Using Short Half-life Nuclide 107Cd for Real-time Imaging and Analysis of Cadmium Dynamics in Cd-Hyperaccumulator Arabidopsis halleri ssp. gemmifera by PETIS System

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Introduction

Positron-emitting tracer imaging system (PETIS), one of the most advanced radiotracer-based imaging methods available today can provide serial time-course images (i.e. animation) of the two-dimensional distribution of a radiotracer within a living organism without contact. Its principle is the same as that of positron emission tomography (PET), which has been widely used for medical diagnosis, but PETIS was specially designed for studying plants and this system enables monitoring of the real-time movement of the tracer in living plants as a video, and also quantitative analysis of the movement of substances by freely selecting a region of interest (ROI) on the image data obtained[1,2].

Cadmium (Cd) hyperaccumulator *Arabidopsis halleri* ssp. *gemmifera* is a perennial weed that spreads widely in Central Europe and East Asia[3]. *A. halleri* ssp. *gemmifera* has a remarkable capacity to uptake Cd. In hydroponic conditions, *A. halleri* ssp. *gemmifera* has been reported to accumulate Cd 2700 mg/kg in shoots without growth inhibition[4]. Also *A. halleri* ssp. *gemmifera* was proved that it had the high accumulation capacity of cadmium in the soil field experiment[5]. For the further phytoremediation application to Cd contamination, Cd uptake and translocation mechanism in *A. halleri* ssp. *gemmifera* need to be clear. The present work aims to visualize the Cd uptake and translocation dynamics in *A. halleri* ssp.*gemmifera* using PETIS and positron-emitting ^{107}Cd tracer.

Methods

Plant cultivation

Seeds of the *A. halleri* ssp. *gemmifera* were germinated and then hydroponically grown in a 250 mL synthetic pot with 1/5 modified Hoagland solution in a growth chamber with the following conditions: 72.9 μmol m^{-2} s^{-1} photon flux density supplied by cool white fluorescent lamps, 60-70% humidity, at 22 °C during a 16:8-h light and dark photoperiod. The culture solution was renewed every week. Approximately 2-month-old plant was used in the whole plant cadmium imaging experiment.

^{107}Cd tracer and PETIS Imaging

^{107}Cd radioisotope was produced as follows[1)]: A natural, 1-mm-thick silver foil was bombarded for 120 min with a 17-MeV proton beam at a current of 3 μA delivered from a cyclotron at Cyclotron and Radioisotope Center, Tohoku University. The irradiated target was dissolved in 4 mL of concentrated nitric acid and 2 mL of water in a glass beaker on a heater. After adding 20 mL of warm water, hydrochloric acid with gradually increasing concentrations from 0 to 2 M was added slowly to the solution to precipitate the silver gently but completely. This gradient was made by adding 10 mL of 2 M hydrochloric acid to 30 mL of water little by little. The supernatant was filtered with a 0.22-mm filter and dried out by heating in a new glass beaker. ^{107}Cd on the bottom of the beaker was dissolved in an appropriate volume of the culture solution containing a designed concentration of nonradioactive Cd.

For *Arabidopsis halleri* ssp. *gemmifera* imaging, the roots of an intact *A. halleri* ssp. *gemmifera* plants were inserted in a 5 mL plastic disposable open root cell (KGS 1509-F01-07,Kumikouki Co., Gunma, Japan), and the shoots were fixed to an acrylic board. The acrylic board was placed in the field of view of the PETIS (PPIS-4800; Hamamatsu Photonics, Hamamatsu, Japan). Open root cell was supplied with 5 mL of 1/5 Hoagland solution containing concentrations 10 μM of $CdSO_4$ labeled with 30 MBq (1.68 pmol) ^{107}Cd. The movement of ^{107}Cd in the plants, including the roots and shoots, was monitored by the PETIS every 10 min for 36 h. The solution was continuously stirred with gentle aeration in order to maintain a uniform composition in each compartment of the root cell. All imaging experiments were conducted in a growth chamber with continuous light at a density of 72.9 μmol m^{-2} s^{-1}. The time course data of the Cd amount (mol) in the regions of interest in the images were calculated by the values of the signal intensity (cps) extracted

using the NIH Image J 1.50 software (http://rsb.info.nih.gov/ij/), counting efficiency of the system (cps Bq^{-1}) and molar activity (Bq mol^{-1}).

Quantitative Determination and Decay Correction

In the feeding experiments, the indicated amounts of nonradioactive Cd were mixed with measured activities of pure ^{107}Cd at a certain time before feeding to the plants. Therefore, the amount of total Cd (i.e. sum of radioactive and nonradioactive Cd) corresponding to the radioactivity of ^{107}Cd at a given time can be easily determined. The graphs shown in this paper indicate the relative amounts of total Cd (%), not just the intensities of ^{107}Cd signal.

Results and Discussions

The tracer solution containing ^{107}Cd was administered to *A. halleri* ssp. *gemmifera* plant and the dynamics of ^{107}Cd in intact whole plant was monitored by PETIS (Figs. 1a and 2a). As a result, obvious clear serial images of ^{107}Cd distributions from the roots to the shoots were successfully obtained for 36 h (Fig. 1b). Because the amount of non-radioactive cadmium labeled with ^{107}Cd was calculated by ^{107}Cd radioactivity, Fig. 2b represents the time course of cadmium. The amount of cadmium in the hydroponic solution decreased rapidly for the first 2 h and slowly after 3 h. The amount of cadmium in the roots increased rapidly for the first 6 h and reached a plateau of approximately at 12 h after feeding. The time-course curves of Cd in shoots showed opposite trends to solution values. It was estimated that at the end of the PETIS experiment 50% of Cd absorbed by the roots from solution was transported into the shoots in plants. This is the first reported study to visualize Cd movement non-invasively in an intact Cd hyperaccumulator, *A. halleri* ssp. *gemmifera*. In comparison with the previous studys[1, 2], the imaging data from this study also can be applied for quantitative analysis of the dynamics and kinetics of Cd uptake and transport in *A. halleri* ssp. *gemmifera*. The further research is required to investigate the Cd dynamics and kinetics in *A. halleri* ssp. *gemmifera* with different Cd conditions.

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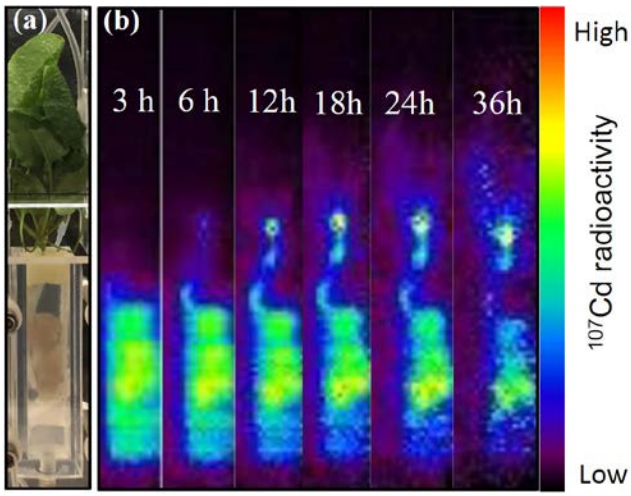

Figure 1. Serial images of ^{107}Cd movement in *A. halleri* ssp. *gemmifera* plant. **(a)** Photograph of test plant in the experimental apparatus. **(b)** Serial images of the whole plant (0–36 h). Each frame was created from the integration of 18 (b) original images collected every 10 min.

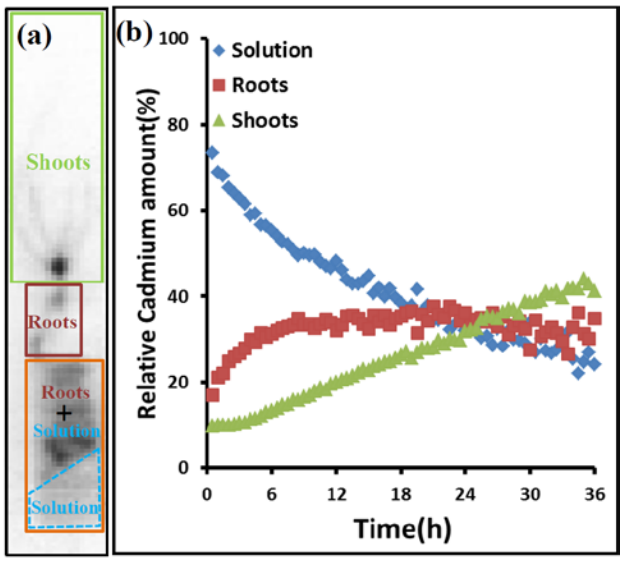

Figure 2. Time course of Cd amount in *A. halleri* ssp. *gemmifera* plant. **(a)** Examined regions of the plant. The blue dotted rectangle indicates the region of the solution, the orange solid rectangle that of the solution and the root, the red solid rectangle that of the root above the solution and the green solid rectangle that of the shoots. **(b)** Time course of the relative cadmium amount in the solution, roots and shoots.