Observation of Arsenic Transfer in Leaf Tissue of Hyperaccumulator Fern by Utilizing Synchrotron Radiation Micro-XRF Imaging

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We have directly observed the early process of arsenic transfer in the leaf tissue of hyperaccumulator fern (*Pteris vittata* L.) within 12 h after the arsenic feeding, by utilizing a synchrotron radiation micro-XRF imaging. This study visually revealed for the first time that arsenic transferred from the root to the marginal part of the leaf within 1 h after feeding. Arsenic accumulated in the region of the vascular bundle and transferred to the paraphysis prior to the sporangium.

Pteris vittata L. is a fern plant well known as an arsenic hyperaccumulator. After the first report by Ma et al.[1] about the high ability of this fern to remove arsenic from contaminated soils, many studies of this plant have been undertaken.[2–6] Hyperaccumulation of toxic heavy metals consists of the following three processes, i.e., uptake of the elements by the roots of the plant, transfer to the shoots, and storage in the tissue. Furthermore, it is assumed that the accumulated heavy metal was isolated in a certain part of plant tissue to prevent the metal toxicity from influencing the biological activities. Nevertheless, the details of these mechanisms have not yet been clarified.

To reveal the mechanism of hyperaccumulation, each process of metal accumulation must be understood as a continuous flow of the element between the plant tissues and/or individual cells. For this purpose, synchrotron radiation (SR) X-ray fluorescence (XRF) imaging, utilizing an X-ray microbeam is very useful. In our previous studies using this technique, we had for the first time succeeded in observing the cadmium distribution in certain tissue of the hyperaccumulating plant[7] and investigated the distribution and/or chemical status of elements in the tissues of the hyperaccumulators.[7–11] In previous studies, however, there was no attempt to observe the transfer of the heavy metal in the plant tissue as time-dependent data after uptake on a cellular resolution level. In this study, we have established the technique to obtain the dynamic change in the XRF image of the arsenic distribution in the leaf tissue of *P. vittata* L.

P. vittata seedlings, cultivated from spores for 6 months, were transferred to a hydroponic culture. Following 2 weeks of preculture with no arsenic, the nutritional solution was changed to the arsenic-containing solution having a concentration of 50 mg L^{-1} (potassium arsenate was used).

A mature leaf blade was sliced into a piece with an approximately 150-μm thickness using a vertical slicer. Each piece was stuck to an adhesive tape on a plastic plate and then immediately frozen on dry ice. This preparation was made within a few minutes. The plastic plates were soaked in a Dewar flask filled with liquid nitrogen until the XRF measurement to maintain the frozen condition and to prevent frost from accumulating on the surface of the sample which increased the background noise due to X-ray scattering.

The micro-XRF imaging was carried out at BL37XU of SPring-8. X-rays from the undulator were monochromatized to 12.8 keV by the Si(111) double-crystals and were focused to $3 \times 3\,\mu m^2$ (beam size at sample) by the Kirkpatrik–Baez mirror optics which consists of a pair of aspheric total reflection mirrors fabricated by the bent-polishing method.[12] A two-dimensional

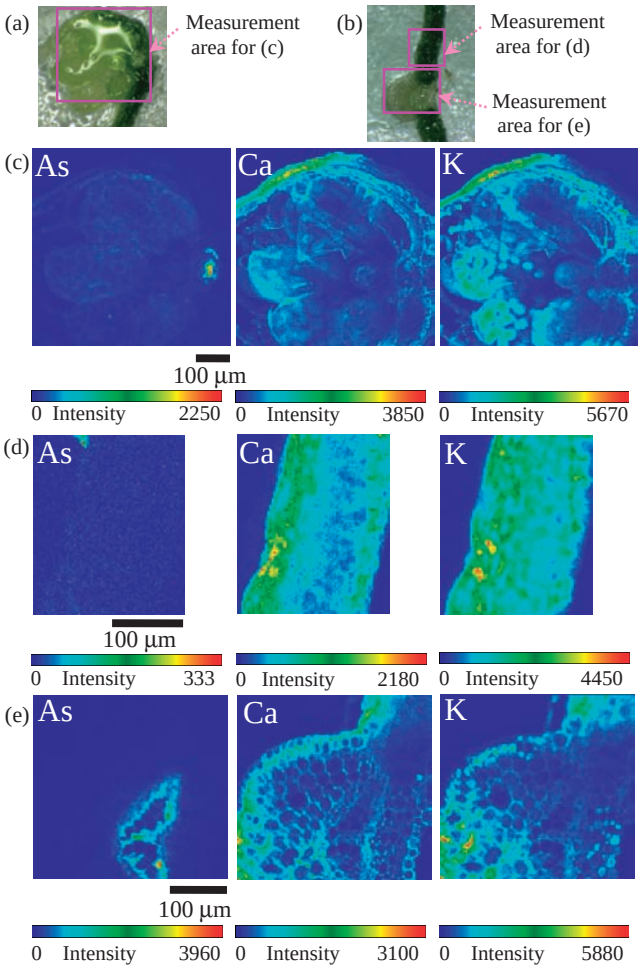

Figure 1. Micro-XRF imaging of leaf at 1 h after arsenic feeding. Photographs of (a) marginal part, and (b) surrounding area of main vein. (c), (d), and (e) are imaging results of elemental distribution for marginal part, mesophyll tissue, and main vein, respectively. X-ray beam size, $3 \times 3\,\mu m^2$; Step size, 3 μm; Measurement time, 0.3 s/point.

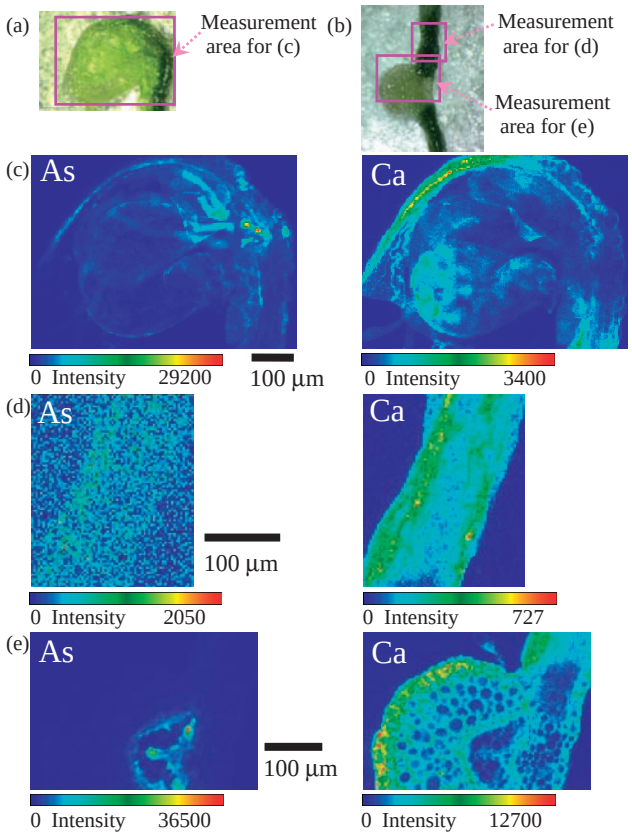

Figure 2. Micro-XRF imaging of leaf at 12 h after arsenic feeding. Photographs of (a) marginal part, and (b) surrounding area of main vein. (c), (d), and (e) are imaging results of elemental distribution for marginal part, mesophyll tissue, and main vein, respectively. X-ray beam size, $3 \times 3\,\mu m^2$; Step size, 3 μm; Measurement time, 0.3 s/point.

(2D) analysis was made by scanning the sample on an X–Y pulse motor controlled XY stage, which was set in a cold flow from a cryojet to keep the frozen condition, and the XRF spectrum was measured by the silicon drift detector. The XRF intensity of each element was normalized by the incident X-ray intensity, which was then scaled to the 256 gradations color scale from red (highest) to blue (lowest) to produce the 2D elemental map.

Figure 1 shows the elemental distributions of the leaf blade cross section at 1 h after the arsenic feeding. The measurement time of each pixel was 0.3 s, and 1 to 5 h was necessary to obtain the whole images. The tissue structures of the leaf were clearly visualized by the calcium maps. Thus, it was confirmed by the calcium distribution in the plant that calcium mainly localizes in the cell walls.[13] On the other hand, potassium has a tendency to exist within the inner cellular region, not only in the cell wall. This feature is quite conspicuous in the epidermal cells (Figure 1e).

As shown in Figure 1c, it is remarkable that arsenic had already reached the marginal part of leaf within 1 h after feeding at the end of the transpiration flow. On the other hand, the storage of arsenic in the mesophyll tissue was not significantly observed (Figure 1d). The arsenic distribution in the tissue of the main vein (Figure 1e) showed that arsenic was localized within the area of a vascular bundle. Furthermore, most of the arsenic had accumulated in its boundary region (probably the parenchyma cells), which had a distinctly smaller cell size, of the vascular bundle. From these observations, it was shown for the first time that arsenic absorbed from the root is rapidly transferred to the marginal area of a leaf blade through a network of vascular bundles in the plant body.

The arsenic distributions at 12 h after feeding are shown in Figure 2. The maximum XRF intensities of arsenic in Figure 2 were six to thirteen times higher than the value in Figure 1, as a result of the arsenic accumulation. The arsenic image in Figure 2c indicated that arsenic is accumulated within the region of the vascular bundles, which are certainly used for supplying water and nutrients to the sporangium and transferred to the paraphysis and the pseudindusium prior to the sporangium.

In this study, we have in detail revealed the process of the arsenic transfer in a leaf tissue of *P. vittata* and determined the specific regions with arsenic localization in the tissue structure. These results explain the early stage of the arsenic localization in the marginal part of the leaf, which was observed in our previous research.[11] It is assumed that isolation of arsenic within the vascular bundle and restriction of transfer into the mesophyll or sporangium are the tactics of *P. vittata* to complete its life cycle with an arsenic hyperaccumulation.

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Published on the web (Advance View) December 1, 2007; doi:10.1246/cl.2008.32