Arsenic Distribution and Speciation in Arsenic Hyperaccumulator Fern by Micro-XRF Imaging and Micro-XANES Analysis

It has been known for a long time that some plants accumulate heavy metal elements such as Mn, Ni, Cu, and Pb. Recently, such heavy-element-hyperaccumulator plants have been attracting much attention because of their application to phytoremediation. Phytoremediation is a technology that uses plants to remove, destroy, or sequester hazardous substances from the environment [1]. It is an emerging technology for environmental remediation that offers promise as a low-cost, versatile technique suitable for use against a number of different types of contaminant in various media. Some specific kinds of plant are known to be heavy metal hyperaccumulators; in particular, the Chinese brake fern (*Pteris vittata* L.) has been reported to accumulate large amounts of arsenic (As: *ca.* 22,000 $\mu g \cdot g^{-1}$ dry weight) in pinnae when it is grown on contaminated soil [2]. It has not yet been determined how this fern efficiently extracts a toxic heavy element such as arsenic from soil into their fronds. As a result, the chemical forms of arsenic and arsenic distribution are being studied with great eagerness [3].

In the present study, we have applied a synchrotron radiation (SR) microbeam to elucidate the elemental distribution and the oxidation state of arsenic in root tissues at sub-cellular levels [4,5]. The XRF imaging of As in a pinna with a nonfocused beam is shown in Fig. 1, which clearly indicated the distribution of As in the fern tissue for the first time. Cells in plant roots are microscopic and fragile (see Fig. 2); therefore, the introduction of a microbeam to XRF imaging and the XANES spectrum of root tissues should provide us new knowledge about the arsenic accumulation mechanism in the Chinese brake fern. Conventional SEM-EDS mapping is not suitable for this purpose because of the low sensitivity of electron beam analysis for heavy elements such as arsenic though SEM is suitable for obtaining clear three-dimensional images of a plant cell.

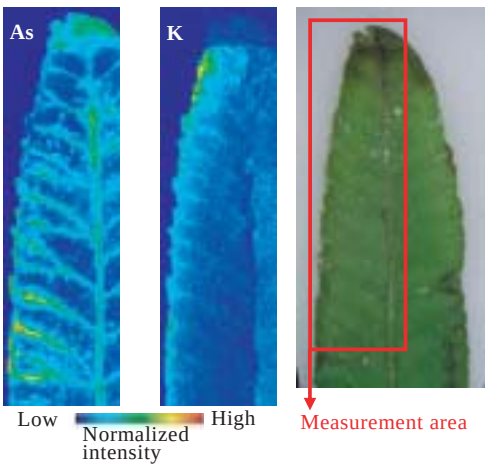

Fig. 1. XRF imaging of As and K in pinna of Chinese brake fern. X-ray energy: 15.0 keV, beam size: 50 μm × 50 μm, measurement points: 60 × 205 points, measurement time: 0.5 s/point.

Pteris vittata seedlings, cultivated from spores for six months on soil, were transferred to hydroponic culture after removing soil from roots carefully. Following two weeks of preculture without arsenic, the nutritional solution was changed to a 10 mg L^{-1} arsenic-containing solution prepared with potassium arsenate. New roots that elongated during the preculture, were used for analyses. Each root cut from the root system of the fern was placed on dry ice immediately and frozen rapidly. Then the root was sliced using a razor blade with *ca*. 200 μm thickness to obtain horizontal cross sections. Sliced root samples were freeze-dried and placed on a mylar film on a sample holder, and then subjected to X-ray microbeam analysis.

SR-μ-XRF imaging and μ-XANES analysis were performed at beamline **BL37XU**. X-rays from the undulator were monochromatized with a Si(111) double crystal monochromator and focused with a Kirkpatrick-Baez (K-B) mirror to 1.1 μm × 1.3 μm at an X-ray energy of 12.8 keV. The sample holder was set on an X-Y-axis stepping-motor-driven stage. X-ray fluorescence intensity was measured with a silicon drift detector (SDD). The step size was set at 1.0 μm, and spectral acquisition times were varied to ensure adequate counting statistics (typically 0.1 s). The measurement was carried out in air. Integrated intensity for each element was calculated from the spectrum, and an elemental image was obtained. XRF intensity was normalized by incident X-ray intensity (I_0).

Fig. 2. SEM image of the root tissue of Chinese brake fern.

After XRF imaging, μ-XANES analysis was carried out to investigate the oxidation state of arsenic at the cellular-level spatial resolution. Arsenic *K*-edge (11.867 keV) X-ray absorption spectra were measured as the fluorescence mode by monitoring the X-ray fluorescence intensities of the As Kα line (10.543 keV). The XANES spectrum of the sample was measured over the energy range from –20 eV to +40 eV from the absorption edge with a step size of 1 eV. The measurement time for each point was varied from 2 to 10 s depending on As level. All data were collected at room temperature in air. The measurement points were selected and controlled precisely using an X-Y-axis stepping motor.

Figure 3 shows the result of the XRF imaging of a cross section of a fern root. Arsenic was detected in the entire root tissue; however, a relatively lower intensity of arsenic signal was observed within a central area (points g and h in Fig. 3 (b)). A comparison of the XRF image with its optical microscope image suggests that this area corresponds to the central cylinder (a tissue which includes the vascular bundle as the pathway of water and substance transportation).

Figure 4 shows a comparison of XANES spectra of eight measured points shown in Fig. 3(b) with those of the reference compounds of $AsIII_2O_3$ and H_3AsVO_4. Although arsenic was added to the culture media as As(V), both As(V) and As(III) exist in root tissues. At points *a* to *d* in the outer cortex, it was found that As(V) was the more dominant species than the As(III) form. It seems to be the general tendency that the As(III)/As(V) ratio increases from the surface to center: the central cylinder and the inner cortex contain a certain amount of As(III). This result indicates that there is reduction activity in the inner cortex or the boundary between the cortex and central cylinder in root tissues.

In conclusion, the arsenic distributions in cross sections were successfully measured by SR-μ-XRF analysis. A comparison of XANES spectra of eight points in the root tissue with high As level with those of the reference compounds (As_2O_3 and H_3AsO_4) revealed that arsenic in root tissues exist as a mixture of As(III) and As(V) and the As(III)/As(V) ratio increases in the inner cortex and the boundary between the cortex and central cylinder.

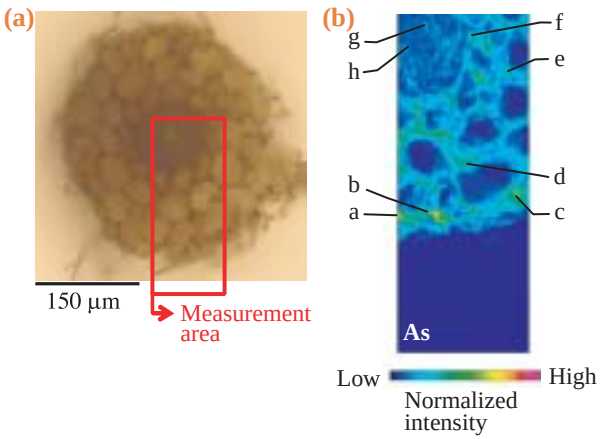

Fig. 3. Optical microscopic image of cross section of fern root **(a)** and μ-XRF imaging of As in root tissue **(b)**. Beam size: 1.1 μm × 1.3 μm, measurement points: 501 × 201 points, measurement time: 0.1 s/point.

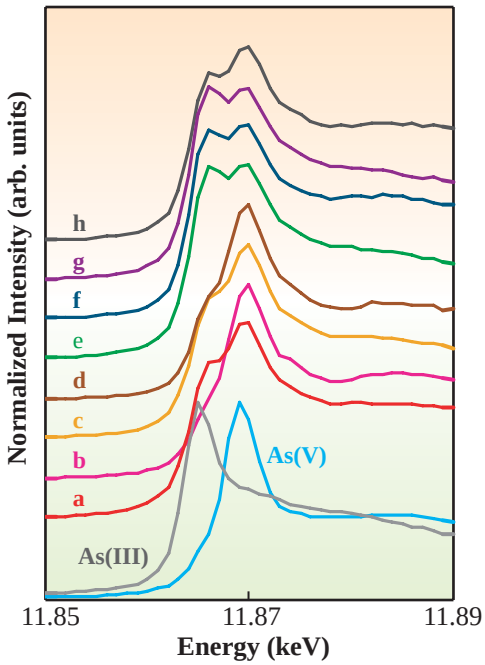

Fig. 4. μ-*K* edge-XANES spectra of As accumulated in root tissue. The measurement points are shown in Fig. 3.

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