PIXE Study on Translocation of Arsenate and Arsenite on Arsenic Hyperaccumulating Fern (Pteris Vittata)

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Introduction

Phytoremediation is a technology for cleaning metal-contaminated soils using plant physiology. *Pteris vittata* is the first plant to be an arsenic hyper-accumulator[1)]. It is reported that arsenate (As(V)) taken-up by the fern root is immediately reduced to arsenite (As(III))[2,3)]. And arsenite is then transported to fern fronds by transpiration stream and accumulated in the fronds[4)]. It is also revealed that arsenate influx into fern roots is faster than that of arsenite[5)]. Different research groups, however, report that arsenate is dominant in the rachis of fern blade and both arsenate and arsenite are contained in xylem vessels of the fern even under the condition of very high concentration of arsenite in a transpiration stream[6-7)]. These findings indicate that arsenic is supplied to the fronds by xylem transport as a mixture of arsenite and arsenate.

To develop practical application of *Pteris vittata* to a phytoremediation technique, it is necessary to explicate how arsenic is distributed in fern frond tissues when either arsenite or arsenate is supplied to fern blades separately without an action of the fern root. It is also important to reveal what kinds of elements essential to the fern metabolism are translocated by accumulation of arsenic of either trivalent or pentavalent state in fern tissues. In this study, the distribution of arsenic of different oxidation states and other elements in fern frond tissues were examined in the procedure of separately feeding arsenate or arsenite to fronds through xylem vessels of the fern by using micro-PIXE analysis.

Material and methods

P. vittata L., a perennial and pinnate fern, which grows naturally in the warm district in southern Japan with long sunshine duration, was used throughout the experiment. Fern with several fronds was cultivated for either one week or three months, and the young ferns with not fully opened fronds and the mature ferns with sporophyte were subjected to an arsenic uptake experiment. A piece of the fern fronds was cut from the stipe and dipped in a solution containing either arsenite or arsenate along with 0.2 mM EDTA for preservation of arsenic species[8]. Concentrations of arsenic in test solutions were 7.5 ppm and 200 mM. After a desired period, a fern pinna was plucked and was sectioned around 25 μm in thickness by a microtome after inserted into a radish block. The sliced pinna section was mounted between tow polycarbonate films of 5 μm thickness and was glued on the sample holder for in-air micro-PIXE analysis.

Analyses of samples were carried out with the in-air micro-PIXE system at Tohoku University. Technical details of the system were presented in our previous papers[9-10]. The analysis was conducted with the proton beam energy of 3 MeV, the beam spot size of 1×1 μm^2 and beam currents of around 50 pA. The beam scanning area was set to 20x20 - 600×600 μm^2[2]. X-ray detector was set in vacuum at 125 degrees with respect to the beam axis. A Mylar filter (250 μm thick) was attached in front of the detector to reduce pile-up events and deformation of the spectrum by recoil protons. Quantitative PIXE analysis was performed using the GeoPIXEII software[11]. In the quantification of elements, the major composition and density of the analyzed layer must be set, so the fern matrix was presumed to be uniform composition of 80% H_2O + 20% $C_4H_6O_3$ with a density of 1.25 g/cm^3 [12].

Results and discussion

Fern fronds with sporophyte (mature stage) were subjected to arsenite (7.5 ppm in 7 hr) and arsenate (7.5 ppm in 2 hr) treatments. Although the results are not shown due to the lack of space, arsenic, irrespective of the oxidation state, was brought into a pinna in the concentration of around 800 ppm, indicating higher arsenic concentration in the sporophyte than in the other pinna regions. Arsenic concentration in the pinna veins was less than 200 ppm. These findings suggest that arsenic of different oxidation states is transported to the sporophyte by transpiration stream for the accumulation. In addition to this, the elemental distribution was well correlated between arsenic, potassium and calcium. Hokura et al. reported that arsenic accumulation was well correlated with potassium but was

anti-correlated with calcium for mature fern pinna with sporophyte[13]. The difference in elemental distribution is ascribed to the inconsistency of growth stage.

The young frond without sporophyte was subjected to7.5-ppm arsenate treatment for 3.5 hr. Arsenic concentrations over 35,000 ppm were detected in whole young pinnae. Figure 1 shows the elemental maps on the young pinna section at three positions as indicated ((a): near midrib, (b): between midrib and edge, (c): edge). The maximum value of colour bar was lined up for each element at three positions. In the region (a), arsenic and potassium show similar distribution and their concentrations are higher in the mesophyll. Phosphorus, sulfur, chlorine, and calcium are uniformly distributed over the whole pinna section. Arsenic concentration is considerably high in the region (b), which was consistent with our previous results[12,14]. In the regions (b) and (c), phosphorus, potassium and arsenic show a tendency to distribute more in the mesophyll than in the epidermis. Since the chemical form between orthophosphate and arsenate is similar, the arsenic distribution is accompanied by transportation of elements needed for keeping cell metabolism. It is reported that arsenic and potassium are localized in pinna epidermis of the fern[15], which is not consistent with the present result, while the correlation in distribution between arsenic and potassium are consistent. The rate of elemental transportation from mesophyll to epidermis might be limited at the initial uptake stage, so the time duration in the present study is considered to be shorter than that in ref.15.

Figure 2 shows elemental distributions on the pinna section of young fern subjected to 7.5-ppm arsenite treatment for 3.5 hr. Arsenic distribution is quite different from that of the other elements and only accumulated in the middle region of the pinna mesophyll. The concentration of arsenic decreased one hundredth of arsenate, indicating the slow uptake rate for arsenite by the fern fronds. These findings infer existence of a certain kind of transportation barrier for arsenite in pinna tissues of the fern. The young frond without sporophyte was treated with 200-mM arsenate solution for 60 hours. Figure 3 shows very different distributions of elements on the sliced pinna section from those shown in Fig. 1, which indicates that high arsenic concentration and long transpiration time results in a fatal damage to the fern tissues. Arsenic of ca. 60,000 ppm is distributed in the upper epidermis and correlated with silicon and calcium that are translocated due to the metabolic change in the pinna tissues. Lombi, et al. reported the translocation of silicon in high concentration for the fern sample containing arsenic by EDXA technique[15]. Calcium is also largely required for keeping a structure and a function of plant cell walls at intense growth phase.

Hence, it is highly probable that silicon and calcium are translocated to maintain the morphology of pinna tissues concentrating actively arsenic unnecessary for vital activity of a leaf. The high-concentration spots of sulfur, chlorine and potassium also indicate a metabolism change of the whole pinna tissues exposed to a transpiration flow of high arsenic concentration, although those elements are translocated differently from arsenic.

Conclusion

The micometer-scale area mapping of elements using the in-air micro PIXE system revealed the arsenic accumulation and translocation of elements essential to a plant metabolism in pinna tissues of living *Pteris vittata* that was supplied with arsenate and arsenite to the frond separately via xylem vessel using transpiration flow. The uptake rate was two orders higher for arsenate than for arsenite. Transportation from mesophyll to epidermis was a rather slow process for arsenite uptake. A high loading of arsenate at longer uptake-time caused the translocation of silicon and calcium accompanying by the arsenic accumulation in the epidermis of fern pinnae, and then the morphology of pinna tissue was maintained satisfactorily in discoloured epidermis with the arsenic-accumulation damage. Hence, the in-air micro-PIXE analysis is an effective measure for undertaking a phytoremediation research of hyper-accumulator plants.

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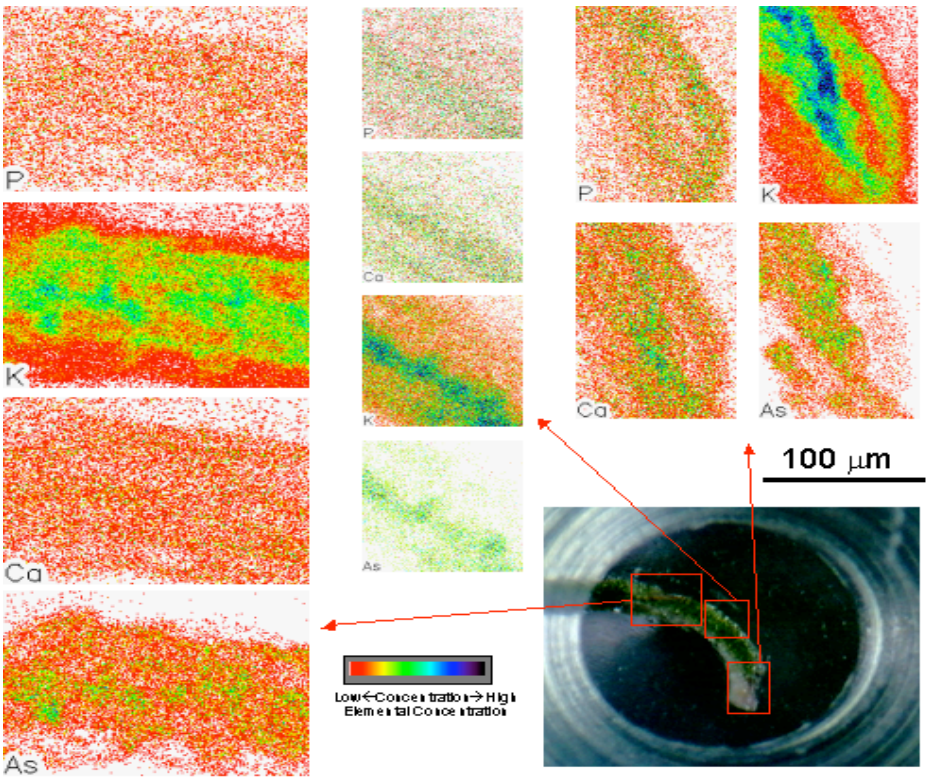

Figure 1. Elemental maps on a young pinna section at three positions, (a):near midrib, (b):between midrib and edge, (c):edge). Young frond was subjected to 7.5-ppm arsenite treatment for 3.5 hours.

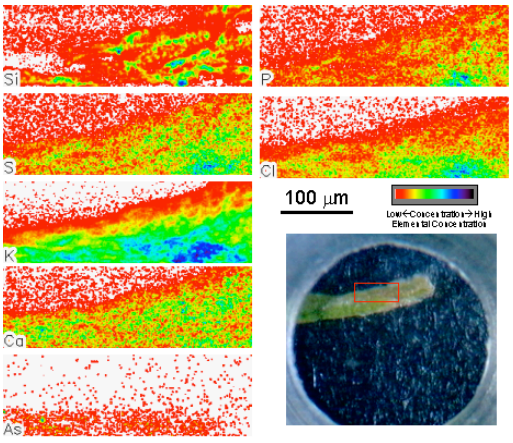

Figure 2. Elemental distributions of pinna section of young frond subjected to 7.5-ppm arsenite treatment for 3.5 hours.

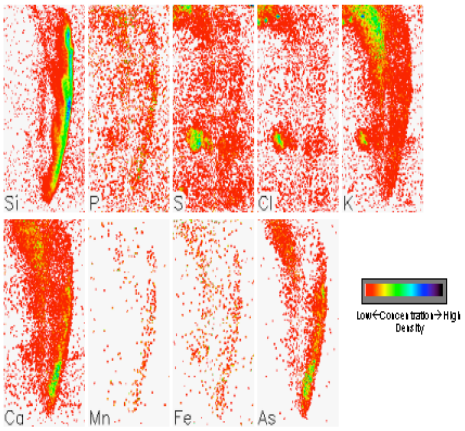

Figure 3. Elemental maps of pinna section treated with 200-mM arsenate solution for 60 hours.