on the permeabilization to H^+ (A) and K^+ (B) by mitochondrial inner membrane. Assays were performed as described in Supplementary materials. Mitochondria (0.5 mg/mL) followed by addition of QDs at concentration: 0 nM (a), 25 nM (b), 50 nM (c) and 500 nM (d).

3.3. Effects of QDs on the respiration of isolated mitochondria

Fig. 3 shows the effect of QDs on the mitochondrial respiratory rate. In the absence of QDs, the rate at high state 3 indicates an intact respiratory chain and ATP synthesis. Meanwhile, low state 4 indicates an intact mitochondrial inner membrane. The effect of QDs on the rate at state 4 respiratory was biphasic: respiration stimulated by low concentration of QDs and inhibited by high concentration of QDs. The effect of QDs on respiratory rate at state 3 and DNP-uncoupled was similar: respiratory rate significantly decreased with the addition of QDs.

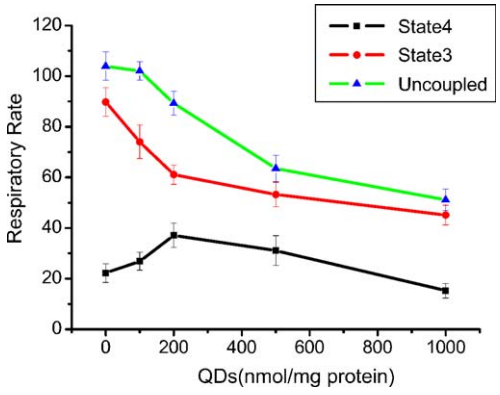

Fig. 3. Effects of QDs on the respiration of isolated mitochondria. Respiration unit represented as nanomole O_2/min/mg protein.

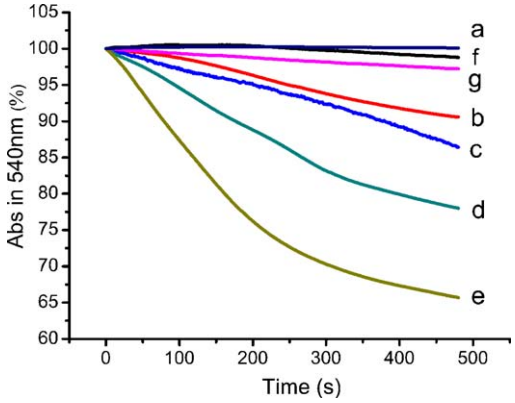

Fig. 4. QDs induce mitochondrial swelling. c_{QDs} (nM), a–e: 0, 25, 150, 100, and 500. Especially, trace f: mitochondria were incubated with BGA (trace f) or CsA (trace g) for 3 min, then QDs (500 nM) were added.

3.4. QDs induce mitochondrial swelling

In order to evaluate the effect of QDs on MPT, we examined whether QDs caused mitochondrial swelling. As shown in Fig. 4, treatment with QDs induced mitochondrial matrix swelling in a time and dose dependent manner. Especially, cyclosporin A (CsA) or bongkrekic acid (BGA) effectively inhibited QDs-induced swelling effectively. The results indicated that QDs induced a classical MPT of mitochondria.

3.5. Effects of QDs on membrane lipid peroxidation

The effects of QDs on ROS production and oxidative damage were assessed by detecting the lipid peroxidation of mitochondrial membrane induced by the pro-oxidant pair ADP/Fe^{2+}. After the addition of ADP/Fe^{2+}, oxygen consumption shows an initial lag phase, followed by a rapid oxygen consumption phase. The lag phase is related to the time required for the generation of the perferryl ion complex, which has been suggested to be responsible for the initiation of lipid peroxidation. The rapid oxygen consumption phase is due to the oxidation of the membrane phospholipids by ROS and consequently, to the propagation phase of lipid peroxidation [23]. As shown in Fig. 5, QDs induce both the initiation and the propagation of lipid peroxidation of mitochondrial membranes.

4. Discussion

Appropriate ion concentration in mitochondria is important for normal function of the organelle. The primary form of energy generated in mitochondria is proton electrochemical potential gradient.

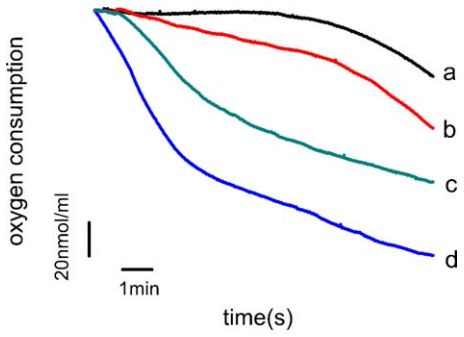

Fig. 5. Effect of QDs on membrane lipid peroxidation of rat liver mitochondria induced by ADP/Fe^{2+}. Trace a: control. Trace b: 0.2 μM QDs. Trace c: 1 μM QDs. Trace d: 5 μM QDs.

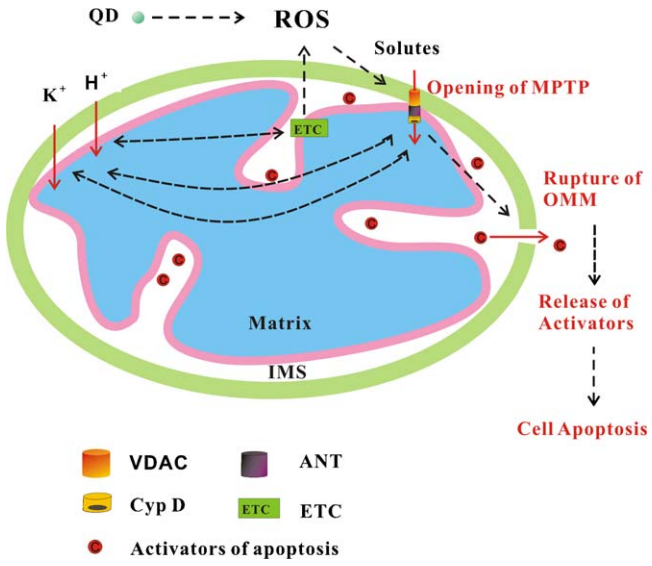

Fig. 6. Proposed mechanism of mitochondrial dysfunction induced by QDs.

Under physiological conditions, the permeability of the inner membrane to protons and other charged species must be controlled by the metabolic condition of mitochondria. Dissipation of the proton gradient will result in a disturbance of mitochondrial bioenergetic and cause mitochondrial poisoning [15,17]. Our results show that QDs can disturb the balance of H^+ between the two sides of mitochondrial matrix and the space between inner and outer membrane (Fig. 2A), indicating the putative mechanism of mitochondrial poisoning induced by QDs.

The K^+ cycle in mitochondria plays a key signal role in regulating cellular bioenergetics [24–26]. The permeabilization of K^+ would change the distribution of charges across inner membrane and induce decrease of proton electrochemical potential gradient. Moving of K^+ into the matrix may cause mitochondrial swelling. The increasing permeabilization of H^+ (Fig. 2A) and K^+ (Fig. 2B) may lead to MPT [26], this is consistent with our dates of swelling (Fig. 4).

In isolated mitochondria the major control over state 4 is the proton leak [27]. The entrance of H^+ across inner membrane will increase oxygen consumption. Taken together the results of state 4 and H^+ permeabilization, we suggest that QDs induced some stimulation of respiration in state 4 due to the permeabilization of mitochondrial inner membrane to H^+. In state 3, most of the control is taken by ETC and substrate transport. QDs causes the increase in state 4 and decrease in state 3, suggesting that QDs affect both ETC and transport of mitochondrial inner membrane. In the uncoupled condition, the change of oxygen consumption was caused by an influence of ETC' component(s) [27]. The ability of QDs to inhibit uncoupled respiration strongly suggested that the site of interaction should be localized in the ETC. This is consistent with our date which shown that high dose of QDs inhibited respiration of state 4. The results are also consistent with the report that the permeabilization of inner membrane to H^+ and K^+ associates with inhibition of ETC [23].

Dose-dependent swelling induced by QDs and protecting effects of swelling by CsA and BGA suggests that QDs induce classical MPT [20,22,23]. Anisotropy experiments (Supplementary materials, Section 12) reinforced the suggestion that QDs are an effective MPT-inducer. QDs-induced swelling was inhibited completely by BGA (Fig. 4, trace g), which suggests that QDs produce the influence on the conformational state of ANT. This is consistent with the report that cyclosporine A causes suppression of MPT due to its effect on the ANT conformation [28]. In addition, MPT can cause permeabilization of the inner membrane to H^+. Meanwhile, the MPT has been shown to regulate the activity of ETC, thus affect respiratory rate [29], which is consistent with our results (Fig. 3).

Oxidative stress has long been known to increase the probability of MPT [22,23,30]. MPTP was formed by the aggregation of misfolded integral membrane proteins that were damaged by oxidative stress [22,31]. QDs can internalize within the mitochondria after incubation (in Supplementary materials, Section 4). As reported, QDs are considered to be energy donors and the possibility for energy transfer between QDs particles and cell molecules has a potential to induce generation of ROS [8,14]. In this situation, the accumulation of ROS leads to thiol oxidation and cross-linkage of membrane proteins, thus initiate the MPT [26,32]. In addition, the oxidation of components of the inner membrane may promote a nonspecific membrane permeabilization [33], which can be manifested by QDs-induced H^+ or K^+ permeabilization in Fig. 2.

5. Conclusions

Our data collected from isolated mitochondria show that QDs cause inhibition of mitochondrial respiration and induce mitochondrial MPT. These results suggest that mitochondria are organelles that are very sensitive to QDs-induced stress and the mechanism of toxicity on mitochondria may be due to MPT. Although the QDs have been capped by antioxidant N-acetylcysteine, which may overcome the toxicity [7], mitochondrial function is still impaired even exposure to very low concentration of QDs. Because the decomposition of QDs and the release of Cd^{2+} were not observed during the incubation process (Supplementary materials, Parts 5 and 6), QDs induce mitochondrial toxicity mainly due to their inherent properties. The possible mechanisms of the mitochondrial dysfunction induced by QDs are summarized in Fig. 6. The emerging picture of QDs toxicity on mitochondria indicates that the QDs require to be further improved before they can be safely used in clinic.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.07.113.

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