2-D X-ray Fluorescence Imaging of Cadmium Hyper-accumulating Plants by Using High-Energy Synchrotron Radiation X-ray Microbeam

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A specific type of plants can grow in contaminated soils and absorb a large amount of heavy elements in their bodies. *Arabidopsis halleri* is known as a cadmium and zinc hyperaccumulator,[1-4] which can contain more than 10000 mg kg^{-1} cadmium and zinc in shoot.[3] This characteristic makes hyperaccumulators highly suitable for phytoremediation, a soft method in which plants are used for the cleanup of heavy metal-polluted soils. However, the cellular distribution of cadmium in the plant and the pathway of transportation remained unknown and the accumulation mechanism has not yet been revealed. The two-dimensional (2-D) analysis of trace cadmium in plant tissues is a key analytical method to investigate such accumulation mechanism. Recent studies using a scanning electron microscope (SEM) with an energy dispersive X-ray spectrometer (EDX) documented the cellular distribution of zinc in the tissues of *A. halleri*.[1-3] In its leaves, zinc had been mostly sequestered in the base of the trichomes and in the mesophyll cells.[2,3] Trichomes are epidermal hairs present at the surface of leaves of *A. halleri*, and their functions are thought to be the exudation of various molecules or the storage of metals, etc. However, conventional SEM-EDX 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 the present study, we have developed an in vivo micro-X-ray fluorescence (μ-XRF) imaging technique utilizing high-energy synchrotron radiation (SR) in order to reveal the distribution of cadmium and zinc in the tissues and cells of the hyperaccumulator plants and to investigate their physiology and accumulation mechanism for heavy elements.

The plant samples of *A. halleri* ssp. *gemmifera*[4] were collected around an abandoned mine site in Hyogo prefecture. The leaves of the plant were subjected to the nondestructive analysis without any sample preparation. Some samples were cut with a vertical slicer, and the thin sections were sealed in a Mylar® plastic bag together with a small piece of moist unwoven paper in order to prevent the sample from drying out.

2-D μ-XRF imaging was carried out at BL37XU of SPring-8. 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 and to minimize overlap of the K-line peak with the Compton scattering peak. The X-ray beam was focused with an FZP to the 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 the appropriate position with respect to the direction of the incoming beam to minimize the scattering. The step size was set to 3 μm and provided some oversampling, ensuring that no area of the target was missed. 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 by an ionization chamber, and then the elemental map was calculated for the measured area.

The μ-XRF imaging was carried out on trichomes prepared from the leaf. The elemental distribution of cadmium, zinc, strontium, and calcium are obtained. The 2-D cellular distribution of cadmium in the trichomes was first observed by in vivo μ-XRF imaging. It is found that both cadmium and zinc highly accumulated in the base of the bifurcation area of the trichomes in the range at 80−140 μm, whereas strontium and calcium were mostly distributed in the whole upper part of the trichomes. These distributions of cadmium and zinc showed a striking sub-cellular compartmentation. On the other hand, the distribution areas of the cadmium and zinc accumulated inside the trichomes were found to be gradually shrinking by a slow drying process. This finding supported that the compartmentation of cadmium and zinc occurs in the vacuole of the trichomes because the vacuole of a living cell contains about 80−90% water. The compartmentation of cadmium and zinc was considered to play an important role in the accumulation process for such elements, consequently, the detailed in vivo analysis should be required in the future.

In the present study, the cellular distribution of cadmium was found to be positively correlated with that of zinc. Since both zinc and cadmium belong to the Group 12 in the periodic table, this finding may suggest that the accumulation of these elements proceeds via similar pathway of transportation in the plants. These results will provide important information for our better understanding of the mechanisms of cadmium hyper-accumulation by plants.

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