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Transformation of ceria nanoparticles in cucumber plants is influenced by phosphate

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ABSTRACT

Transformation is a critical factor that affects the fate and toxicity of manufactured nanoparticles (NPs) in the environment and living organisms. This paper aims to investigate the effect of phosphate on the transformation of CeO_2 NPs in hydroponic plants. Cucumber seedlings were treated with 2000 mg/L CeO_2 NPs in nutrient solutions with or without adding phosphate (+P or −P) for 3 weeks. Large quantities of needle-like $CePO_4$ was found outside the epidermis in the +P group. While in the −P group, $CePO_4$ only existed in the intercellular spaces and vacuole of root cells. X-ray absorption near edge spectroscopy (XANES) indicates that content and percentage of Ce-carboxylates in the shoots of −P group (418 mg/kg, 67.5%) were much higher than those in the +P group (30.1 mg/kg, 21%). The results suggest that phosphate might influence the transformation process of CeO_2 NPs in plants and subsequently their ultimate fate in the ecosystem.

1. Introduction

CeO_2 NPs are increasingly used in a wide variety of diverse applications such as fuel-borne catalysts, UV-blockers, ceramics, solid-state fuel cells and polishing agents due to their unique properties including high oxygen storage and release ability, UV absorption ability, and high hardness and reactivity (Cassee et al., 2011). The estimated production of CeO_2 NPs is around 1000 tons per year in the world (Piccinno et al., 2012). The environmental concentration of CeO_2 will inevitably increase with their continuing use. Harmful effects of CeO_2 NPs to terrestrial plants and plant-associated soil bacteria have been reported by a number of studies recently (Ma et al., 2010; Zhang et al., 2013; Rico et al., 2013; López-Moreno et al., 2010; Bandyopadhyay et al., 2012; Antisari et al., 2011). Plant is the essential component of the environment and food source for animals and human beings. Accumulation of ENPs may not only impair the plant growth, but also potentially contaminate the food chain, posing a threat to environmental safety and human health (Judy et al., 2011).

Uptake and accumulation of CeO_2 NPs in plants including edible tissues were reported in recent studies (Zhang et al., 2012, 2013; Rico et al., 2013; López-Moreno et al., 2010; Bandyopadhyay et al., 2012; Wang et al., 2012). However, there is only limited information on the chemical species of accumulated Ce in plants. Actually, NPs may undergo various kinds of physical, chemical, or biological transformations in environmental and biological conditions (Lowry et al., 2012). These transformations will alter the fate, transport, and toxicity of the NPs. Since CeO_2 NPs are generally considered to be highly stable, transformation of CeO_2 NPs is rarely concerned. However, in a previous study, we found that nano-sized $Ce(IV)O_2$ was reduced to Ce(III) in cucumber plants under hydroponic conditions and biogenic reducing substances and organic acids were

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the key factors involved in the biotransformation process (Zhang et al., 2012). Transformation of CeO_2 NPs in agar and soil cultivated plants was also reported (Zhao et al., 2012; Cui et al., 2014). Recently, we found that the high sensitivity of *Lactuca* plants to the released Ce^{3+} ions caused the species-specific phytotoxicity of CeO_2 NPs (Zhang et al., 2013). These results indicate that the behavior of CeO_2 NPs in plants is highly related to their transformation processes. It can be expected that the alteration of the transformation may affect the uptake, translocation and toxicity in plants.

Phosphates widely exist in environment and phosphorus is one of the essential elements for plant growth. It is also a basic component of buffer solution and culture media in laboratory studies. In particular, phosphate is a strong precipitant for many metals including rare earth elements ($CePO_4$: $K_{sp} = 10^{-24}$) (Byrne et al., 1996). For instance, phosphates can induce the speciation and structural transformation of ZnO NPs by forming $Zn_3(PO_4)_2$ in aqueous environment (Lv et al., 2012). As described earlier, rare earth (RE) oxide NPs (La_2O_3, Yb_2O_3 and CeO_2) can transform to $REPO_4$ in cucumber plants (Ma et al., 2011; Zhang et al., 2011a; 2012).

This paper aims to investigate whether phosphate can affect the transformation and subsequent translocation of the transformation products in hydroponic cucumber plants. Distribution and chemical species of Ce in the plants cultured in nutrient solution with and without phosphate were compared. Multiple analytical methods including transmission electron microscopy (TEM), synchrotron-based scanning transmission soft X-ray microscopy (STXM), and X-ray absorption near edge spectroscopy (XANES) were used. This study will provide an insightful understanding of the transformation and translocation of NPs in plants.

2. Materials and methods

2.1. Chemicals and seeds

All the commercial chemicals were analytical grade. Cucumber (*Cucumis sativus*) seeds (Zhongnong NO.8) were purchased from Chinese Academy of Agricultural Sciences.

2.2. Synthesis and characterization of CeO_2 NPs

CeO_2 NPs were synthesized by a precipitation method (Zhang et al., 2011b). TEM (JEM 200CX, Japan) was used to determine the particle morphology and size. Hydrodynamic size and Zeta potential of the CeO_2 NPs suspension (20 mg/L) in deionized water and nutrient solution were measured by a dynamic light scattering (DLS) system (Malvern, UK).

2.3. Seedling culture and nanoparticle application

Seeds were sterilized by 10% NaClO solution for 10 min and rinsed with deionized water thoroughly. Then the seeds were arrayed on moist filter papers in Petri dishes and placed in an artificial climatic chamber (PRX-450C, Saifu, China) at 25 °C in darkness. After 3 days, uniform seedlings were selected and each seedling was anchored by a plastic foam and transferred into a 250 mL beaker containing 100 mL modified 1/4 strength Hoagland solution. Six replicates were set for each treatment. The seedlings were allowed to grow in the climate chamber with 16-h photoperiod (light intensity of 1.76×10^4 μmol/m^2 s), 25 °C/18 °C day/night temperature and 50%/70% day/night humidity for 10 days before CeO_2 NP exposure.

Modified 1/4 strength Hoagland nutrient solution with 0.25 mM PO_4^{3-} (called "+P NS") and without PO_4^{3-} (called "−P NS") were prepared. KCl was added in −P solution to a concentration of 0.25 mM to compensate for the reduced potassium nutrient (Stewart et al., 2001). CeO_2 NPs were then added into the +P NS and −P NS to a concentration of 2000 mg/L followed by ultrasonic pretreatment for 15 min. A high exposure concentration can lead to a proper accumulative concentration of Ce in plant shoots (>10 ppm) which is necessary for collection of XANES spectra with a good signal quality (Zhang et al., 2012). The seedlings were then transferred into the beakers with CeO_2 suspensions and allowed to grow in the chamber for three weeks. Each beaker contained 100 mL CeO_2 NP suspension with one seedling. A constant volume (100 mL) in each beaker was maintained by replenishing with fresh nutrient solution every 2 days. To maintain the required phosphors for the normal growth of plants, 1 mM KH_2PO_4 solution (~10 mL for each plant) was sprayed on foliage of plants in −P groups every two days. Plants in +P and −P NS without CeO_2 NPs were set as controls.

2.4. Biomass and Ce content determination

After treatment for 21 days, plants were harvested and washed by flowing tap water and then deionized water thoroughly. Roots and shoots were separated, lyophilized and weighed. The dry samples were then ground to fine powders and digested with a mixture of HNO_3 and H_2O_2 on a heating plate (80 °C for 1 h, 120 °C for 3 h, and 160 °C for 0.5 h). The digestive residues were diluted with deionized water and the Ce contents were analyzed by ICP-MS (Thermo X7, USA). Bush branches and leaves (GBW07602) were also digested and analyzed by ICP-MS as standard references. Indium of 20 ng/mL was used as an internal standard to compensate for the matrix suppression and signal drifting. The linearity ranged from 0.1 to 50 ng/mL, Recovery from the standard reference was 99.1%. Spike recovery was 101%. Relative standard deviation was 1.5% and the detection limit is 0.01 ng/mL. Translocation factors (TF) of Ce in plants were calculated by comparing Ce contents in shoots to that in roots.

2.5. TEM observation

After growing for 21 days, plant roots were washed with tap water and deionized water thoroughly and the root apexes were cut and fixed in 2.5% glutaraldehyde solution. Then they were dehydrated and embedded in Spurr's resin. Ultrathin sections of 90 nm were cut by an UC6i ultramicrotome (Leica, Austria) with a diamond knife and collected on copper grids. To avoid the image illusion that may be induced by high metal stain, uranyl acetate and lead citrate that are commonly used in TEM sample preparation were not used here. Sections were observed on a JEM-1230 (JEOL, Japan) transmission electron microscope operating at 80 kV.

2.6. In situ speciation of Ce in roots by STXM

Root sections of cucumber were analyzed by STXM to determine the chemical species of Ce *in situ*. STXM analyses were performed on the beamline BL08U1 at Shanghai Synchrotron Radiation Facility. CeO_2 NPs, $CePO_4$ and $Ce(CH_3COO)_3$ were chosen as the reference materials. The reference materials were ultrasonically dispersed in ethanol and deposited on a TEM grid. Root sections with a thickness of 1.5 μm were prepared by the same protocols as the TEM sample preparation. For STXM analyses, a dual-energy method was performed on the chosen regions of the sample and a Ce element map was derived by calculation to ensure the existence of the Ce-component in the regions. Then, image sequences (called "stack") were acquired at energies spanning the relevant element absorption edge (from 884 to 915 eV for Ce $M_{4,5}$ edge) and aligned via a spatial cross correlation analysis method. Finally, XANES spectra were