Micro X-Ray Fluorescence Imaging and Micro X-Ray Absorption Spectroscopy of Cadmium Hyperaccumulating Plant, *Arabidopsis halleri* ssp. *gemmifera*, using High-Energy Synchrotron Radiation

Arabidopsis halleri grows in Europe and is known to contain more than 10,000 mg kg^{-1} of cadmium and zinc in its shoot [1,2]. This *A. halleri* trait has the potential to be exploited in phytoremediation, a soft method in which plants are used for the cleanup of heavy metal-polluted soils. A Japanese native subspecies of this plant, *Arabidopsis halleri* ssp. *gemmifera* of which an old scientific name is *Arabis gemmifera*, is also known as a cadmium and zinc hyperaccumulator [3]. Since Cd is a highly toxic metal for plants, it is interesting to reveal the mechanism involved in the Cd accumulation of this plant.

The objectives of the present study is to determine the distribution of Cd in the leaves of *A. halleri* ssp. *gemmifera* and also to determine the chemical form of Cd accumulated in the trichomes [4]. The distribution of Cd in the leaves and trichomes was investigated by μ-XRF analysis with the Kirkpatrick-Baez (K-B) mirror. The chemical form of Cd was then studied by Cd *K*-edge (26.7 keV) μ-XANES analysis.

A. halleri ssp. *gemmifera* (Fig. 1) was used in the present study. The plants were cultivated in a nutrient solution of 20 μM Cd. Analytical grade $Cd(NO_3)_2 \cdot 4H_2O$ was used. To reveal the chemical form of Cd in a living plant, the sample with minimal preparation was required for the μ-XANES analysis. Therefore, a thin freeze-dried section of the sample was prepared in order to maintain its tissue structure and chemical state.

The μ-XRF and Cd *K*-edge μ-XANES measurements of the plant leaves were performed using beamline **BL37XU** operated at 8 GeV and *ca*. 100 mA. The X-rays from an undulator were monochromatized by a Si(111) double-crystal monochromator to 37 keV in order to excite the K-lines of cadmium. The beam was focused to a spot size of 3.8 μm (horizontal, H) × 1.3 μm (vertical, V) using a pair of elliptical mirrors in the K-B configuration. The sample on the acryl board was mounted on an x-y translation stage. Fluorescence X-rays were measured using a Si(Li)-SSD in air at room temperature. Cd *K*-edge μ-XANES spectra were measured at the accumulating points of Cd, which were revealed by μ-XRF imaging, in the fluorescence mode. A monochromator stabilization (MOSTAB) system [5] was used to stabilize the X-ray beam position. The XANES spectra of the cadmium sulfate (CdS), cadmium oxide (CdO), cadmium acetate (AcCd), hexakis(imidazole)cadmium(II) nitrate ($(Im)_6Cd(NO_3)_2$), phytochelatin-Cd (PC-Cd) and metallothionein-Cd (MT-Cd) were measured as references.

Fig. 1. Cadmium hyper-accumulating plant, *A. halleri* ssp. *gemmifera*.

The cellular distributions of Cd, Zn, Mn and Ca present in the trichomes were successfully revealed for the first time. The distributions of Cd and Zn are shown in Fig. 2 together with a photograph taken by a digital microscope. High accumulations of Cd, Zn and Mn were found at the base of the trichomes. There is a strong positive correlation between the XRF intensities of Cd and Zn ($r^2 = 0.8674$, $n = 438$, where r and n are the correlation factor and number of data, respectively). In contrast, Ca was highly enriched in the upper part of the trichomes, particularly at the tip, while little was found in the base of the trichomes where Cd highly accumulated. Cd and Zn accumulated in a ring shape at the border between the upper and lower parts of the trichomes. These results indicate that a striking subcellular compartmentalization of Cd and Zn occurs in the vacuole of the trichomes, and this compartmentalization plays an important role in the hyperaccumulating process of this plant.

The Cd *K*-edge μ-XANES spectrum of the plant was successfully measured for the first time by utilizing the K-B mirror for high-energy X-ray focusing and the MOSTAB system. The Cd *K*-edge μ-XANES spectra of the trichomes and reference materials are shown in Fig. 3. The spectrum of reference samples above the absorption edge (26.72–26.76 keV) shows a characteristic difference in shape between Cd with the S ligand and that with the O/N ligand. Thus, the spectrum of Cd with the S ligand (CdS, PC-Cd, MT-Cd) can be distinguished from those of Cd with the O ligand (CdO, $Cd(NO_3)_2$aq, AcCd) and the N ligand ($(Im)_6Cd(NO_3)_2$). The Cd *K*-edge spectra

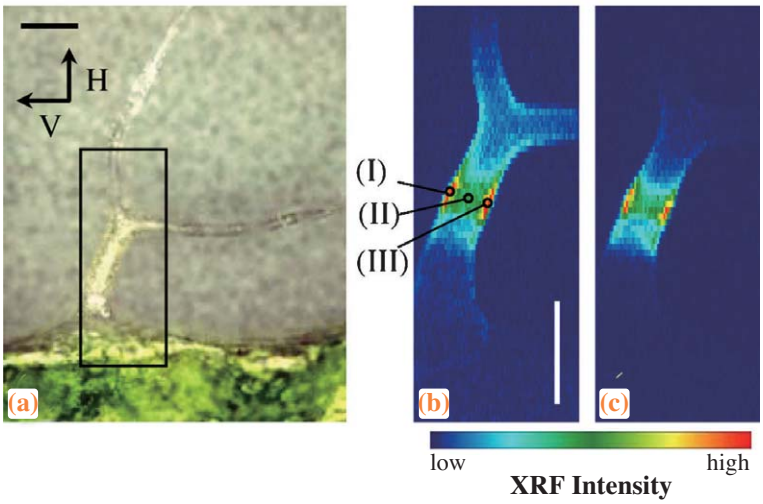

Fig. 2. μ-XRF imaging of trichomes. **(a)** photograph of measured samples showing imaging area, **(b)** Cd, and **(c)** Zn. For the maps, an imaging area of 204 μm (H)×81 μm (V), a beam size of 3.8 μm (H)×1.3 μm (V), a step size of 3 μm (H)×1 μm (V), and a dwell time of 0.3 s/point were used. (I)-(III) show measurement points of Cd μ-XANES. The scale bar is 50 μm [4].

of points (I), (II), and (III) (Fig. 2(b)) in the trichomes are shown in Figs. 3(a)–3(c). It was found that these spectra are similar in shape to those of Cd with the O or N ligand. Therefore, in these specialized cells, the μ-XANES results showed that the majority of Cd exists as a divalent state and binds to the O or N ligand, not the S ligand.

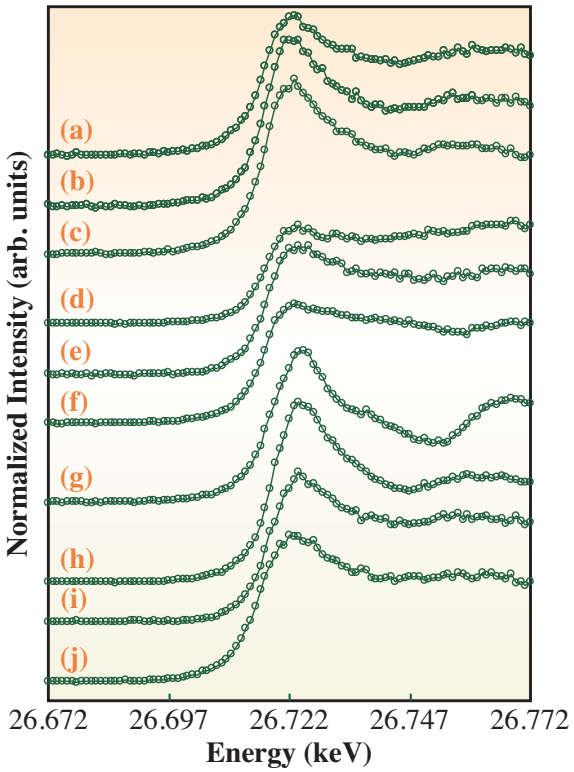

Fig. 3. Cadmium *K*-edge μ-XANES spectra measured at points in Fig. 2 and reference compounds. **(a)** Point I, **(b)** point II, **(c)** point III, **(d)** PC-Cd, **(e)** MT-Cd, **(f)** CdS, **(g)** CdO, **(h)** $Cd(NO_3)_2aq$, **(i)** AcCd and **(j)** $(Im)_6Cd(NO_3)_2$. Beam size: 3.8 μm×1.3 μm, energy step: 1 eV, and dwell time: 1 – 4 s/point [4].

This study has demonstrated the performance of Cd *K*-edge μ-XANES spectroscopy, which was applied here for the first time to plant samples. The combination of μ-XRF and μ-XANES has proved to be an indispensable tool for the study of Cd accumulation in biological samples on a cellular scale. In future research, we will investigate changes in the distribution and chemical form of Cd through the transportation process of Cd.

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