Study of Mechanisms for Transportation and Storage of Heavy Metals in Cadmium Hyperaccumulator Plant Using High-energy μ-XRF Analysis

Specific types of plant can grow in contaminated soils and absorb a large amount of heavy elements [1]. *Arabidopsis halleri* is known as a cadmium and zinc hyperaccumulator [2,3], and it can accumulate more than 10000 mg·kg^{-1} cadmium and zinc in its shoot [2]. This characteristic makes it highly suitable for phytoremediation, a soft method in which plants are used to clean up heavy metal-polluted environments [1]. However, the cellular distribution of cadmium and the mechanisms of cadmium transportation and accumulation in the plant have not yet been elucidated. The two-dimensional (2D) analysis of trace cadmium in plant tissues is a key method used to investigate such accumulation mechanism. Recent studies using scanning electron microscopy (SEM) with energy dispersive X-ray spectrometry (EDS) documented the cellular distribution of zinc in the tissues of *A. halleri* [2]. In its leaves, zinc was mostly sequestered in the base of trichomes and in mesophyll cells [2]. Trichomes are epidermal hairs present at the surface of the leaves of *A. halleri*, and their functions are thought to be the exudation of various molecules or the storage of substances such as metals. However, conventional SEM-EDS mapping is not suitable for the analysis of cadmium due to the low sensitivity of the electron beam excitation for heavy elements. Furthermore, the detection of the Cd L-line is also difficult because the line overlaps with the K-line peak of potassium, which is an essential element for plants.

In this study, we have developed an in vivo micro-X-ray fluorescence (μ-XRF) imaging technique utilizing high-energy synchrotron radiation (SR) to determine the distribution of cadmium and zinc in the tissues and cells of hyperaccumulator plants and to investigate their physiology and mechanism of accumulating cadmium [4].

Plant samples of *A. halleri* ssp. *gemmifera* [3] were collected around an abandoned mine site in Hyogo prefecture. The leaves of the plant were subjected to nondestructive analysis without any sample preparation. Some samples were cut with a vertical slicer, and thin sections were sealed in a Mylar® plastic bag together with a small piece of moist unwoven paper to prevent the sample from drying out.

2D μ-XRF imaging was carried out at beamline **BL37XU**. An X-ray beam from an undulator was monochromatized by a Si(111) double-crystal monochromator to 37 keV to effectively excite the K-lines of cadmium and to minimize the overlap of the K-line peak with the Compton scattering peak. The X-ray beam was focused with an FZP to a beam size of *ca.* 3 × 3 μm^2. The FZP was produced by the sputtered-slice manufacturing method [5]. A Si(Li) solid-state detector was placed in an appropriate position. Samples were placed on the x-y stepper motorized stage, which was step scanned to obtain a 2D image. The step size was set to 3 μm. The integrated XRF intensity of each line, e.g., Cd Kα, was calculated from the spectrum and normalized by that of the incident beam I_0, which was measured using an ionization chamber, and then an elemental map of the measured area was calculated.

Before the microbeam analysis, the elemental distribution at the plant organ level was investigated using a non-focused beam at BL37XU. The X-ray beam was adjusted using horizontal and vertical slits, allowing us to obtain a beam size of 50 × 50 μm^2. The other experimental conditions, including the excitation energy, were almost the same as those used for μ-XRF imaging.

Elemental maps of Cd, Zn, Rb, and Sr are presented in Fig. 1, in which the normalized X-ray fluorescence intensities are scaled to red (maximum) and blue (minimum). Each image indicates the relative distribution of the specific element; thus, the concentration scale varies for each image. The distribution of cadmium in the leaves has been very clearly revealed by the *in vivo* monitoring of the Cd Kα line for the first time. These images can be

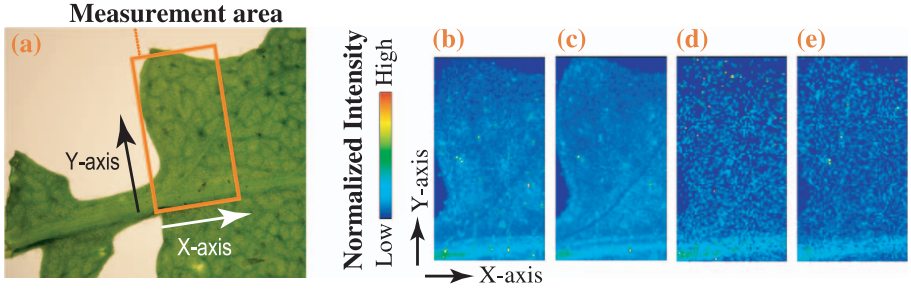

Fig. 1. XRF imaging of leaf of *A. halleri* ssp. *gemmifera*. **(a)** Image of leaf, and distribution of **(b)** Cd, **(c)** Zn, **(d)** Rb, and **(e)** Sr. X-ray beam size, 50 × 50 μm^2; scan step, 50 μm; measurement time, 1 s/pixel; image size, 66 × 132 pixels.

directly used to correlate the distribution of various elements with plant tissue structure. As can be seen in Figs. 1(b) and 1(c), cadmium and zinc show a wide distribution in mesophyll tissues and the main vein of leaves. The distribution of cadmium was found to positively correlate with that of zinc. Both elements preferentially accumulated in specific tissues on the leaf, namely, the trichomes, which are epidermal hairs present on the surface of the plant leaves.

μ-XRF imaging was carried out on trichomes prepared from the leaves. The elemental distributions of cadmium, zinc, strontium, and calcium are presented in Fig. 2 together with photographs taken under an optical microscope and an SEM. The 2D cellular distribution of cadmium in the trichomes was first observed by *in vivo* μ-XRF imaging. It was found that both cadmium and zinc accumulated to a high degree in the base of the bifurcation area of the trichomes, whereas strontium and calcium were mostly distributed throughout the entire upper part of the trichomes. These distributions of cadmium and zinc showed striking subcellular compartmentation.

The distribution areas of cadmium and zinc accumulated inside the trichomes were found to shrink gradually as the result of slow drying. This finding supported the notion that the compartmentation of cadmium and zinc occurs in the vacuole of the trichomes because 80-90% of the vacuole of a living cell is composed of water. The compartmentation of cadmium and zinc was considered to play an important role in the accumulation process of the elements; consequently, a detailed *in vivo* analysis should be carried out in the future.

In conclusion, we have for the first time succeeded in determining the 2D cellular distribution of cadmium in hyperaccumulating plant tissue using a high-energy X-ray microbeam. Data were obtained using a living sample. The cellular distribution of cadmium was found to correlate positively with that of zinc. Because both zinc and cadmium belong to Group 12 in the periodic table, this finding may suggest that the accumulation of these elements proceeds via similar transportation pathways in the plants. These results will provide important information for a better understanding of the mechanism of cadmium hyperaccumulation by plants.

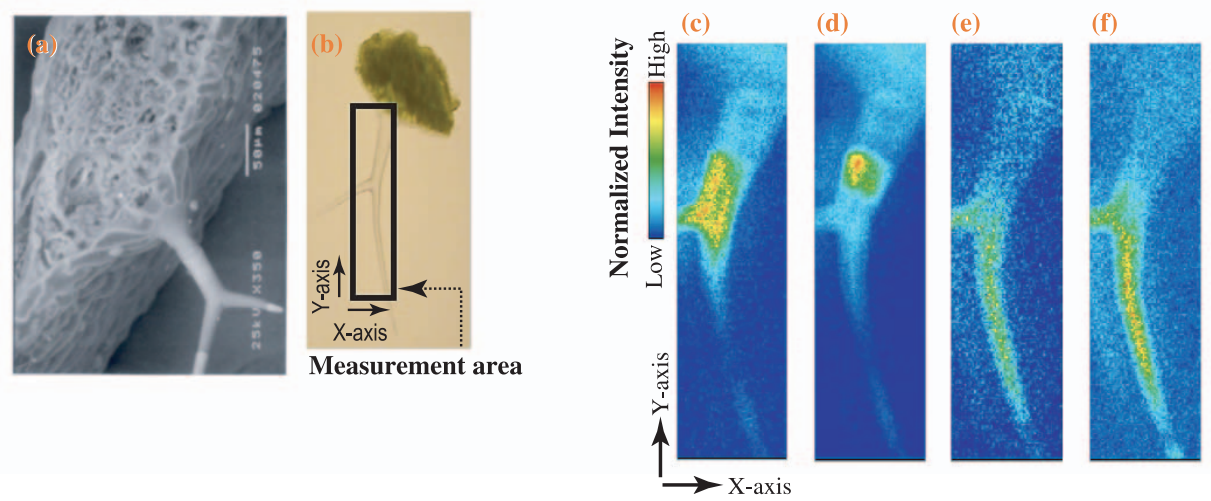

Fig. 2. Micro-XRF imaging of trichome taken from leaf. **(a)** SEM image, **(b)** image of trichome, and distribution of **(c)** Cd, **(d)** Zn, **(e)** Sr, **(f)** Ca. X-ray beam size, 3 × 3 μm^2; scan step, 3 μm; measurement time, 0.5 s/pixel; image size, 59 × 226 pixels.

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