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Influence of phosphate on phytotoxicity of ceria nanoparticles in an agar medium☆

Guohua Wang [a, 1], Yuhui Ma [a, 1], Peng Zhang [a], Xiao He [a], Zhaohui Zhang [b], Meihua Qu [c], Yayun Ding [a], Junzhe Zhang [a], Changjian Xie [a], Wenhe Luo [a], Jing Zhang [d], Shengqi Chu [d], Zhifang Chai [a], Zhiyong Zhang [a, e, *]

[a] Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China
[b] School of Public Health, University of South China, Hunan, 421001, China
[c] Weifang Medical University, Shandong, 261042, China
[d] Beijing Synchrotron Radiation Facility, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China
[e] School of Physical Sciences, University of Chinese Academy of Sciences, Beijing 100049, China

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ABSTRACT

Fate and toxicity of manufactured nanoparticles (NPs) in the living organisms and the environment are highly related to their transformation. In the present study, the effect of phosphate on the phytotoxicity and transformation of CeO_2 NPs was investigated in an agar medium using head lettuce plants that are sensitive to Ce^{3+} ions. Plants were treated by CeO_2 NPs with or without phosphate for 10 days. Results suggest that the treatments of P deficiency (P(-)) and CeO_2 NPs (P(+)&Ce) could separately induce significant inhibition on the growth of lettuce seedlings and cause oxidative stress, but the inhibition was the most serious when the two conditions were combined (P(-)&Ce). In the absence of phosphate, more CeO_2 NPs were transformed to Ce(III) in the roots and more Ce^{3+} ions were translocated to the shoots, which induced higher toxicity to head lettuce. Phosphates could alleviate the phytotoxic effect of CeO_2 NPs through the precipitation of dissociated Ce^{3+} ions. Considering the wide existence of phosphate in the environment, phosphate-related transformation may be a critical factor in evaluating the toxicity and fate of many other metal-based NPs.

1. Introduction

Engineered nanoparticles (NPs) exhibit many novel physicochemical properties, which differ greatly from their bulk or ionic counterparts. However, accompanying the ever-expanding applications is the growing anxiety about their potential to affect the environment. Current literature reveals that some of the NPs used in consumer products are detected in the environment (Benn and Westerhoff, 2008; Brar et al., 2010; Gottschalk et al., 2009), yet their risk assessment, including phytotoxicity, is still greatly insufficient. Previous research had shown that many engineered NPs were able to be absorbed and accumulated in plants (Zhang et al., 2015a). For example, once inside the root of woody poplar, Au NPs can be transferred from one cell to another through plasmodesmata (Zhai et al., 2014). Several studies had demonstrated that NPs taken up by roots can be translocated to the above-ground parts (Zhang et al., 2011, 2017b; Rui et al., 2014; Ma et al., 2015a). Through food consumption, NPs may be transmitted to herbivorous consumers, as well as human beings (Koo et al., 2014). Consequently, the impacts of NPs on edible plants and the possible effects in the whole ecosystem have become a serious concern.

Ceria (CeO_2) NPs, as one of the 10 most produced NPs in the world (Piccinno et al., 2012), are widely used in catalysts, polishing agents, fuel cells industries, and so on (Yamamoto et al., 2014; Yang et al., 2016; Yu et al., 2008). Given the widespread use, it is urgent to understand their biological effects and potential risk. A number of studies have reported the phytotoxicity

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* Corresponding author. Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China.
E-mail address: zhangzhy@ihep.ac.cn (Z. Zhang).

[1] The two authors contributed equally to this article.

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of CeO_2 NPs in plants. Andersen et al. (2016) found that the root lengths of several agronomic plant species examined were significantly decreased by CeO_2 NPs treatment at 1000 mg/L. Wang et al. (2013) investigated the *trans*-generational toxicity of CeO_2 NPs on tomato plants, in which the second generation seedlings grown from treated seeds were weaker and smaller with extensive root hairs compared with the control second generation seedlings. A recent study conducted by Ma et al. (2016) demonstrated that *Brassica rapa* made different responses to CeO_2 NPs exposure with varying levels of hydrogen peroxide (H_2O_2) in tissues at the vegetative stage, the floral stage and the seed ripening stage, indicating that the toxicity of CeO_2 NPs to *Brassica rapa* was growth-stage dependent. In addition, the genotoxicity of CeO_2 NPs in plants was also studied. Mattiello et al. (2015) investigated early genotoxic effect of CeO_2 NPs on seedlings of *Hordeum vulgare* L. Results illustrated that modifications in the chromatin aggregation in the nuclei of shoot and root parenchymal cells were induced and mitotic index in root tips cells was reduced after treatment with high concentration of CeO_2 NPs, which demonstrated the genotoxic effect of CeO_2 NPs treatment on the cell cycle. Tumburu et al. (2015) demonstrated that part of genes in *Arabidopsis thaliana* were significantly regulated by exposure to CeO_2 NPs. As a result, a range of metabolic processes, including deoxyribonucleic acid (DNA) metabolism, may be affected.

However, the toxicity mechanisms of CeO_2 NPs on plants are still not well understood. Previous studies had confirmed that part of $Ce(IV)O_2$ NPs can be transformed to Ce(III)-containing compounds at the root surface in hydroponic plants (Zhang et al., 2012b; Ma et al., 2015b; Dan et al., 2016). Similarly, the dissolution and transformation of CeO_2 NPs in soybeans and corn cultivated in soil were also reported (Hernandez-Viezcas et al., 2013; Zhao et al., 2012). The release of Ce^{3+} ions was proved to be the main reason for toxicity of CeO_2 NPs to *lactuca* plants in the aqueous suspensions (Zhang et al., 2015b), agar media (Cui et al., 2014), or potting soil (Gui et al., 2015). Meanwhile, the transformation process of CeO_2 NPs could be influenced by phosphates (Rui et al., 2015), which widely exist in environment and are basic components of many culture media used in the toxicity testing. One of our recent studies has also shown that the phytotoxicity of CeO_2 NPs to romaine lettuce was determined by phosphates in a sand culture (Zhang et al., 2017a). On the other hand, culture medium is also an important factor to affect the toxicity of NPs. For instance, at the seed germination stage, the toxicity of CeO_2 NPs to asparagus lettuce in aqueous suspensions was higher than in agar media at the same exposure concentration (Cui et al., 2014). Considering that the behavior and toxicity of CeO_2 NPs are culture media and growth stage dependent, more studies are needed to understand the interaction between CeO_2 NPs and plants.

In the present study, at relatively low concentration (200 mg/L) and longer exposure time (10 d), we investigated how phosphate influences the transformation and phytotoxicity of CeO_2 NPs in an agar medium on head lettuce, which is a popular vegetable and is among the plant species for assessing the phytotoxicity recommended by US EPA (1996). To better characterize the interaction between lettuce and NPs in the presence or absence of phosphate, root/shoot lengths, biomass, and enzyme activities were measured. A combination of comprehensive analytical techniques, namely transmission electron microscopy (TEM), X-ray absorption near-edge structure (XANES) and linear combination fitting (LCF) were adopted to study the distribution and speciation of Ce in plant tissues. The results of this study will provide an insightful understanding of the phytotoxicity of CeO_2 NPs in the natural environment.

2. Materials and methods

2.1. CeO_2 NPs synthesis and characterization

All the reagents used for synthesis were of analytical grade. CeO_2 NPs with the size of ca. 7 nm were prepared according to the approach described by Zhang et al., (2011). CeO_2 NPs stock solution was kept in deionized water at room temperature.

The crystal form of the CeO_2 NPs was analyzed by X-ray diffractometer (XRD, X'Pert PRO MPD, Netherlands). The images of TEM were obtained using a Tecnai G2 20 S-Twin transmission electron microscope 119 (FEI Company, Japan). The homogeneity of the CeO_2 NPs in the agar media was observed with a dark-field microscope (Olympus BX51, Olympus Cooperation, Japan).

2.2. Plant culture and nanoparticles exposure

Modified Hoagland nutrient solutions with 1 mM PO_4^{3-} and without PO_4^{3-} were referred to as P(+) NS and P(-) NS, respectively. Each culture medium contained 40 mL 1 mM P(+) NS or P(-) NS with 1% agar inside and was heated at 120 °C for 2 h. Then CeO_2 NPs and equal amounts of deionized water were added into the hot agar solution to obtain 200 mg/L suspensions or to obtain solutions without NPs. After a 15-min ultrasonication (100 W, 40 kHz) at 60 °C, the four different kinds of agar solutions were quickly solidified in 90 mm × 18 mm Petri dishes at −20 °C. Accordingly, four experimental groups were set in this study. Plants cultured in +P NS-prepared agar media without CeO_2 NPs was taken as the control group (CT). The other three groups were referred to as P(-) group (plants cultured in P(-) NS-prepared agar media without CeO_2 NPs inside), P(+)&Ce group (plants cultured in P(+) NS-prepared agar media containing 200 mg/L CeO_2 NPs) and P(-)&Ce group (plants cultured in P(-) NS-prepared agar media containing 200 mg/L CeO_2 NPs), respectively.

The seeds of head lettuce (*Lactuca sativa* L. var. *capitata* L., purchased from the Chinese Academy of Agricultural Sciences) were sterilized by 3% (v/v) H_2O_2 solution for 30 min and then washed with deionized water. After being soaked in ultrapure water for 2 h, 50 seeds were planted 1 μm deep below the surface of the agar in each Petri dish, and then they were covered and sealed. Five replicates were set for each group. After a 1-day vernalization period at 4 °C, the media were placed to darkness at a constant temperature of 20 °C for two days. Then the media were transferred to a controlled environment cabinet (PRX 450D, Saifu Laboratory Instrument Cooperation, China) with 16/8 h of light/dark at 22/18 °C. After 10 days of growth, the plants were harvested from the agar. Lengths and dry weights of shoots and roots were measured from each replicate dish.

2.3. Influence of phosphate on the uptake of cerium

Shoots and roots of P(+)&Ce and P(-)&Ce groups were washed thoroughly with deionized water and then lyophilized. The dried tissues were digested using a mixture of plasma-pure HNO_3 and H_2O_2 (v/v: 4/1) at 160 °C. After cooling down, the clear solutions obtained were diluted and quantified by inductively coupled plasma mass spectroscopy (ICP-MS, Thermo, X7, USA) for cerium content. As an internal standard, indium (20 ng/mL) was added to compensate for matrix suppression and signal drifting.

2.4. Antioxidative enzyme activities

After 10 days of growth, the plants of four different groups were harvested and then were washed with deionized water thoroughly. Plant tissues (shoots or roots, 100 mg) were homogenized in 0.9 mL

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