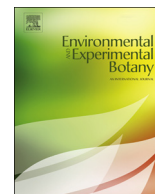




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journal homepage: www.elsevier.com/locate/envexpbotThallium-induced oxalate secretion from rice (*Oryza sativa* L.) root contributes to the reduction of Tl(III) to Tl(I)Yan Yao^a, Fuyi Zhang^a, Moyun Wang^a, Fang Liu^a, Wenfeng Liu^b, Xiuwuan Li^b, Dongdong Qin^b, Xinhua Geng^b, Xuexia Huang^c, Ping Zhang^{b,*}^a School of Life Science, Key Laboratory for Functional Study on Plant Stress-Resistant Genes, Guangzhou University, Guangzhou, China^b School of Chemistry and Chemical Engineering, Guangzhou University, Guangzhou, China^c School of Environmental Science and Engineering, Guangzhou University, Guangzhou, China

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ABSTRACT

Thallium (Tl) is a non-essential, highly toxic element having Tl(I) and Tl(III) two redox states. Tl(III) is more toxic to organisms than Tl(I) but is less of attention. The objective of this study was to investigate the reason of Tl(III) instability in the presence of plant. The content of Tl(III) was rapidly reduced from 100% to 16.1% in Tl solution cultivating rice seedlings while content of Tl(I) was increased from 0 to 83.8% within 8 h. High performance liquid chromatography (HPLC) analysis showed that organic acids exudates in rice roots caused the pH decline in rhizosphere, but only oxalate was the specific organic acid in root exudates under Tl stress. The anion channel inhibitor, nifumic acid (NIF), could efficiently inhibit the oxalate exudation but had no effect on oxalate content in root cytosol compared with Tl treatment. The exogenous oxalate, malate and citrate significantly contributed the reduction of Tl(III) to Tl(I). It was concluded that the contact of Tl(III) with organic acids in plant cells or in root exudates promoted the instability of Tl(III) with plants. Meanwhile, the presence of DTPA could effectively restrain the conversion of Tl(III) to Tl(I). This study provided better understanding of the basic biochemical mechanism for the reduction of Tl(III) in contact with plants. It will contribute to controlling relevant factors to stabilize Tl(III) for further toxic assessment on organism and to studying Tl(III) behavior in ecological system.

1. Introduction

Thallium is a non-essential, highly toxic heavy metal element. The major sources of natural thallium are deposits of sulphide ores and the extensive mining increases the amount of thallium into the environment (Krasnodębska-Ostreęa et al., 2005; Nelson and Chen, 2017). The areas with high amount of Tl are located in Poland (Cabala and Teper, 2006), Spain (Marqués et al., 2001), Turkey (Sasmaz et al., 2007), Switzerland (Voegelin et al., 2015) and China (Xiao et al., 2012). Tl is also found in the Tamar estuary, South-West England (Anagboso et al., 2013). Thallium adsorbed by plants and living organisms to the food chain can cause serious toxicity problems (Huang et al., 2016; Liu et al., 2017).

Thallium occurs in the environment in two redox states, namely Tl(I) and Tl(III). Tl(I) is thermodynamically more stable and less reactive compared to Tl(III) and is the dominant form in the environment. However, Tl(III) was still found in notable amount in river, lake, ocean, tap water and in plants (Lin and Nriagu, 1999; Mazur et al., 2016;

Sadowska et al., 2016; Belzile and Chen, 2017; Campanella et al., 2018). Mediated biological activity, biomethylation and oxidation were proved responsible for Tl(I) oxidation into Tl(III) (Huber and Kirchmann, 1978; Campanella et al., 2018; Schedlbauer and Heumann, 2000; Twining et al., 2003). Although both forms are dangerous for humans, plants and animals, Tl(III) is a few thousand times more toxic than Tl(I) (Lan and Lin, 2005; Ralph and Twiss, 2002). But there are few studies have been focused on Tl(III) (Belzile and Chen, 2017).

The information of Tl(III) remains limited due to the instability of Tl(III) in the environment. The extremely fast, spontaneous reduction of Tl(III) to Tl(I) is much faster than the complexation of Tl(III) (Sadowska et al., 2016). The partial reduction of Tl(III) to Tl(I) is always observed (Karlsson et al., 2006; Krasnodębska-Ostreęa et al., 2012). Sadowska et al. (2016) also noticed that it was not possible to totally avoid the reduction of Tl(III) to Tl(I) in contact with plant matrix. Belzile and Chen (2017) found that biotic and abiotic processes regulated the conversion in the complex interaction between thallium and plants (plant matrix rich in organic substances). Till now, there is no more

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investigation to confirm what the exact organic substances are in plant matrix causing the reduction of Tl(III). Meanwhile, many studies proved Tl(III) could be more stable when the complexes of Tl(III) with organic ligands such as ethylene diamine tetraacetic acid (EDTA, log K = 22.5), diethylene triamine pentacetate acid (DTPA, log K = 46) and diethyldithiocarbamate (DDTC) (Sadowska et al., 2016; Zembrzuska and Karbowska, 2017).

Low-molecular weight organic acids, mainly citrate, malate and oxalate, are prevailing constituents of all living cells (Zhu et al., 2011). Organic acids are also the important root exudates in rhizosphere during plant growth, which not only play a crucial role in osmoregulation, pH adjustment and ionic balance in vivo (Ma et al., 2011), but also in forming metal-metabolite complexes to regulate nutrient availability and in detoxifying undesirable metal pollutants, such as cadmium (Cd), aluminum (Al), gallium (Ga), copper (Cu), manganese (Mn), and lead (Pb) (Chen et al., 2017). At the same time, organic acids acting as reducing agents are secreted in roots to reduce the high oxidation state of some metal elements, e.g. iron Fe(III) to Fe(II) and chromium Cr(VI) to Cr(III), which are easy for plant absorption and utilization (Brumbarova et al., 2015; Romheld and Marschner, 1986; Rajniak et al., 2018; Zeng et al., 2008). Meanwhile, the type of organic-acid secretion was affected by plant nutritional stress and plant species (Chen et al., 2017). Aluminum (Al) could independently induce citrate secretion in rice root (Yokosho et al., 2011), and could induce malate and citrate from wheat root (Ryan et al., 2009; Sasaki et al., 2004). Yang et al. (2000) first found that lead (Pb) could increase oxalate content in rice root cells and in rhizosphere. The types of organic acid secreted by different plant species can be explained by the presence of specific membrane-localized transporters and anion channels being responsible for organic acid secretion (Chen and Liao, 2016). Many studies proved the anion channel inhibitors, such as niflumicalate acid (NIF) would affect the function of organic acid anions and the secretion of organic acids in root (Ryan et al., 2001; Zhu et al., 2011).

Rice (*Oryza sativa* L.) is the most important grain with regard to human nutrition and caloric intake in the world. Though several studies have investigated the uptake and accumulation of Tl in rice plants, they all focused on total Tl [mainly Tl(I)] (Huang et al., 2016, 2018). Compared to Tl(I), the effect of Tl(III) on plant is stronger due to the toxicity. Hence, our study will focus on (1) examining the stability of Tl(III) in contact with rice plants; (2) investigating the exact organic substances causing the reduction of Tl(III) in plants; and (3) identifying ways to avoid the reduction of Tl(III). This study will contribute to a better understanding of the mechanisms of interaction between thallium and plants and the behavior of Tl(I) and Tl(III) in plants.

2. Materials and methods

2.1. Plant materials and growth condition

For hydroponic experiments, seeds of rice (*Oryza Sativa* L.) cultivar of Huajinxian were fully imbibed with sterile water and then germinated at 26 °C in the dark. After 5 days, the germination seeds were transplanted into 1.1 L plastic pots (four holes per seedling holder, and one seedling per hole) containing aerated nutrient solution. The nutrient solution was prepared according to the method of Yoshida et al. (1976) with the following salts: NH_4NO_3 2.9 mM, NaH_2PO_4 0.32 mM, K_2SO_4 1.0 mM, CaCl_2 1.0 mM, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.7 mM, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 9.1 μM , $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ 0.52 μM , H_3BO_3 18 μM , $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.15 μM , $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.16 μM , $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 36 μM . The solution was adjusted to pH 6.0 and half concentration of the nutrient solution was applied for the first 3 days and then changed to full nutrient solution renewed every three days. 15-day-old seedlings were placed in 3.0 mM $\text{Ca}(\text{NO}_3)_2$ and 1.5 mM MgSO_4 solution (Renkema et al., 2015) overnight and then were subjected to different treatments. All the experiments were carried out in a controlled incubator with 12 h light period and 25 °C / 22 °C day/night temperatures, light intensity of 350 $\mu\text{mol m}^{-2}\text{s}^{-1}$

photon flux density and 75% relative humidity.

2.2. Preparation of Tl(III) solution

The solution of 25 μM Tl(III) was prepared as follows. 0.1246 g $\text{Tl}(\text{NO}_3)_3 \cdot 3\text{H}_2\text{O}$ was dissolved in 100 mL sterile deionized water, and diluted 100-fold at pH 6.0 for experiment. The solution of 25 μM Tl(III) + DTPA was prepared as follows. 0.1246 g $\text{Tl}(\text{NO}_3)_3 \cdot 3\text{H}_2\text{O}$ was dissolved in 100 mL 0.1 mM DTPA solution and diluted 100-fold at pH 6.0 according to the method of Sadowska et al. (2016) with some modifications.

2.3. The stability of Tl(III) in the presence or absence of rice seedlings

To study the stability of Tl(III) in the presence or absence of rice seedlings, 15-day-old seedlings were subjected to Tl(III) 25 μM treatment and Tl(III) 25 μM solution without seedling was used as control treatment. The concentrations of Tl(III) (% of total Tl) solutions in two treatments were determined in 0, 0.25, 0.5, 1, 2, 4, 6, and 8 h. Tl(III) and Tl(I) in the samples were separated using Chelex-100 resin according to the method of (Lin and Nriagu, 1999), and the contents of Tl were determined by flame atomic absorption spectrometry (AA6300, Shimadzu, Japan). Three replications were undertaken for each treatment (Fig. 1).

2.4. Visualizing in situ the changes of pH in rhizosphere

In order to visualize in situ the changes of pH in rhizosphere under three treatments, namely, control (deionized water), Tl and Tl adding NIF treatments, a modified agar plate technique was used (Calba et al., 1996; Pang et al., 2010). For Tl treatment, bromocresol purple, an indicator of pH, was added to agar medium and the final medium were agar 0.7%, bromocresol purple 0.015%, $\text{Ca}(\text{NO}_3)_2$ 3.0 mM and Tl(III) 25 μM (pH 6.0). The control medium was the same substances except Tl(III), and Tl adding NIF treatment was 50 μM NIF adding to Tl treatment. The rice roots embedded by media were placed in a smart light-temperature incubator (26 °C) and were observed the color change in 3 h and 6 h. Each treatment was three replicates, each replicate plate embedded four rice roots. The color of bromocresol purple indicator showing pH is from 3.5 to 10, i.e. yellow at pH 3.5–4.8, orange at pH 4.8 to 5.5, red at pH 5.5–6.4, and deep purple at pH 6.4–10 (Pang et al., 2010).

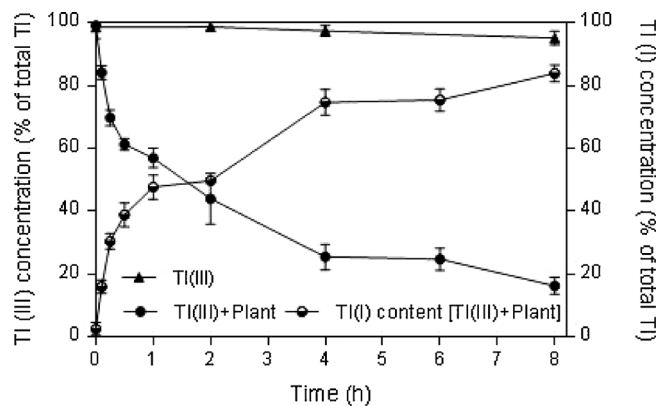


Fig. 1. Effects of rice seedlings on stability of Tl(III) in hydroponics system The concentrations of Tl(III) in Tl(III) with rice plant treatment and without rice plant treatment were determined from 0 to 8 h. Especially in Tl(III) with rice plant treatment, the concentrations of Tl(I) were determined either. Data are means \pm SD ($n = 3$).

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