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Kinetics of phyto-accumulation of hexavalent and trivalent chromium in rice seedlings

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ABSTRACT

Phyto-accumulation kinetics of chromium (Cr) was investigated using hydroponic experiments with excised roots or intact rice seedlings exposed to potassium chromate Cr(VI) or chromium nitrate Cr(III). Results showed that the accumulation kinetics was all described by *Michaelis-Menten* function, whereas very distinct half-saturation constant (K_M) and maximal accumulation capacity (v_{max}) was obtained for the respective Cr species. The comparison of the results from intact rice seedlings to detached roots indicated that transpiration has a stronger influence on Cr(III) transport by rice seedlings. Rice seedlings exposed to Cr(III) showed significantly higher potential for Cr accumulation in plant tissues than these exposed to Cr(VI). Although roots were the major site for Cr accumulation in both Cr treatments, restriction of root-to-shoot Cr translocation was more evident in Cr(III) treatments than Cr(VI) treatments. A remarkable difference in subcellular distribution of Cr in plant materials was observed between the two Cr treatments. Cell walls of rice seedlings exposed to Cr(III) accumulated more Cr in both roots and shoots than organelles and cytosol, whereas cytosol fraction was the largest in roots from the Cr(VI) treatments. Our results suggest that significant differences in uptake, accumulation and translocation pathways for Cr(VI) and Cr(III) exist in rice seedlings.

1. Introduction

Chromium (Cr) is one of most commonly found heavy metals in the environment because it easily can be accumulated in the living tissues and food webs (Ontañon et al., 2014; Jitar et al., 2015). The bioaccumulation of Cr in food webs not only threatens directly the biodiversity, but sometimes may cause adverse health effects to human (Ren et al., 2014; Chang et al., 2013; Jitar et al., 2015; Han et al., 2016). It is known that natural sources of Cr have been widely observed in the environment. However, the majority that influences the health of the environment is anthropogenic, derived from uncontrolled releasing from industrial activities and extensive use of Cr-containing materials in agriculture, including fertilizers, pesticides and sewage sludge (Zayed and Terry, 2003; Kuo et al., 2006; Zeng et al., 2011a; Tiwary and Dubey, 2016). It has been estimated that cumulative Cr production was approximately 105.4 million tons globally in 2000 and has been significantly increased since the 1950s (Han et al., 2002). Currently, the discharge of hexavalent (Cr(VI)) to surface water is regulated below 0.05 mg/l by the U.S. EPA, and total Cr including trivalent (Cr(III)), Cr(VI) as well as its other forms is regulated below 2.0 mg/l (Park et al., 2004; Han et al., 2007).

Previous studies have proven that Cr(VI) and Cr(III) are the most common and stable species in the environment. Because the dissimilarities that do exist between the two Cr species are particularly in chemical properties, occurrence, behavior, and biological effects, a number of toxicity studies have been conducted with different living organisms. Nutritionally, Cr(III) in small amounts is an essential component of a balanced human and animal diet for preventing adverse effects in the metabolism of glucose and lipids (Zayed and Terry, 2003). However, Cr(VI) is regarded as the most hazardous to animals and humans, leading to a variety of clinical problems as well as carcinogenic and mutagenic effects (Dixit et al., 2002; Damodaran et al., 2013). Phytotoxicity of Cr has been extensively studied in many plants. Indeed, over accumulation of Cr in plants inhibits seed germination and plant growth, impairs

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nutrient balance and transpiration, degrades photosynthetic pigments, and reduces mitochondrial electron transport and activities of antioxidant enzymes (Dixit et al., 2002; Shanker et al., 2005; Panda, 2007; Zeng et al., 2011a). Additionally, an increasing number of studies have measured transport and accumulation of Cr from different compartments by different plants from different climate zones; however, these studies have focused on either emphasizing uptake potential or screening hyperaccumulators. Rice is a waterlogged plant, which may directly serve as a heavy metal receptor for accumulation, especially in/or near mining areas. Recently, the food safety issue of agricultural crops produced from contaminated sites has resulted in a growing public concern. Rice is the staple crop for 3 billion people, second only to wheat in its importance as a food cereal in the human diet, especially in Asia (Ren et al., 2014; Zhang et al., 2014). Although uptake and translocation of Cr by rice plants has been reported, there is little information available on the kinetics involved in Cr phyto-accumulation. Difference in the uptake pathway between the two Cr species was also still unclear. Therefore, the overall goal of this study was to investigate the accumulation and translocation of Cr by rice seedlings exposed to Cr(VI) and Cr(III). Our specific goals were to: 1) determine the *Michaelis-Menten* kinetics involved in Cr accumulation; 2) investigate Cr subcellular distribution in rice seedlings.

2. Materials and methods

2.1. Preparation of rice seedlings

The procedure of cultivation of rice seedlings was identical to our previous work (Zhang et al., 2014). Briefly, seeds of rice (*Oryza sativa* L. cv. BX139) were planted in sandy soils under laboratory condition at 25 °C. They were irrigated with 25%-strength Hoagland's nutrient solution. After 20 d of growth, young seedlings were collected and rinsed with distilled water and incubated in a pre-treated solution containing 1 mM $CaCl_2$ + 2 mM MES-Tris buffer (pH 6.0) for 4 h to remove the ions from the cell wall space (Ebbs et al., 2008). All pre-treated young seedlings were used for the subsequent experiments. Both Cr uptake tests were conducted in a plant growth chamber with constant temperature of 25 ± 0.5 °C and a relative humidity of 60 ± 2% under continuous artificial light. Four independent biological replicates were performed for each treatment.

2.2. Cr uptake kinetics by detached rice roots

Exposure regime of Cr uptake by detached roots was identical to our previous work (Yu et al., 2011). Plant roots approximately 8 cm in length were excised from the root tip of pre-treated young seedlings. Excised roots (1.0 g fresh weight) were placed in 50 ml solution containing 0, 1.0, 2.0, 4.0 and 8.0 mg Cr/l Cr(VI) (potassium chromate) or Cr(III) (chromium nitrate). After different periods of incubation, excised roots were collected and carefully rinsed with distilled water. Dried plant materials were digested with 4:1HNO_3-$HClO_4$ solution. The precise concentration of total Cr in roots was measured at 1, 3, 5, 7, 9, 12, 16 and 24 h by inductively coupled plasma atomic emission spectrometry (ICP-AES).

2.3. Cr uptake by rice seedlings

One gram of pre-treated young rice seedlings with similar height and weight were exposed to 50 ml Cr-spiked solution containing 0, 1.0, 2.0, 4.0 and 8.0 mg Cr/l Cr(VI) or Cr(III). After exposure in different intervals, seedlings were collected and divided into roots and shoots. The remaining procedure was identical to our previous work (Zhang et al., 2014). The concentration of total Cr in roots and shoots of rice seedlings was also measured by ICP-AES at same interval as the Cr uptake test by detached roots.

2.4. Subcellular distribution of Cr in rice seedlings

Subcellular distribution was determined by gradient centrifugation technique (Ren et al., 2014) with slight modifications. The initial concentrations of Cr in treatments spiked with Cr(III) (chromium nitrate) were 2.0, 4.0, 8.0, 16.0, 24.0, and 32.0 mg Cr/l, while the initial Cr concentrations in treatments amended with Cr(VI) (potassium chromate) were 1.0, 2.0, 4.0, 8.0, 12.0 and 16.0 mg Cr/l. After exposing to Cr(VI) or Cr(III) for 3 days, rice seedlings were rinsed with distilled water and divided into roots and shoots. Fresh plant materials were precisely weighted and homogenized in a triturator with 10 ml grinding medium containing 50 mM MES-Tris buffer (pH 7.8), 0.25 mM sucrose, 1 mM $MgCl_2$ and 10 mM cysteine at 4 °C. The remaining procedure was identical to previous work described by Ren et al. (2014). Finally, each collected supernatant representing cellwall, cytosol and organelle fractions was all digested using 4:1HNO_3-$HClO_4$ solution and measured by ICP-AES.

2.5. Statistical analyses

Analysis of variance (ANOVA) and Tukey's multiple range test was used to determine the statistical significance at 0.01 or 0.05 level between plant performances.

3. Results and discussion

3.1. Time-dependent and kinetics of Cr(VI) accumulation

To better clarify possible mechanisms involved in Cr accumulation, we used excised roots and intact rice seedlings to determine kinetics in related to Cr accumulation. Results of time-dependent accumulation of Cr(VI) showed that the accumulation increased linearly within 5 h, after which Cr accumulation started to slow down (Fig. 1a) in all Cr(VI)-treated groups with detached roots. All curves shown in Fig. 1a displayed exponential accumulation kinetics. Therefore, the accumulation rates of Cr(VI) were determined from the slope of the plot of the amounts of total Cr detected in roots (μg/g DW) versus time (h). The fit was obtained with linear regression (figures not shown), judged by the regression coefficient (R^2). Estimation was all based on the data from the exposure period from 0 to 5 h. Kinetics of Cr accumulation by detached roots of rice was adequately described by the *Michaelis-Menten* equation (Fig. 2). Subsequently, kinetics of K_M and v_{max} was calculated by the analysis of initial accumulation rates at a serious of Cr(VI) concentrations using non-linear regression treatments. The calculated v_{max} for the Cr(VI)-treatments with detached roots was 67.21 μg/g DW. h, and K_M was 0.92 μg/g (R^2, 0.991).

Changes of total Cr content (μg/g DW) in plant materials of rice seedlings exposed to Cr(VI) with time (h) are given in Table 1. No Cr above the detection limit was detected in roots and shoots from non-treated plants, while significant amounts of Cr was detected in both parts of rice seedlings from all treatment groups with Cr(VI), indicating uptake and translocation of Cr(VI). It is interesting to note that the distribution of Cr in different parts of plant materials was variable, majority being in roots rather than shoots. Indeed, the Cr detected from roots accounted for 70.3% (S.D. 1.03, n = 4) of the total Cr detected in biomass of rice seedlings, whereas 29.7% (S.D. 1.03, n = 4) was detected in shoots. A similar estimation was conducted to calculate the accumulation rate for the Cr(VI) treatments with intact rice seedlings based on the data of Cr accumulation in plant materials from the exposure period from 0 to 5 h (Fig. 1b).

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