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Zinc adsorption and desorption characteristics in root cell wall involving zinc hyperaccumulation in *Sedum alfredii* Hance*

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Abstract: Radiotracer techniques were employed to characterize ^{65}Zn adsorption and desorption in root-cell-wall of hyperaccumulating ecotype (HE) and non-hyperaccumulating ecotype (NHE) species of *Sedum alfredii* Hance. The results indicated that at the end of a 30 min short time radioisotope loading period, comparable amounts of ^{65}Zn were accumulated in the roots of the two ecotypes *Sedum alfredii*, whereas 2.1-fold more ^{65}Zn remains in NHE root after 45-min desorption. At the end of 60 min uptake period, no difference of ^{65}Zn accumulation was observed in undesorbed root-cell-wall of *Sedum alfredii*. However, 3.0-fold more ^{65}Zn accumulated in desorbed root-cell-wall of NHE. Zn^{2+} binding in root-cell-wall preparations of NHE was greater than that in HE under high Zn^{2+} concentration. All these results suggested that root-cell-wall of the two ecotypes *Sedum alfredii* had the same ability to adsorb Zn^{2+}, whereas the desorption characteristics were different, and with most of ^{65}Zn binding on root of HE being available for loading into the xylem, as a result, more ^{65}Zn was translocated to the shoot.

Key words: Adsorption, Desorption, Hyperaccumulator, *Sedum alfredii* Hance, Zn
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INTRODUCTION

Phytoremediation has emerged as an alternative technique for removing toxic metals from soil and offers the benefits of being in situ, cost-effective and environmentally sustainable (Raskin *et al*., 1997; McGrath and Zhao, 2003; Santos *et al*., 2006; Bañuelos, 2006). Plants with high metal uptake and accumulation capacity have been termed as hyperaccumulator species. An understanding of the physiological mechanism of metal tolerance and hyperaccumulation is very important for exploring new metal hyperaccumulating plant species that are tolerant to high levels of metal, have large biomass, extensive adaptation, and easy propagation (Ebbs and Kochian, 1997; Salt *et al*., 1998; Yang *et al*., 2005).

Sedum alfredii has been identified as a new Zn-hyperaccumulator plant native to China (Yang *et al*., 2002). However, the mechanisms responsible for its Zn hyperaccumulation remain unknown. Root system is the main interface of material exchange between plants and their environment, and plays an important role in metal uptake and transport in plant (Marschner, 1995; Fritioff and Greger, 2006). In a previous study we reported that at the end of a 24-h radioisotope-loading period, comparable amounts of ^{65}Zn were accumulated in the roots of the two ecotypes of *S. alfredii*. However, after a long-term (48 h) efflux experiment, approximately 6.8-fold more ^{65}Zn was translocated from the root to the shoot of hyperaccumulating ecotype (HE), as compared with non-hyperaccumulating ecotype (NHE). However, during the same period, 3.7-fold more ^{65}Zn remained in NHE roots (Li *et al*., 2005; Yang *et al*., 2006). In both ecotypes roots, ^{65}Zn mainly located in the root cell wall (56%~64%) which had metal-sequestering properties (Volesky and Holan, 1995). Thus we hy-

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pothesized that the difference of Zn biosorption on root-cell-wall may play an important role in controlling the difference of Zn translocation and storage in the shoot. In this study radiotracer techniques were employed to investigate the characteristics of Zn adsorption and desorption in the root of *S. alfredii* using hydroponically grown seedlings. The results should be helpful in elucidating the Zn uptake and transport mechanisms of this Zn hyperaccumulating *S. alfredii*.

MATERIALS AND METHODS

Plant origins and culture

Plants were cultivated according to Li *et al*.(2005). Twenty-day old seedlings were used in all radiotracer studies. One day prior to the uptake experiment, seedlings roots were immersed in aerated pretreatment solution consisting of 2 mmol/L Mes-Tris buffer (pH 6.0) and 0.5 mmol/L $CaCl_2$.

^{65}Zn desorption from roots of *S. alfredii*

Roots of HE and NHE *S. alfredii* were immersed in 3 L of aerated uptake solution containing 2 mmol/L Mes-Tris (pH 6.0), 0.5 mmol/L $CaCl_2$, and 10 μmol/L ^{65}Zn (45 kBq/L). After 30 min, the radioactive uptake solution was replaced with 2 °C solution containing 5 mmol/L Mes-Tris (pH 6.0), 5 mmol/L $CaCl_2$, and 100 μmol/L $ZnCl_2$. At different times (0~45 min), seedlings were harvested and the roots were then blotted to remove adhering solution and the excised roots were oven-dried at 65 °C and weighed, and root radioactivity of ^{65}Zn was measured using a gamma detector (GR2519, Canberra Co., USA). The complete experiment was repeated four times.

^{65}Zn accumulation in roots of *S. alfredii*

Roots of HE and NHE *S. alfredii* were immersed in 3 L of aerated uptake solution containing 2 mmol/L Mes-Tris (pH 6.0), 0.5 mmol/L $CaCl_2$, and 10 μmol/L $^{65}Zn^{2+}$ (45 kBq/L). At different times (0~60 min), one plant of each ecotype was harvested and the roots were briefly rinsed in deionized water or/and desorbed for 20 min in 2 °C solution containing 5 mmol/L Mes-Tris (pH 6.0), 5 mmol/L $CaCl_2$, 100 μmol/L $ZnCl_2$ and then separated from shoots. The excised roots were blotted, roots and shoots were oven-dried at 65 °C and weighed, and ^{65}Zn radioactivity was quantified. The complete experiment was repeated four times.

^{65}Zn adsorption in root-cell-wall preparations of *S. alfredii*

These root-cell-wall preparations were obtained by Hart's method (Hart *et al*., 1992) with some modification. Roots of intact HE and NHE *S. alfredii* were immersed in an MC (methanol/chloroform=2:1, v/v) solution for 3 d, then the root-cell-wall preparations were washed in a series of deionized water for 8 h. Roots of either intact or MC-treated seedlings were incubated in 1 L solution containing 2 mmol/L Mes-Tris (pH 6.0), 0.5 mmol/L $CaCl_2$, and 10 μmol/L ^{65}Zn (45 kBq/L) for time periods between 1 and 45 min, and then either briefly rinsed in deionized water (undesorbed) or desorbed in 1 L of desorption solution for 20 min. Subsequently, root-cell-wall preparations were excised, blotted, oven-dried at 65 °C and weighed, and ^{65}Zn radioactivity was quantified. The complete experiment was repeated four times.

Concentration-dependent kinetics of ^{65}Zn binding in root-cell-wall preparations of *S. alfredii*

MC-treated roots were incubated in 500 ml radiolabeled uptake solution (45 kBq/L ^{65}Zn, 0.5 mmol/L $CaCl_2$, and 2 mmol/L Mes-Tris, pH 6.0) at concentrations of 1, 2.5, 5, 10, 25, 50, 100, 200 μmol/L Zn^{2+} in Plexiglas uptake apparatus. After a 30-min uptake, uptake wells were refilled with 2 °C desorption solution. Following a 20-min desorption period, seedlings were harvested and their roots excised, blotted, oven-dried at 65 °C and weighed, and ^{65}Zn radioactivity was quantified. The experiment was randomly arranged with each treatment replicated for four times.

RESULTS

Time course of ^{65}Zn desorption from roots of *S. alfredii*

At the end of a 30 min short time radioisotope-loading period, comparable amounts of ^{65}Zn were accumulated in the roots of the two *S. alfredii* ecotypes. The desorption curves were similar for the two *S. alfredii* ecotypes (Fig.1). At the end of a

45-min desorption period in a solution containing high levels of Zn^{2+} (100 μmol/L) and Ca^{2+} (5 mmol/L), 87.1% and 72.9% of the ^{65}Zn accumulated in HE and NHE *S. alfredii* root during the uptake period was desorbed into the external solution respectively. ^{65}Zn release into the external solution was initially very rapid, and followed by a slower stage of desorption. There were 2.1-fold more ^{65}Zn remaining in NHE root after 45-min desorption, as compared with HE. However, during the 45-min desorption, about 1.9-fold more ^{65}Zn was accumulated in the shoot of HE, as compared with NHE, these results indicated that the HE has an extraordinary ability to transport ^{65}Zn to the shoot.

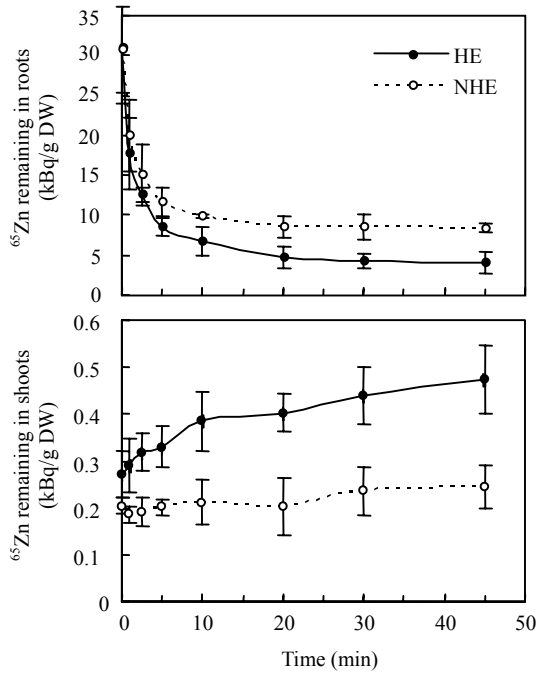

Fig.1 Time course of ^{65}Zn desorption from roots of *S. alfredii*. Data points and error bars represent means±*SE* of four replications

Time-course of ^{65}Zn accumulation in roots of *S. alfredii*

^{65}Zn accumulation in undesorbed root of both *S. alfredii* ecotypes was biphasic, with the initial 10 min rapid phase followed by slower linear stage of accumulation over the subsequent 50 min (Fig.2). At the end of 60 min uptake period, no difference of ^{65}Zn accumulation was observed in root of *S. alfredii*. In desorbed roots, however, Zn^{2+} accumulation was linear, and the slope of the curve was similar to that of the slower phase for ^{65}Zn accumulation in the undesorbed roots. At the end of 60-min uptake, 2.0-fold more ^{65}Zn accumulated in NHE root as compared with HE.

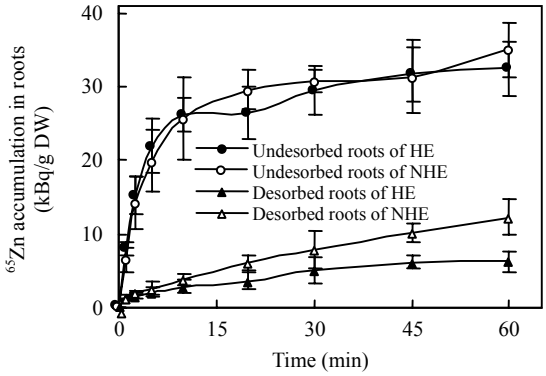

Fig.2 Time course of ^{65}Zn accumulation in roots of *S. alfredii*. Data points and error bars represent means±*SE* of four replications

Time course of ^{65}Zn adsorption in root-cell-wall preparations of *S. alfredii*

^{65}Zn accumulation in both desorbed and undesorbed root-cell-wall of both ecotypes was linear (Fig.3). At the end of 60 min uptake period, no difference of ^{65}Zn accumulation was observed in undesorbed root-cell-wall of both *S. alfredii* ecotypes. In desorbed root-cell-wall, however, 3.0-fold more ^{65}Zn accumulated in NHE as compared with HE at the end of 60-min uptake.

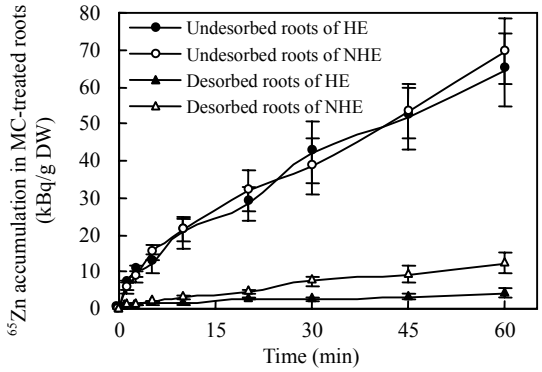

Fig.3 Time course of ^{65}Zn accumulation in root-cell-wall preparations of *S. alfredii*. Data points and error bars represent means±*SE* of four replications

Concentration-dependent kinetics of ^{65}Zn binding in root-cell-wall of *S. alfredii*

Concentration-dependent uptake kinetics for Zn^{2+} in root-cell-wall preparations of *S. alfredii* was

linear (Fig.4). In both ecotypes root-cell-wall preparations, Zn^{2+} accumulation increased with increasing Zn^{2+} concentration. Zn^{2+} binding in MC-treated roots of NHE was greater than that in HE under high Zn^{2+} concentration (>100 μmol/L).

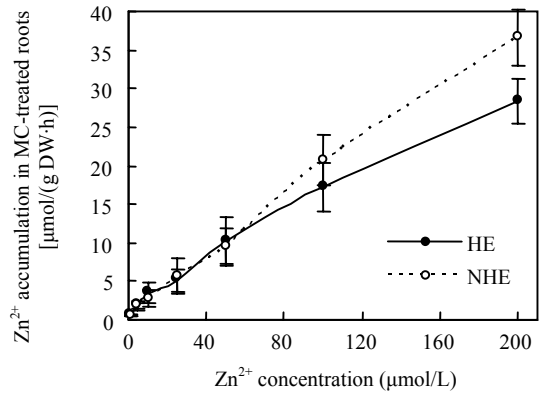

Fig.4 Concentration-dependent kinetics of Zn^{2+} binding in MC-treated roots of *S. alfredii*. Data points and error bars represent means±*SE* of four replications

DISCUSSION

Previous investigations showed that heavy metals bind to natural cellulosic materials (Bryant *et al*., 1992; Shukla and Sakhardande, 1992; Prasad and Freitas, 2000). This phenomenon, known as biosorption, has attracted increasing research interests recently. Cell walls may represent a significant portion of the surface area exposed to both surface waters (Geesey *et al*., 1977) and soil fluids (Mahmood and Rama, 1993), the effects of cell walls on metal mobilities in these systems have been reported (de Lurdes *et al*., 1987; Chen, 1997; Zakir Hossain *et al*., 2006), however the role in controlling metal translocation and storage in hyperaccumulator are still poorly understood. At the end of a 30 min short time radioisotope loading period, comparable amounts of ^{65}Zn were accumulated in the roots of the two *S. alfredii* ecotypes. However, at the end of a 45-min desorption period, there were 2.1-fold more ^{65}Zn remaining in NHE root (Fig.1). In the time-course of ^{65}Zn accumulation in roots of *S. alfredii*, no difference of ^{65}Zn accumulation was observed in undesorbed root, whereas 2.0-fold more ^{65}Zn accumulated in desorbed root of NHE (Fig.2). These results indicated that ^{65}Zn bind loosely to root of HE *S. alfredii* and could be desorbed easily.

Desorbed Zn^{2+} could have originated from the cell walls and/or from the cytosol via efflux across the plasma membrane back into the external solution. Radiotracer studies on *Thlaspi caerulescens* demonstrated that most Zn^{2+} removed during desorption period originated from the cell wall (Lasat *et al*., 1996). In this study, ^{65}Zn accumulation in undesorbed intact root of both *S. alfredii* ecotypes were biphasic (Fig.2), the initial rapid phase presumably was due to diffusion into the free space and subsequent binding to root cell walls, the slower linear stage of accumulation over the subsequent 50 min was believed to represent transport across the plasma membrane. For root-cell-wall preparations, however, ^{65}Zn accumulation was monophasic and rapid, and was much higher in undesorbed root-cell-wall than that in undesorbed intact root, in contrast, less ^{65}Zn accumulated in desorbed MC-treated roots, compared with desorbed intact root (Fig.3). These results support the hypothesis that in intact roots, most ^{65}Zn removed during desorption period originated from the cell wall. On the other hand, Zn^{2+} accumulation in root-cell-wall preparations increased with increasing Zn^{2+} concentration, and Zn^{2+} binding in MC-treated roots of NHE was greater than that in HE under high Zn^{2+} concentration (Fig.4). These results suggested that the affinity of root cell wall of the two *S. alfredii* ecotypes for ^{65}Zn were different, and that most of this binding ^{65}Zn on root of HE are available for loading into the xylem, as a result, more ^{65}Zn was translocated to the shoot.

From the results of the present study, root cell wall of the two *S. alfredii* ecotypes had the same adsorption ability on Zn^{2+} whereas the desorption characteristics were different. Some studies reported that cell wall composition could affect the total metal upake (Nishizono, 1987). Hence, further analysis of cell wall structural components of HE *S. alfredii* would clarify the role of root cell wall in the zinc hyperaccumulation mechanism.

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Correcting Note:

The phrases of "*LeERT*" and "*ERT*" appearing in "**Characteristics of transgenic tomatoes antisensed for the ethylene receptor genes *LeERT1* and *LeERT2***" published in "*Wang et al. / J Zhejiang Univ SCIENCE B 2006 7(7):591-595*" should be corrected to "*LeETR*" and "*ETR*".