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Toxicological effects of CdSe/ZnS quantum dots on marine planktonic organisms

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ABSTRACT

Quantum dot nanoparticles (QDs) are proposed as novel materials for photovoltaic technologies, light emitting devices, and biomedical applications. In this study we investigated the effect of CdSe/ZnS QDs on the growth rate of four microalgae: the diatom *Phaeodactylum tricornutum*, the cryptophyte *Rhinomonas reticulata*, the prymnesiophyte *Isochrysis galbana* and the green alga *Dunaliella tertiolecta*. In addition we analyzed the effect of QDs on the copepod *Acartia tonsa*. A classical acute test (48-h) with embryos was carried out to evaluate naupliar survival. Moreover, a 4-day chronic test with adult copepods was conducted to evaluate their fecundity (embryos $f^{-1}day^{-1}$) and egg hatching success. QDs in the range from 1 to 4 nM gradually inhibited the growth rate of *P. tricornutum*, *I. galbana*, *R. reticulata* and *D. tertiolecta* with an EC_{50} of 1.5, 2.4, 2.5 and 4.2 nM, respectively. Acute tests with *A. tonsa* (QD concentration tested from 0.15 to 1.5 nM) showed an increased naupliar mortality in response to QD treatment, exhibiting an EC_{50} of 0.7 nM. Chronic test showed no negative effect on egg production, except on the last two days at the highest QD concentration (2.5 nM). No significant reduction of the percentage of egg hatching success was recorded during the exposure. Toxicity assessment of QDs was also investigated at the molecular level, studying heat shock protein 70 gene expression (*hsp 70*). Our results indicate that *hsp70* was upregulated in adults exposed 3 days to 0.5 nM QDs. Overall, these results suggest that species unable to swim along the water column, like *P. tricornutum* and early hatched copepods, could be more exposed to toxic effects of QDs which tend to aggregate and settle in seawater.

1. Introduction

Recent developments in the field of nanotechnology have led to an increased interest to understand the effect of nanomaterials on the human health and on natural environment (Handy et al., 2008; Corsi et al., 2014). Nanoparticles can reach estuarine and coastal waters and undergo physicochemical transformation affecting their fate, behaviour and toxicity towards aquatic organisms at different levels of the food chain (Matranga and Corsi, 2012). In particular, in the marine environment the high ionic strength of sea water facilitates aggregation of nanoparticles, thereby favouring settlement on the sediment or mixing with the water column, following marine movements (Klaine et al., 2008). In this scenario, marine organisms will be exposed to nanoparticles through different routes of exposure, depending on the physiology of the marine organisms. As a consequence, the extent to which benthic rather than pelagic organisms can be exposed to nanoparticles needs to be investigated.

Quantum dots (QDs) are semiconductor nanocrystals, exhibiting unique photophysical properties, very attractive for biomedical applications as well as for manufactured products, including image sensors, emitting materials in LEDs and solar cells (Winnik and Maysinger, 2013). Due to their growing industrial production, increasing amounts of QDs are expected to enter the aquatic ecosystems where their effects on biological communities are poorly understood (Hardman, 2006). There is a growing body of literature regarding the effects of QDs on aquatic organisms, such as bacteria, microalgae, invertebrates and fishes (Gagnè et al., 2008; Domingos et al., 2011; Zhang et al., 2012; Yang et al., 2012; Ambrosone et al., 2012; Leigh et al., 2012). Although it has been reported that QDs can partially degrade in aqueous environments

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and release free Cd, it seems that QDs cause greater and specific toxicity than equivalent concentrations of dissolved Cd (Domingos et al., 2011; Ambrosone et al., 2012; Morelli et al., 2012). So far, few studies have investigated the effects of QDs on marine organisms, such as planktonic algae, bivalves, polychaetes and crustaceans (Jackson et al., 2012; Zhang et al., 2013a; Mouneyrac et al., 2014; Rocha et al., 2014).

The aim of the present paper was to assess toxicological effects of QDs on planktonic organisms representing the first level of the marine food chain. We analyzed different biological parameters in phyto and zooplanktonic organisms to individuate sensitive endpoints which could be suitable to describe the impact of QDs in marine environment.

Phytoplankton represents the first trophic level of the marine food web, providing food for zooplankton with the potentiality to transfer nanoparticles or other accumulated toxic substances to the food chain. The inhibition of algal growth can have detrimental effects on zooplankton, with a consequent reduction of herbivore grazers, such as copepods, the dominant zooplanktonic group in marine ecosystem. QDs might also directly affect copepod survival, reducing their viability and/or fecundity, with consequent effects at the higher trophic levels.

Here we studied the effect of CdSe/ZnS QDs on the growth of four different marine phytoplanktonic unicellular algae (*Phaeodactylum tricornutum, Dunaliella tertiolecta*, *Rhinomonas reticulata* and *Isochrysis galbana*) belonging to main taxonomic groups, world-wide distributed in the marine ecosystem, and on the reproduction and larval viability of the calanoid copepod *Acartia tonsa,* a marine and brackish water herbivore used as a model organism in ecotoxicology (Gorbi et al., 2012).

Several authors demonstrated that QDs induce formation of reactive oxygen species (ROS) with a consequent oxidative stress in aquatic and terrestrial organisms (Von Moss and Slaveykova, 2014; Ambrosone et al., 2012; Buffet et al., 2014). Mechanisms of defence against oxidative stress, as well as specific gene expression stimulation (i.e. caspase activity, heat shock proteins) have been proposed as effective biomarkers of nanoparticle exposure. We investigated, for the first time, the modulation of the heat shock protein 70 gene expression (*hsp 70*) in *A. tonsa* adults exposed to QDs. This heat shock protein is generally considered a good potential stress biomarker in copepods exposed to heavy metals, since it is involved, as a molecular chaperone, in protein repair (Rhee et al., 2009; Lauritano et al., 2012; Kim et al., 2014).

2. Materials and methods

2.1. Preparation of aqueous suspensions of quantum dots

Lumidot® orange quantum dots (QDs) emitting at 590 nm, constituted by a CdSe core with a ZnS shell and stabilized by hexadecylamine (HDA), were purchased by Sigma-Aldrich (Milan, Italy). QDs were encapsulated with the amphiphilic polymer poly (styrene-co-maleic anhydride) terminated with cumene (PSMA) and ethanolamine (EA) and transferred in deionized water following the procedure reported in (Morelli et al., 2012).

The same protocol was performed with HDA, PSMA and EA solutions without QDs, in order to obtain a blank medium (named HDA/PSMA), useful to evaluate if these organic constituents of QDs affected algae and copepods. Nanoparticle concentration was measured by Jasco V-550 UV/vis spectrophotometer (Lecco, Italy) using the molar absorption coefficient ($\varepsilon = 1.6 \times 10^5\ M^{-1}\ cm^{-1}$) provided by Sigma-Aldrich. Total Cd was measured by Atomic Absorption Spectrometry equipped with a graphite furnace (AAS, Perkin Elmer, Ueberlingen, Germany), after acidification with HNO_3 (0.3% v/v). QD and total Cd concentrations in the stock suspension used in this study were 1.23 μM and 590 ± 40 μM ($n=4$), respectively. The size of the water-soluble QD suspension was 17 ± 3 nm, as stated by fluorescence correlation spectroscopy measurements (Morelli et al., 2013). Stable suspensions of water-soluble QDs were stored in the dark at 4 °C for a maximum of 3 months and used for exposure experiments (Fig. 1 SI).

2.2. Microalgal cultures and growth rate

The marine microalgae *Phaeodactylum tricornutum* (bacillariophyceae, strain CCAP 1052/1A) and *Dunaliella tertiolecta* (chlorophyceae, strain CCAP 19/27) were obtained from the Culture Collection of Algae and Protozoa, Dunstaffnage Marine Laboratory, UK and cultured at CNR (Pisa, Italy). The marine microalgae *Rhinomonas reticulata* (cryptophyceae, strain FE208) and *Isochrysis galbana* (prymnesiophyceae, strain FE207) were provided by Zoological Station Anton Dohrn (Naples, Italy) and cultured at ISPRA (Leghorn, Italy). Stock cultures were grown at 21 ± 1 °C at a fluorescent daylight (100 μmol photons × m^{-2} × s^{-1}) in a 16:8 light-dark cycle photoperiod. Culture medium (natural seawater diluted with deionized water to obtain salinity 30 psu) was enriched with f/2 medium and sterilized by filtration on 0.2 μm membrane filters (Millipore, Milan, Italy). Seawater was collected in an uncontaminated area, three miles offshore from the island of Capraia (Tyrrhenian Sea, Italy), filtered through 0.2 μm membrane filters and stored in the dark at +4 °C. Stock cultures were maintained in exponential growth by inoculating cells weekly into a new sterilized medium.

Exposure experiments were carried out by inoculating algae from a stock culture to a new medium at the initial cell density of 3 to 5 × 10^4 cells mL^{-1} (Table 1) (ISO 10253-2006). Cultures were distributed in 2.5 mL multiwell plates and spiked with suitable amounts of QDs to obtain final nominal concentrations ranging from 0.5 to 4 nM. Cell density was measured at the third day of growth by recording the absorbance of chlorophyll at 680 nm (Jasco V-550 UV/vis spectrophotometer). Standard equations were established between optical density at 680 nm (OD 680) to cell number per mL (Fig. 2 SI) using a haemocytometer, under a light microscopy (Zeiss, Oberkochen, Germany). Finally, the specific growth rate (μ) was calculated by using the equation $\mu = \ln(N_3 - N_0)/(t_3 - t_0)$, where N_3 and N_0 are the cell number mL^{-1} at the third day (t_3) and initial day (t_0) of the exponential growth phase. Control cultures, without QDs, were always carried out. Moreover, we checked the possible toxicity of the blank medium on algae by adding the same volume of HDA/PSMA used for the highest QD concentration, to algal cultures. All experiments were carried out in triplicate.

2.3. Acartia tonsa cultures and bioassays

Acartia tonsa copepods were reared at the ISPRA laboratory in Leghorn (Italy) in a 25 L tank containing 0.22 μm mesh net filtered

Table 1
Growth rate and EC_{50} of marine microalgae exposed 3 days to increasing QD concentrations and to the blank medium HDA/PSMA. EC_{50} is the QD concentration causing 50% of growth inhibition (C.I.=Confidence Interval). Experiments were carried out in triplicate.

Microalgae	Inoculum (10^4 cells mL^{-1})	Growth rate (day^{-1}) Control	HDA/PSMA	[QDs], nM EC_{50}	C.I.
P. tricornutum	5.0 ± 0.8	1.12 ± 0.01	1.04 ± 0.03	1.54	1.47–1.61
I. galbana	3.1 ± 0.4	0.77 ± 0.03	0.77 ± 0.06	2.42	2.13–2.71
R. reticulata	3.0 ± 0.5	0.97 ± 0.01	0.94 ± 0.01	2.47	2.41–2.53
D. tertiolecta	3.0 ± 0.4	0.65 ± 0.01	0.63 ± 0.02	4.20	3.81–4.59