Ecotoxicology and Environmental Safety 152 (2018) 78–90

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Toxicological effects of CdSe nanocrystals on the marine diatom *Phaeodactylum tricornutum*: The first mass spectrometry-based proteomic approach

Isabelle Poirier[a,b,*], Marie Pallud[a,c], Lauriane Kuhn[d], Philippe Hammann[d], Arnaud Demortière[e,f,g], Arash Jamali[e], Johana Chicher[d], Christelle Caplat[h], Régis Kevin Gallon[a,b], Martine Bertrand[a,b]

[a] *Institut National des Sciences et Techniques de la Mer, Conservatoire National des Arts et Métiers, 50103 Cherbourg en Cotentin Cedex, France*
[b] *Laboratoire Universitaire des Sciences Appliquées de Cherbourg, EA4253, Normandie Université, UNICAEN, 50130 Cherbourg en Cotentin, France*
[c] *IFREMER, LEAD NC, Equipe Ecophysiologie Station aquacole de Saint Vincent, Boulouparis, 98897 Nouvelle Calédonie Cedex, France*
[d] *Plateforme Protéomique Strasbourg Esplanade, CNRS FRC 1589, Institut de Biologie Moléculaire et Cellulaire, 67084 Strasbourg Cedex, France*
[e] *Laboratoire de Réactivité et Chimie des Solides, CNRS UMR 7314, Université de Picardie Jules Verne, 80039 Amiens Cedex 1, France*
[f] *Réseau sur le Stockage Electrochimique de l'Energie (RS2E), CNRS FR 3459, 80039 Amiens Cedex 1, France*
[g] *Center for Nanoscale Materials, Argonne National Laboratory, Argonne, IL 60439, United States*
[h] *UMR BOREA, UCBN, MNHN, UPMC, CNRS-7208, IRD-207, Institut de Biologie Fondamentale et Appliquée, Normandie Université, UNICAEN, 14032 Caen Cedex 5, France*

ARTICLE INFO

Keywords:
Marine microalgae
Nanoparticles
Toxicity
Physiological responses
Proteomics
Oxidative stress

ABSTRACT

In the marine environment, benthic diatoms from estuarine and coastal sediments are among the first targets of nanoparticle pollution whose potential toxicity on marine organisms is still largely unknown. It is therefore relevant to improve our knowledge of interactions between these new pollutants and microalgae, the key players in the control of marine resources. In this study, the response of *P. tricornutum* to CdSe nanocrystals (CdSe NPs) of 5 nm (NP5) and 12 nm (NP12) in diameter was evaluated through microscopic, physiological, biochemical and proteomic approaches. NP5 and NP12 affected cell growth but oxygen production was only slightly decreased by NP5 after 1-d incubation time. In our experimental conditions, a high CdSe NP dissolution was observed during the first day of culture, leading to Cd bioaccumulation and oxidative stress, particularly with NP12. However, after a 7-day incubation time, proteomic analysis highlighted that *P. tricornutum* responded to CdSe NP toxicity by regulating numerous proteins involved in protection against oxidative stress, cellular redox homeostasis, Ca^{2+} regulation and signalling, S-nitrosylation and S-glutathionylation processes and cell damage repair. These proteome changes allowed algae cells to regulate their intracellular ROS level in contaminated cultures. *P. tricornutum* was also capable to control its intracellular Cd concentration at a sufficiently low level to preserve its growth. To our knowledge, this is the first work allowing the identification of proteins differentially expressed by *P. tricornutum* subjected to NPs and thus the understanding of some molecular pathways involved in its cellular response to nanoparticles.

Significance: The microalgae play a key role in the control of marine resources. Moreover, they produce 50% of the atmospheric oxygen. CdSe NPs are extensively used in the industry of renewable energies and it is regrettably expected that these pollutants will sometime soon appear in the marine environment through surface runoff,

Abbreviations: NPs, nanoparticles; CdSe NPs, cadmium-selenium colloidal nanocrystals; NP5, cadmium-selenium colloidal nanocrystals of 5 nm in diameter; NP12, cadmium-selenium colloidal nanocrystals of 12 nm in diameter; QDs, quantum dots; TOPO, trioctylphosphine oxide; ODPA, octadecylphosphonic acid; TOP, trioctylphosphine; TEM, transmission electron microscopy; STEM, scanning transmission electron microscopy; HAADF-STEM, high angle annular dark field in scanning transmission electron microscopy; EDX-STEM, scanning transmission electron microscopy energy dispersive X-ray spectroscopy; ROS, reactive oxygen species; $[Cd]_{eq.}$, equivalent Cd concentration; $[Se]_{eq.}$, equivalent Se concentration; PAR, photosynthetically active radiation; AAS, atomic absorption spectrometry; DCF-DA, dichlorofluorescein diacetate; DCF, dichlorofluorescein; DDA, data-dependent acquisition mode; CID, collision-induced dissociation; nanoLC-MS/MS, nanoscale liquid chromatography coupled to tandem mass spectrometry; MS, mass spectrometry; AGC, automatic gain control; HCD, higher energy collisional dissociation; PSM, peptide spectrum matches; GLM, generalized linear model; FDR, false-discovery rate; XIC, extracted ion chromatograms; PCA, principle component analysis; EC50, median effective concentration; GPX, glutathione peroxidase; SeGPX, selenium-dependent glutathione peroxidase; PCD, programmed cell death; MASP, mucin-associated surface protein; SUMO, small ubiquitin-like modifier; TRD, tryptophan rich domain

* Corresponding author at: Institut National des Sciences et Techniques de la Mer, Conservatoire National des Arts et Métiers, 50103 Cherbourg en Cotentin Cedex, France.
E-mail addresses: isabelle.poirier@lecnam.net (I. Poirier), Marie.Pallud@ifremer.fr (M. Pallud), l.kuhn@ibmc-cnrs.unistra.fr (L. Kuhn), p.hammann@ibmc.u-strasbg.fr (P. Hammann), arnaud.demortiere@u-picardie.fr (A. Demortière), arash.jamali@u-picardie.fr (A. Jamali), j.chicher@ibmc-cnrs.unistra.fr (J. Chicher), christelle.caplat@unicaen.fr (C. Caplat), regis.gallon@lecnam.net (R.K. Gallon), martine.bertrand@lecnam.net (M. Bertrand).

https://doi.org/10.1016/j.ecoenv.2018.01.043
Received 8 November 2017; Received in revised form 17 January 2018; Accepted 19 January 2018

urban effluents and rivers. Since estuarine and coastal sediments concentrate pollutants, benthic microalgae which live in superficial sediments will be among the first targets of nanoparticle pollution. Thus, it is relevant to improve our knowledge of interactions between diatoms and nanoparticles. Proteomics is a powerful tool for understanding the molecular mechanisms triggered by nanoparticle exposure, and our study is the first one to use this tool to identify proteins differentially expressed by *P. tricornutum* subjected to CdSe nanocrystals. This work is fundamental to improve our knowledge about the defence mechanisms developed by algae cells to counteract damage caused by CdSe NPs.

1. Introduction

In the past two decades, research efforts have resulted in the gradual introduction of nanoparticles in numerous industrial fields, because of their unique optical, mechanical, electrical and magnetic properties (Stark et al., 2015). Nanoparticles containing a cadmium selenide core have recently attracted attention as promising photovoltaic devices (Hetsch et al., 2011) and their use in solar panel design is expanding (Wong et al., 2014). The cadmium selenide core is usually surrounded by a surface layer of organic and/or inorganic molecules named shell, but some environmental conditions can lead to a shell dissolution and a release of the CdSe core (Morelli et al., 2012). Toxicological effects of CdSe/ZnS quantum dots (QDs) on marine planktonic organisms have been the subject of several studies (Morelli et al., 2015; Scebba et al., 2016; Zhou et al., 2016), but the impact of the CdSe core alone has never been studied. The toxicity of uncoated metallic nanoparticles has been discussed in several reports and often deriving from dissolution of metal ions (Kirchner et al., 2005; Rzigalinski and Strobl, 2009; Baker et al., 2014). Previous studies have shown that the CdSe core dissolution is accelerated by its surface oxidation generated by aerobic conditions (Derfus et al., 2004; Zeng et al., 2015) or by light (Derfus et al., 2004). Moreover, Morelli et al. (2012) have shown that bare CdSe QDs, lacking the ZnS shell, underwent a salinity-dependent degradation process. Since CdSe nanocrystals are currently extensively used in the industry of renewable energies, it is regrettably expected that these new pollutants will sometime soon appear in the marine environment through surface runoff, urban effluents and rivers, and more particularly in estuarine and coastal sediments impacted by anthropogenic activities. Marine organisms living in estuarine and coastal sediments will be the first targets of these new pollutants. Among them, benthic microalgae play a key role in the control of marine resources and in global biogeochemical cycles; it is therefore relevant to improve knowledge about interactions between these microorganisms and nanoparticles. The diatom *P. tricornutum* appears as a good model to study these interactions for several reasons, (i) diatoms are the dominant microalgae in most marine ecosystems (Walsh, 1993), (ii) *P. tricornutum* has been completely sequenced (Bowler et al., 2008), (iii) this species has a high tolerance to cadmium (Brembu et al., 2011) and it was commonly used for assessing effects of nanoparticles in recent studies (CdSe/ZnS quantum dots: Morelli et al., 2015; Scebba et al., 2016; Zhou et al., 2016; TiO_2 NPs: Wang et al., 2016; Deng et al., 2017; Minetto et al., 2017; CeO_2 NPs: Deng et al., 2017; Sendra et al., 2017a; Ag NPs: Schiavo et al., 2017; Sendra et al., 2017b; CuNPs: Zhu et al., 2017 and ZnO NPs: Castro-Bugallo et al., 2014; Li et al., 2017). The authors focused on physiological and biochemical responses and highlighted the following major impacts: a growth inhibition (Zhou et al., 2016; Deng et al., 2017; Schiavo et al., 2017; Zhu et al., 2017), an increase in reactive oxygen species (ROS) production causing oxidative stress (Deng et al., 2017; Peng et al., 2017; Sendra et al., 2017a, b; Zhu et al., 2017), direct interactions between NPs and algae cells leading to membrane damages (Wang et al., 2016; Li et al., 2017; Schiavo et al., 2017; Sendra et al., 2017a, b), an increase in activity of antioxidant enzymes (Morelli et al., 2015; Wang et al., 2016; Deng et al., 2017) and a loss of photosynthetic pigments (Castro-Bugallo et al., 2014; Sendra et al., 2017b; Zhu et al., 2017). To our knowledge, only two previous studies used proteomics to analyse the impact of CdSe NPs on *P. tricornutum* (Morelli et al., 2015; Scebba et al., 2016). These studies have shown that CdSe/ZnS quantum dots can induce numerous proteome changes in *P. tricornutum*, but authors did not identify involved proteins. In our study, we have examined the response of *P. tricornutum* to CdSe nanocrystals through microscopic, physiological, biochemical and proteomic approaches. Proteins differentially expressed by algae cells submitted to NPs are identified by mass spectrometry analyses in order to elucidate strategies developed by this marine diatom to counteract the toxicity of NPs.

2. Materials and methods

2.1. Synthesis of CdSe colloidal nanocrystals

In previous studies, NPs with a diameter less than 30 nm were commonly used (Rocha et al., 2017). We have therefore chosen to test CdSe colloidal nanocrystals (CdSe NPs) with diameters of 5 nm and 12 nm (named NP5 and NP12, respectively, in the manuscript) (She et al., 2011, 2013). In addition, these two sizes would display different degrees of toxicity based on their volume and metal content (Morones et al., 2005; Monrás et al., 2014). CdSe NPs were synthesized in a 50 mL three-neck flask using a Schlenk-line approach. TOPO (3.0 g, Sigma-Aldrich-99%), ODPA (0.308 g, PCI Synthesis-97%), and CdO (0.060 g, Sigma-Aldrich-99%) were mixed, heated up to 150 °C, and kept under vacuum for 2 h. The reaction solution was then heated up to 300 °C under nitrogen at approximately 7 °C/min. Next, 1.5 g of TOP was rapidly injected into the reaction flask. TOP-Se solution (0.058 g Se, Aldrich-98% + 0.360 g TOP, Sigma-Aldrich, 90%) was then also injected of 360 °C and 330 °C for the synthesis of NP5 and NP12, respectively. For NP5, the reaction was quenched 60 s after the TOP-Se injection by the injection of 5 mL of room-temperature toluene. For NP12, the reaction solution was kept at high temperature for 180 s. After the solution cooled down to room temperature, the CdSe NPs were precipitated by adding ethanol and centrifuging; this washing step was repeated twice. Finally, the CdSe NPs were re-dissolved in toluene and stored inside a glove box under nitrogen atmosphere. The size measurement of the newly synthesized pristine nanoparticles was based on a transmission electron microscopy (TEM) image analysis using an automatic procedure (analyse particles) in the FIJI software. The study of their behaviour in aqueous solution, by TEM image analysis, highlighted a satisfactory stability (good dispersion and no meaningful change in size). The properties and characteristics of CdSe NPs are described in Supplementary Table 1.

2.2. Test species and culture conditions

The experiments were performed with the marine diatom *P. tricornutum* BOHLIN COUGHLAN/−632 axenic strain from the algal culture collection of the Göttingen University (SAG: Sammlung von Algenkulturen der Universität Göttingen), Germany. *P. tricornutum* was cultured in 250-mL flasks containing 100 mL of autoclaved f/2 medium realized with filtered natural seawater. Culture medium was inoculated at the cell density of about 8×10^5 cells/mL from a 7-day-old mother culture.

To determine the effect of CdSe NPs on growth, oxygen production, intracellular ROS level and proteome evolution of *P. tricornutum*,