Environmental Pollution 230 (2017) 902–910

Contents lists available at ScienceDirect

Environmental Pollution

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

Abnormal pinocytosis and valence-variable behaviors of cerium suggested a cellular mechanism for plant yield reduction induced by environmental cerium☆

Lihong Wang [a, b], Jingfang He [a], Qing Yang [a], Xiaofen Lv [b], Jigang Li [c], David D.Y. Chen [d], Xiaolan Ding [e], Xiaohua Huang [a, *], Qing Zhou [b, **]

[a] Jiangsu Collaborative Innovation Center of Biomedical Functional Materials, Jiangsu Key Laboratory of Biomedical Materials, School of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210046, China
[b] State Key Laboratory of Food Science and Technology, Jiangsu Key Laboratory of Anaerobic Biotechnology, School of Environment and Civil Engineering, Jiangnan University, Wuxi 214122, China
[c] State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing 100193, China
[d] Department of Chemistry, University of British Columbia, Vancouver V6T 1Z4, British Columbia, Canada
[e] State Key Laboratory of Biomembrane and Membrane Biotechnology, Department of Biological Science and Biotechnology, Tsinghua University, Beijing 100084, China

ARTICLE INFO

Article history:
Received 3 February 2017
Received in revised form
9 July 2017
Accepted 11 July 2017

Keywords:
Rare earth elements
Cerium
Plant cell
Life cycle
Valence-variable behavior

ABSTRACT

The environmental safety of cerium (Ce) applications in many fields has been debated for almost a century because the cellular effects of environmental Ce on living organisms remain largely unclear. Here, using new, interdisciplinary methods, we surprisingly found that after Ce(III) treatment, Ce(III) was first recognized and anchored on the plasma membrane in leaf cells. Moreover, some trivalent Ce(III) was oxidized to tetravalent Ce(IV) in this organelle, which activated pinocytosis. Subsequently, more anchoring sites and stronger valence-variable behavior on the plasma membrane caused stronger pinocytosis to transport Ce(III and IV) into the leaf cells. Interestingly, a great deal of Ce was bound on the pinocytotic vesicle membrane; only a small amount of Ce was enclosed in the pinocytotic vesicles. Some pinocytic vesicles in the cytoplasm were deformed and broken. Upon breaking, pinocytic vesicles released Ce into the cytoplasm, and then these Ce particles self-assembled into nanospheres. The aforementioned special behaviors of Ce decreased the fluidity of the plasma membrane, inhibited the cellular growth of leaves, and finally, decreased plant yield. In summary, our findings directly show the special cellular behavior of Ce in plant cells, which may be the cellular basis of plant yield reduction induced by environmental Ce.

1. Introduction

Environmental pollution of heavy metal elements and their effects in plants have attracted great attention from scientists worldwide, especially metal ions in trivalent or higher valences, due to their toxicity and susceptibility to the environment (Abdolali et al., 2015; Araújo and Cedeño-Macias, 2015; Guo and Yang, 2016; Wu et al., 2016). Rare earth elements (REEs), specifically, fifteen lanthanides, are a set of heavy metal elements in trivalent or higher states (Redling, 2006). They have excellent magnetic susceptibility, radiant energy absorption, luminescence, chemical bonding behavior, and other advantages (Redling, 2006). Because of these special properties, REEs have been widely used in electronics, optics, communications, lasers, agriculture, medicine (such as phosphate binders, treatment of burns, diagnosis and treatment of cancer, and contrast agents), environmental protection, and many other fields (Redling, 2006; Stone, 2009; Tyler, 2004). These uses have led to over-accumulation of REEs in the environment (Biasioli et al., 2012; Gonzalez et al., 2014; Kulaksiz and Bau, 2011; Moreno et al., 2008; Wiseman et al., 2015). In soils worldwide, researchers estimated that the content of REEs has reached 1000 mg kg^{-1}

☆ This paper has been recommended for acceptance by Dr. Chen Da.
* Corresponding author.
** Corresponding author.
E-mail addresses: huangxiaohuanjnu@yahoo.com (X. Huang), qingzhou510@yahoo.com (Q. Zhou).

http://dx.doi.org/10.1016/j.envpol.2017.07.034
0269-7491/

(Biasioli et al., 2012; Gonzalez et al., 2014). In the atmospheric particulate matter of Mexico and Tokyo, the REE content is approximately 10 ng m^{-3} (Moreno et al., 2008) and 44.1 μg m^{-3}, respectively. In the water of Rhine River rainwater, Germany, the REE content could be up to 610 ng kg^{-1} (Kulaksiz and Bau, 2011). Of all REEs, cerium (Ce) ($4f^{1}5d^{1}6s^{2}$) is widely used in industry, agriculture and medicine because it has two stable valence states [trivalence, Ce(III) and quadrivalence, Ce(IV)] in chemical reaction and relatively low toxicity in living organisms (Kostova et al., 2005; Redling, 2006). Therefore, Ce has become the most abundant REE in the environment and in living organisms (Biasioli et al., 2012; Kato et al., 2011; Kulaksiz and Bau, 2011; Moreno et al., 2008; Ni, 1995; Suzuki et al., 2011; Wiseman et al., 2015). It is noteworthy that excess Ce can cause detrimental effects on living organisms from plants to humans that include yield reduction for plants (Redling, 2006). Plants are the primary producer in ecosystems, and Ce is not essential for many physiological processes in plants. Therefore, to protect the ecosystem, it is urgent to elucidate the mechanism of Ce(III) actions in plants to establish a maximum permissible limit for REEs in plants as soon as possible.

The study on the toxic effect of Ce(III) in plants dates back to 1917 (Chien and Ostenhout, 1917). Many advances have been achieved in recent years (Guo and Huang, 2007; Redling, 2006; Schwabe et al., 2015). At the level of whole plant, the major symptom of Ce toxicity is global decreases in plant yield and growth (Guo and Huang, 2007; Redling, 2006; Schwabe et al., 2015). At the physiological level, inhibitory effects of Ce(III) on photosynthesis, respiration, mineral nutrient uptake and metabolism, and hormonal balance are often reported (Guo and Huang, 2007; Redling, 2006; Schwabe et al., 2015). Within cells, Ce toxicity is associated with oxidative stress, membrane damage and alteration of numerous enzymatic activities (Guo and Huang, 2007; Redling, 2006; Schwabe et al., 2015). Despite almost a century of research, the cellular mechanisms remain largely unknown (Guo and Huang, 2007; Redling, 2006; Schwabe et al., 2015) because investigators have known little about the life cycle and behaviors of Ce(III) in plant cells. By using electron microscopic autoradiography (EMARG), we previously observed that Ce(III) can enter the cells of the horseradish leaf (Guo and Huang, 2007). However, in the study of the behaviors of Ce(III) in plant cells, some key questions are still unclear: How does Ce(III) enter plant cells? How does Ce(III) remain in plant cells? Which valence state of Ce deposits in plant cells? How does Ce(III) regulate cellular responses?

In this study, we reported some special cellular behaviors of Ce(III) in plant cells by using interdisciplinary methods of EMARG, X-ray photoelectron spectroscopy (XPS), confocal laser scanning microscopy (CLSM), gas chromatography (GC), and scanning electron microscope (SEM), etc. Our study provides a new opportunity to probe into the mechanism of Ce(III) action in plants.

2. Materials and methods

2.1. Materials

The $^{141}CeCl_3$ solution was prepared by dissolving $^{141}Ce_2O_3$ powders (Beijing Atom High Tech Co. Ltd.) in the concentrated HCl and H_2O_2. The nuclear emulsion was purchased from the Technical Institute of Physics and Chemistry, Chinese Academy of Sciences. $CeCl_3$ (purity>99.99%) and other chemical reagents were purchased from Sigma-Aldrich China, Inc. (Shanghai, China).

2.2. Plant culture and treatment

Horseradish [*Armoracia rusticana* (Lam.) Gaerth.] is an important commercial crop, and it is a typical representative of edible plants. It is widely used as a flavouring in Japan, Korea, Europe, America, etc. because it contains specific pungency (Veitch, 2004). In addition, horseradish is rich in horseradish peroxidase, which is an important industrial product (Veitch, 2004). Horseradish tubers were obtained from the Planting Base of Exported Horseradish in Dafeng City, Jiangsu Province, China, in which the Ce content in the airborne particulate matter of this base was 30 mg kg^{-1}, determined by inductively coupled plasma mass spectrometry (VG Plasma Quad Plus, Fisons Instruments, UK). Horseradish culture and treatment with Ce(III) were performed based on the previous reports (Wang et al., 2009, 2014). Horseradish tubers were planted in 30 × 30 cm^2 pots without any fertilizer. The basic properties of the soil were as follows: sandy, pH 6.56, 15.71 me 100 g^{-1} cation exchange capacity, 0.81% soil organic matter, 0.031 μg g^{-1} Ce. The horseradish plants were cultured in a glasshouse at the following condition: 25 ± 1 °C, 16 h light: 8 h dark cycle, 300 μmol m^{-2} s^{-1} light, and 70% relative humidity. At the four-leaf stage, some horseradish plants were evenly sprayed with 30, 80 or 300 μM $CeCl_3$ solution until the drops began to fall, meanwhile, the soil was covered with plastic wrap and filter paper, which were called the $CeCl_3$-treated plants; some horseradish plants was prayed with the deionized water, which were called as the control plants. The amount of $CeCl_3$ solution sprayed on each horseradish plant was approximately 50 mL. Eight days after being sprayed, some $CeCl_3$-treated and control plant leaves were collected for determining the net photosynthesis rate (P_n) [by a portable gas exchange system (CIRAS-1, PP Systems International Ltd, UK)], the chlorophyll content (Lichtenthaler, 1987), cell growth, fatty acids in the plasma membrane. The determination methods of the P_n and chlorophyll content were shown in the Supporting Information (SI).

2.3. Cell growth

To observe the size of the plant leaf cells, 100 mM Na-phosphate buffer (pH 7.0) containing 4% glutaraldehyde was used for fixing the fourth fully expanded rosette leaves of the plant. After fixation, the leaf samples were put into a series of concentrations of ethanol for dehydration. After infiltration, embedding, and cutting into sections, the plant leaf cells were observed under a microscope (Wang et al., 2014).

The protoplasts of horseradish leaves were isolated based on previously reported methods (Benoit et al., 2009; Wang et al., 2005). After fixation with 1% glutaraldehyde in 50 mM buffer solution (0.8 M mannitol and 50 mM Tris-HCl, pH 6.8) for 2 h at 4 °C, the protoplasts were washed several times with the above-mentioned buffer solution. After critical point drying, the protoplasts were mounted over the stubs with double-sided conductivity tape, and a thin layer of gold metal was applied over the samples using an automated sputter coater (K500X, Quorum Technologies Ltd, United Kingdom). The samples were examined under the condition of low vacuum with a secondary electron detector at 15 kV using SEM (JSM-5600LV, JEOL, Japan) at various magnifications (Wang et al., 2010a).

2.4. EMARG observation of subcellular distribution of Ce(III)

Horseradish leaves were treated with 0, 30 or 80 μM $CeCl_3$ solution that contained $^{141}CeCl_3$, respectively, and the radioactivities of these solutions were all 75 μCi mL^{-1}. After treating with ^{141}Ce(III)-contained Ce(III) solution for 12 h, 48 h and 8 d, the leaves were sampled and then cut into regular ultra-thin (~60 nm thickness) sections of 1.5 × 2 mm size by using Reichert Ultracut E ultramicrotome. These ultra-thin sections were painted with nuclear emulsion, and these sections were subsequently developed and fixed. The subcellular distribution of ^{141}Ce(III) was subsequently