Environmental Pollution 228 (2017) 363–369

Contents lists available at ScienceDirect

Environmental Pollution

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

Silica nanoparticles alleviate cadmium toxicity in rice cells: Mechanisms and size effects☆

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ARTICLE INFO

Article history:
Received 20 October 2016
Received in revised form
3 May 2017
Accepted 4 May 2017

Keywords:
Heavy metals
Genetic variation
Silicon nanoparticles
Cadmium
Size effects
Cell wall

ABSTRACT

Although it was recently determined that silicon can alleviate cadmium (Cd) toxicity in rice, the effects of silicon properties and the molecular mechanisms are still unclear. Here, the effect of silica nanoparticles (SiNPs) on Cd toxicity in rice was examined using cells cultured in suspension in the presence or absence of SiNPs (19 nm, 48 nm and 202 nm). The results showed that the presence of SiNPs substantially enhanced the proportion of live cells to 95.4%, 78.6% and 66.2%, respectively, suggesting that the extent of alleviation of Cd toxicity decreased gradually with size of SiNPs. The morphological results showed that dramatic damage and severe structural changes in the organelle integrity of cells occurred in the absence of SiNPs, whereas the cells exposed to the SiNPs remained nearly intact even in the presence of high concentrations of Cd. Furthermore, the SiNPs accumulated on the surface of the rice cells. Using inductively coupled plasma mass spectroscopy, Cd accumulated preferentially in plant cells with cell walls. In addition, noninvasive microtest technology showed that the average Cd^{2+} influx in those treated with SiNPs (19 nm, 48 nm and 202 nm) decreased by 15.7-, 11.1- and 4.6-fold, respectively. The gene expression of Cd uptake and transport (*OsLCT1* and *OsNramp5*) was inhibited by SiNPs, but the gene expression of Cd transport into vacuole (*OsHMA3*) and Si uptake (*OsLsi1*) was enhanced by the SiNPs. These results indicate that the presence of SiNPs increased at least 1.87-fold the Si uptake capacity and inhibited the Cd uptake capacity, which together resulted in the alleviation of the toxicity of Cd in rice. This study provided a molecular-scale insight into the understanding of the SiNPs-induced alleviation of the toxicity of Cd in rice.

1. Introduction

Rapid industrial development has resulted in the serious problem of contamination of the soil by toxic heavy metals (Aziz et al., 2015; Lu et al., 2009; Xiao et al., 2013). Of the heavy metals, cadmium (Cd) is one of the most toxic (Das et al., 1997). The annual release of Cd into the water and soil reached 22,000 tons by the 1990s (Liu et al., 2007). It has been reported that more than 1.3×10^5 km^2 of soil has been contaminated with Cd (Clemens et al., 2013). The contamination results in increased Cd accumulation in rice, which inhibits some physiological processes including respiration, photosynthesis, oxidative stress, and nitrogen metabolism (Wang et al., 2015a). Rice is a staple food crop for more than two billion people (Li et al., 2009b). Cd-polluted rice can cause long-term health risks including effects on bone mineralization and cancer (Nawrot et al., 2010). Therefore, it has become a public health issue to reduce the Cd accumulation and alleviate the toxicity of Cd in rice. The Cd concentration of soil is more than 0.2 mg/kg, which is the World Health Organization's regulatory level. To control pollution sources and remediate Cd-contaminated soil, many remediation techniques have been used including soil moisture management (Li and Xu, 2015), chemical cleanup (Xu et al., 2014), and phytoremediation (Li et al., 2009a) and screening of rice varieties with a low uptake of Cd (Wang et al., 2011). However, chemical cleanup methods can destroy the soil physicochemical properties and produce secondary pollution. The phytoremediation technique is a long-term process that requires changes in the traditional mode of planting.

Silicon (Si), which is the second most abundant element in soils,

☆ This paper has been recommended for acceptance by B. Nowack.
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http://dx.doi.org/10.1016/j.envpol.2017.05.014
0269-7491/

is able to protect plants from multiple abiotic and biotic stresses, improve the resistance of plants to diseases, and minimize transpiration losses of crops (Liang et al., 2007; Ma, 2004; Nawrot et al., 2010). Rice is one of the most efficient Si accumulators (up to 10% of total dry weight) (Epstein, 1994). It was recently reported that the application of Si fertilizer can alleviate Cd toxicity to crops (Zhang et al., 2008; Shi et al., 2005). Furthermore, Si can minimize the uptake and transport of Cd in rice seedlings and maintain the cell wall stability during its division (Liu et al., 2013). Recently, Wang et al. used suspension cultures of rice cells to explore the Si-cadmium interactions and found that most of the Si accumulated in the cell walls via a co-complexation mechanism that reduced the Cd uptake into cells (Liu et al., 2013). In addition, the role of Si in Cd resistance in rice was analyzed using a proteomic approach, in which 50 proteins including the low-affinity cation transporter (*OsLCT1*) and natural resistance-associated macrophage protein (*Nramp*) family were found to be regulated by Si and Cd-induced proteins (Ma et al., 2006; Sasaki et al., 2012; Uraguchi et al., 2011).

Nanotechnology has received increasing attention in the context of agriculture and environmental protection in recent years due to the unique chemical and physical properties of the nanomaterials. The applications of these materials range from crop improvement applications to fertilizer formulations. Nanoscale silicon fertilizer demonstrates extraordinary increases in bioavailability and solubility in comparison to the conventional Si fertilizers (Suriyaprabha et al., 2014). Therefore, nanoscale silicon fertilizer is absorbed rapidly by plants. Wang et al. found that a silicon nanoparticles (SiNPs) hydrosol alleviated Cd toxicity in rice by decreasing the Cd accumulation and the malondialdehyde level and by increasing the content of some mineral elements and the antioxidant capacity (Wang et al., 2015b). Our previous study also reported that foliar application of SiNPs hydrosol could significantly increase the dry weights of grains and shoots of rice grown in Cd-contaminated soil, and the Cd content of the grains and shoots were clearly decreased in parallel. The application of a SiNPs hydrosol provides an economically and environmentally friendly strategy to reduce cadmium accumulation in rice (Liu et al., 2009).

Although the impact of SiNPs on the alleviation of Cd toxicity at the whole-plant and tissue levels has been demonstrated, the underlying mechanisms involved in the Si NPS-induced alleviation of the toxicity of Cd remains unclear. To avoid the complexity of the whole plants or tissues, we used suspension cultures of rice cells as a model system to investigate the cellular and chemical mechanisms of the SiNPs-induced alleviation of Cd toxicity using a combination of plant cell nutritional, molecular biological and physical techniques. While the soluble form of Si ($[Si(OH)_4]$) was previously used to investigate the molecular mechanisms by which Si alleviates the Cd toxicity in suspension cells (Adrees et al., 2015; Ma et al., 2006), the size effects and the mechanisms of the SiNPs-induced alleviation of Cd toxicity are still not understood. Therefore, the aims of this study were to elucidate the molecular mechanisms involved in the SiNPs-induced alleviation of the Cd toxicity. The observations from this study will be helpful in understanding the underlying molecular mechanism responsible for the SiNPs-Cd interaction in rice and may potentially provide a new strategy for developing effective fertilizers to alleviate Cd toxicity in rice.

2. Materials and methods

2.1. Cultures of cells and protoplasts

Rice suspension cells of Nipponbare (*Oryza sativa* L. *japonica*) were incubated according to the reported literature (Liu et al., 2013; Thomas et al., 1989). Briefly, rice seeds were washed with water. The seeds were sterilized using ethanol (75%) and mercuric chloride (0.1%) for 10 min, and then cultured at 28 °C in the modified N6 medium (Table S1). After incubation of the seeds for 1 month in dark, the formed calli were transferred to a plastic flask. Rice liquid medium (Table S2) was added into a plastic flask and placed at 28 °C in dark (Liu et al., 2013). The suspension cells were cultured at 5 d intervals. The rice suspension cells were collected into the plastic flask in the exponential growth phase. And the part of incubated suspension cells was transferred and added into the fresh medium. The suspension cells were sealed to avoid contamination from dust. All solutions were made by ultra-high-purity water. The ultrapure water (18 MΩ.cm) was from the purification device (Milli-Q, Billerica, MA, USA). To produce protoplasts, the suspension cells were harvested by centrifugation and incubated in an enzyme solution to digest the cell walls. The protoplasts were added into 10 mL of enzyme solution containing cellulase (2.5%), hemicellulase (1%), mannitol (0.4 M), $CaCl_2$ (80 mM), $MgCl_2$ (0.125 mM) and 0.5 mM of 2-(N-morpholino) ethanesulfonic acid (MES). After incubation for 12 h, the protoplasts were obtained from the undigested suspension cells by centrifugation at 800×*g* for 10 min. The final protoplasts were washed three times with ultra-high-purity water and re-suspended in the medium solution (Liu et al., 2013). SiNPs with average sizes of 19 nm, 48 nm, and 202 nm were used as the Si source. The SiNPs was prepared through sol-gel method (Stöber et al., 1968). The detailed procedures in detail were described in SI-1. The SiNPs were characterized by transmission electron microscopy (Fig. S1), particle size distribution (Fig. S2), and Fourier-transform infrared spectroscopy (Fig. S3) as described in SI-2. The concentration of SiNPs is 1.0 mM. Different concentrations of Cd^{2+} (0, 10, 20, or 40 μM) were added into culture solutions for the following experiments.

2.2. Cell viability assay

Qualitative analysis of cell viability was performed using flow cytometry technique as previously described (Shi et al., 2007). Propidium iodide (PI) can pass through dead cell membranes and be formed a PI-nucleic acid conjugate (Liu et al., 2013). 1 μL PI solution was added into 99 mL of ultrahigh purity water and used fresh solution each time. PI can be excited by light at a wavelength 488 nm, and the emission light can be collected by the FL3 channel of the flow cytometer (Becton Dickinson, CA, USA). For this experiment, the rice cells were incubated in the absence or presence of SiNPs (1.0 mM) at a Cd concentration of 40 μM in the culture solution. In order to incorporate into the PI, the PI solution was added to 1.0 mL of each culture and incubated at 37 °C in the dark for 10 min. The final samples were washed three times in order to remove no-conjugated PI, and detected using flow cytometry. The excitation wavelength sets at 488 nm and the emission wavelength at 630 nm. Cell viability was detected by the fluorescence intensity of 20,000 cells. And the raw data were analyzed by CELLQuest software.

2.3. Cell morphology

The suspended rice cells were cultivated in the absence and presence of 20 nm SiNPs (1.0 mM) and/or 40 μM Cd^{2+}. The rice cells were grown on the surface of Si chip. 5 mL medium was added into the petri dish. After incubation for 48 h, the cells were harvested and then washed three times with buffer to remove the medium. Subsequently, the samples were fixed in 2.5% glutaraldehyde and OsO_4, and dehydrated in ethanol solutions (30, 50, 70, 95 and 100% v/v). Finally, the samples were embedded in Epon/Araldite resin (polymerization at 60 °C for 48 h). 90 nm thin sections were cut by ultramicrotome (Zeiss, Germany) and were stained with 4% uranyl acetate (1:1 acetone/water) and 0.2% lead citrate. The obtained