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Subcellular partitioning kinetics, metallothionein response and oxidative damage in the marine mussel *Mytilus galloprovincialis* exposed to cadmium-based quantum dots

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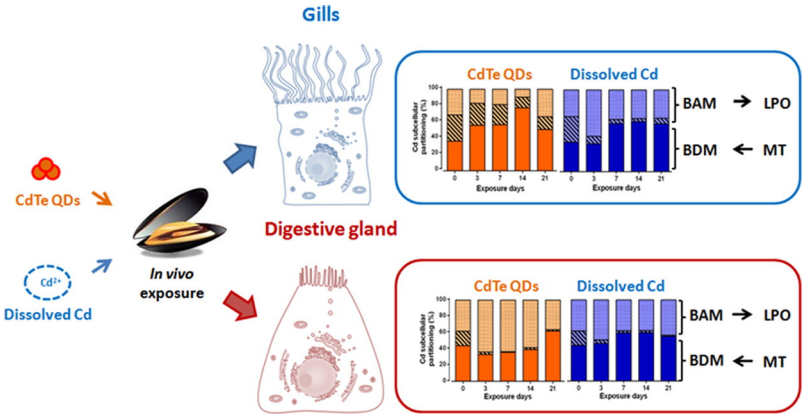
HIGHLIGHTS

- Subcellular partitioning and MT response are Cd form, tissue and time dependent.
- Tissue specific metabolism of Cd-based quantum dots (QDs) in marine mussels.
- QDs are slower biologically detoxified when compared to dissolved Cd.
- Subcellular partitioning and biomarker responses indicate nano-specific effects.
- Subcellular partitioning is potential tool to assess nanomaterial ecotoxicity.

GRAPHICAL ABSTRACT



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ABSTRACT

The environmental health impact of metal-based nanomaterials is of emerging concern, but their metabolism and detoxification pathways in marine bioindicator species remain unclear. This study investigated the role of subcellular partitioning kinetics, metallothioneins (MTs) response and oxidative damage (lipid peroxidation – LPO) in the marine mussel *Mytilus galloprovincialis* exposed to CdTe quantum dots (QDs) in comparison with its dissolved counterpart. Mussels were exposed to QDs and dissolved Cd for 21 days at 10 μg Cd L^{-1} followed by a 50 days depuration. Higher Cd concentrations were detected in fractions containing mitochondria, nucleus and lysosomes, suggesting potential subcellular targets of QDs toxicity in mussel tissues. Tissue specific metabolism patterns were observed in mussels exposed to both Cd forms. Although MT levels were directly associated with Cd in both forms, QDs subcellular partitioning is linked to biologically active metal (BAM), but no increase in LPO occurred, while in the case of dissolved Cd levels are in the biologically detoxified metal (BDM) form,

Abbreviations: 4-HNE, 4-hydroxyalkenals; BAM, biologically active metal; BCF, bioconcentration factor; BDM, biologically detoxified metal; C_B, Cd concentration in the subcellular fractions; C_{B0}, initial Cd concentration; Cd-total, total Cd concentration; C_W, Cd concentration in seawater; ENP, engineered nanoparticle; GPx, glutathione peroxidase; GST, glutathione-S-transferase; HMW, high molecular weight proteins; Ht, whole tissue homogenate; IF, insoluble fraction; K_a, accumulation rate; K_l, loss rate; LMS, lysosomal membrane stability; LMW, low molecular weight protein; LPO, lipid peroxidation; MDA, malondialdehyde; MT, metallothionein; NP, nanoparticle; PCA, principal component analysis; QDs, quantum dots; SOD, superoxide dismutase; $t_{1/2}$, half-life time.

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indicating nano-specific effects. Mussel gills showed lower detoxification capability of QDs, while the digestive gland is the major tissue for storage and detoxification of both Cd forms. Both mussel tissues were unable to completely eliminate the Cd accumulated in the QDs form (estimated half-life time > 50 days), highlighting the potential source of Cd and QDs toxicity for human and environmental health. Results indicate tissue specific metabolism patterns and nano-specific effects in marine mussel exposed to QDs.

1. Introduction

Cadmium (Cd) is a metal with no known biological function widespread in estuarine and coastal environments, which at environmentally relevant concentrations (0.5–10 μg Cd L^{-1}) induces several toxic effects in marine organisms (e.g. Soto et al., 2003; Eisler, 2007; Serafim and Bebianno, 2007; Macías-Mayorga et al., 2015). Ecotoxicity of Cd was extensively studied in marine bivalves, but few studies exist on the ecotoxicological impact of Cd-based engineered nanoparticles (ENPs), such as quantum dots (QDs) (Katsumiti et al., 2014; Munari et al., 2014; Rocha et al., 2014; Rocha et al., 2015a, 2015b, 2015c). Due to the increasing production and use of QDs (e.g. electronic, chemistry, nanomedicine, cell and molecular biology), the impact of Cd concentration from QDs core and their potential environment risk need to be investigated.

Marine bivalves are useful for characterizing the environmental impact of ENPs in the marine environment (Moore, 2006; Canesi et al., 2012; Rocha et al., 2015b). Among bivalve species, *Mytilus galloprovincialis* have the capacity to accumulate high QDs concentrations, which can induce several cellular damage, such as decrease of lysosomal membrane stability (LMS), genotoxicity on hemocytes, production of reactive oxygen species (ROS) and changes in antioxidant capacity in the gills and digestive gland after *in vivo* exposure (CdTe QDs 6 nm; 10 μg C d L^{-1}; 14 days) (Rocha et al., 2014; Rocha et al., 2015c). CdS QDs toxicity was also associated with extra- and intracellular release of Cd^{2+} ions and oxidative damage in *M. galloprovincialis* hemocytes and gills cells after *in vitro* exposure (5 nm; 10^{-4}–10^{2} mg Cd L^{-1}; 24 h) (Katsumiti et al., 2014). In freshwater mussel *Elliptio complanata*, oxidative stress and genotoxicity were observed after exposure to CdTe QDs (1.6–8 mg L^{-1}; 24 h) (Gagné et al., 2008a, 2008b; Peyrot et al., 2009), while larger CdS-CdTe QDs aggregates were more immunotoxic than smaller ones (1–10 nm; 0.05–2.7; μg L^{-1}; 21 h) (Bruneau et al., 2013).

The mode of action (MoA) and toxicity of metal-based ENPs in bivalve cells depend on their subcellular localization and interaction to organelles and/or ligands (Rocha et al., 2015b). However, few studies have addressed subcellular localization of metal-based ENPs in bivalves and their metabolism and detoxification pathways remain unclear. On the other hand, the MoA, toxicity and detoxification mechanisms of dissolved metals in marine organisms are related to their subcellular partitioning and threshold concentration in the biologically active metal form (BAM) or in the biologically detoxified metal form (BDM) (Wallace et al., 2003; Ng and Wang, 2005; Campana et al., 2015).

Cd subcellular partitioning is related to metallothioneins (MTs) which are cysteine-rich proteins with the ability to bind up to 7 divalent or up to 20 monovalent metal ions. In invertebrates, MT molecule can bind at least six Cd atoms (Bebianno and Langston, 1989) and play an important role in the regulation of essential metals (Cu, Zn), detoxification of non-essential metals (Ag, Cd and Hg), scavenging of free radicals and protection against oxidative stress (Palmiter, 1998; Gagné et al., 2008a, 2008b). Furthermore, MTs induction is recognized as a biomarker of metal exposure in monitoring environmental quality (Geret et al., 2003; Cravo et al., 2009). Recent studies highlighted the MT role in metal-based ENPs metabolism and protection against oxidative stress in *M. galloprovincialis* exposed to CuO NPs (<50 nm; 10 μg L^{-1}; 15 days) (Gomes et al., 2011, 2012) and to Ag NPs (<100 nm; 10 μg L^{-1}; 15 days) (Gomes et al., 2014).

The impact of QDs in aquatic organisms has been associated with QDs dissolution, release of metal ions (e.g. Cd^{2+}), ROS production, oxidative stress and genotoxicity (Gagné et al., 2008a, 2008b; Peyrot et al., 2009; Rocha et al., 2014; Rocha et al., 2015c; Buffet et al., 2015). On the other hand, oxidative damage at DNA level (nuclei and mitochondria) was more sensitive to QDs toxicity than to lipid membrane (Gagné et al., 2008a, 2008b). Therefore, no increase in lipid peroxidation (LPO) was reported in the marine clam *Scrobicularia plana* (Buffet et al., 2015), nor in the freshwater fish *Fundulus heteroclitus* (Blickley et al., 2014) or in *Oncorhynchus mykiss* (Gagné et al., 2008a, 2008b) after exposure to different QDs, indicating that the mechanisms of defense and detoxification is robust enough to prevent QDs-induced oxidative damage according to concentration and exposure time.

In this context, the hypothesis of this study was to assess the subcellular partitioning kinetics, BAM and BDM concentrations of Cd-based QDs, MTs response and oxidative damage to describe the MoA and to predict the ecotoxicological effects of Cd-based ENPs in marine organisms. Accordingly, the aim of this study was to analyze Cd subcellular partitioning in different tissues (gills and digestive gland) of the marine mussel *M. galloprovincialis* exposed to CdTe QDs and to its dissolved counterpart during a 21 days exposure period followed by a 50 days depuration period. Furthermore, the relationships between Cd subcellular partitioning, MTs response and oxidative damage (LPO) in mussels exposed to both Cd forms (nanoparticulate and dissolved form) were examined to identify which subcellular fraction was more important in the accumulation, detoxification and toxicity of Cd-based QDs.

2. Materials and methods

2.1. Experimental design

Mussels *M. galloprovincialis* (60 ± 5 mm shell length) were collected in the Ria Formosa Lagoon (Portugal) and acclimated during 14 days in static tanks containing seawater from the sampling site (salinity = 36.3) at 16 °C and constant aeration. CdTe QDs (6 ± 1 nm) and dissolved Cd ($Cd(NO_3)_2 \cdot 4H_2O$) stock solutions were prepared in ultrapure water and characterized according to the method described by Sousa and Teixeira (2013). Data on characterization and behaviour of CdTe QDs in seawater were previously reported in Rocha et al. (2014, 2015a) and are summarized in the Supporting information (Table S1).

After the acclimation period, ninety mussels were placed in 30 L tanks filled with 25 L of seawater (3.6 mussels L^{-1}) and exposed to 10 μg Cd L^{-1} of CdTe QDs and to the same concentration of their soluble counterpart, jointly with a control group kept in clean seawater in a duplicate design (2 tanks per treatment) for 21 days (exposure period). Seawater was changed daily with redosing of the QDs and dissolved Cd concentration, as previously described by Rocha et al. (2015a). At the end of exposure period, mussels were transferred to clean seawater for 50 days (depuration period). Mussels were only fed with natural seawater providing animals with food to avoid starvation and any effects resulting from the interaction of QDs and food (Rocha et al., 2015a).

Ten mussels from each experimental condition were collected at the beginning of the experiment and after 3, 7, 14 and 21 days of exposure and 15, 20, 30 and 50 days of depuration. Experiments were conducted in a static-renewal condition under 12 h:12 h light/dark cycles and abiotic parameters was analyzed daily by measuring salinity (36.3 ± 0.07),