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Title

Role of Thiol Compounds in Arsenic Tolerance in *Pteris vittata*

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Introduction

Arsenic (As) contamination in soils is a serious environmental and agricultural problem worldwide. Phytoremediation by using As-hyperaccumulator plants has been proposed as an effective and environmental-friendly tool to eliminate As in the contaminated soils. Whereas all known As hyperaccumulator species belong to the genus *Pteris* and its related species, the detailed mechanisms of As tolerance and hyperaccumulation in these species have not been well elucidated. In higher plants, it has been suggested that thiol compounds (e.g. glutathione (GSH) and phytochelatin (PC)) are related to the internal detoxification of As (Tripathi et al., 2007). Meanwhile, there is no report showing a strong contribution of thiol compounds to As tolerance and accumulation in As hyperaccumulator ferns. Therefore, role of thiol compounds in As tolerance in *P. vittata* was examined in this study.

Materials and Methods

Chemical form and localization of arsenic in *Pteris vittata*

Chemical forms of As in pinnae extract of *P. vittata* were deduced from ^{75}As NMR and gel-filtration chromatography. Additionally, distribution of As were compared with that of thiol compounds using segmentalized fronds. Concentrations of As and thiol compounds were determined by ICP-MS and HPLC with fluorescence detector after the derivatization with monobromobimane (MBrB), respectively.

Arsenic-induced thiol synthesis in *Pteris vittata*

Response of thiol synthesis to As exposure in *P. vittata* was investigated, and compared with that to other oxidative stresses (cadmium (Cd) and H_2O_2) or with that in an As non-hyperaccumulator fern, *Nephrolepis exaltata*. Moreover, *PvECS* encoding γ -glutamylcysteine synthetase (γECS) was isolated. Then, As-induced changes in expression of *PvECS* were determined by real-time PCR. Also, concentration of thiol compounds was determined.

Results and Discussion

Multinuclear NMR spectroscopy is useful for identifying the form of element in intact plant tissue. However the pinnae extract did not give significant resonance peak in ^{75}As NMR analysis (data not shown). Since the ^{75}As nucleus is quadrupolar ($\text{spin}=3/2$), the signals may become too broad to observe due to asymmetric As molecules in plant tissue. When applying gel-filtration chromatography to As fractionation in pinnae extract, high-molecular-weight As compounds (>700 Da) containing thiol compounds were detected in addition to inorganic As species (data not shown).

As concentration was the highest in the apical pinna and decrease with getting lower in position

of pinna (Fig. 1a). In one pinna, As concentration was the highest in the edge. These trends in distribution may be resulted from transpiration. Meanwhile, localization of GSH and PC2 showed similar trends to that of As (Fig. 1bc). When determining correlation between As and thiol (GSH + PCn) concentrations in segments, significant positive correlation was observed (Fig. 1d). The regression line indicated the molar ratio of As : thiol was ca. 1 : 2.97, which was nearly equal to the ideal molar ratio of As: thiol when making As-thiol complexes in solution (1 : 3). These results strongly suggest that As makes complex with thiol compounds in *P. vittata*.

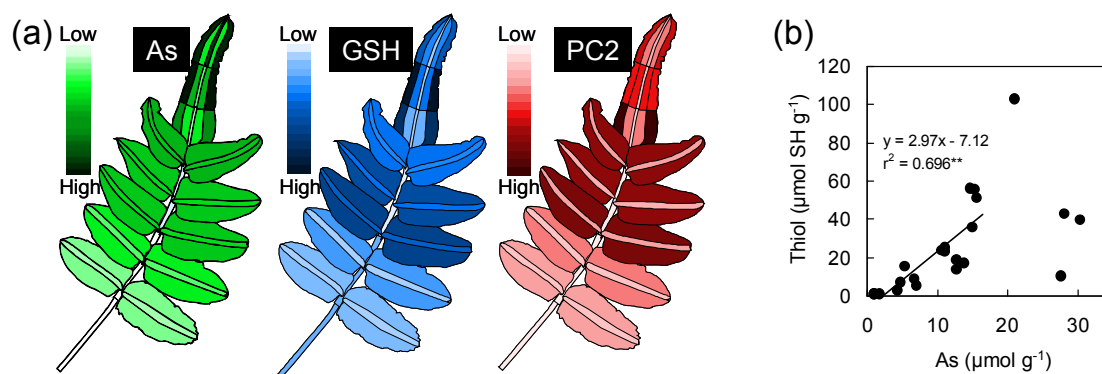


Fig. 1. Relationship between As, GSH, and PC2 accumulations in fronds of *Pteris vittata*. (a) localization of As, GSH, and PC2 in a frond; (b) correlation between As and thiols (GSH+PC2). The regression line was calculated using the concentrations in each segment with As concentration of < 20 $\mu\text{mol g}^{-1}$.

Then, synthesis of these thiol compounds were characterized in *P. vittata*. First, response of thiol synthesis to As exposure in *P. vittata* was compared that in *N. exaltata*, a non-As accumulator fern. Concentrations of GSH in frond and roots of *N. exaltata*, were relatively higher than that of *P. vittata* in the absence of As, and decreased with increasing As concentrations in the medium (data not shown). By contrast, GSH concentrations in *P. vittata* were drastically increased by As exposure, especially in fronds (data not shown).

It is well known that oxidative stress is one of the primary factors contributing to As toxicity in plant, and thiol compounds can alleviate the oxidative damage (Leustek et al., 1999; Requejo and Tena, 2005). Therefore, it is possible that the As-induced oxidative stress enhances the thiol synthesis. To clarify whether As directly induces thiol synthesis in *P. vittata*, effect of As, Cd, and H_2O_2 applications on the induction of thiol synthesis and lipid peroxidation was examined. Although all treatments induced more or less oxidative stress (lipid peroxidation) in roots, only the As application induced thiol synthesis (data not shown), indicating that the synthesis of thiol compounds was not caused indirectly by oxidative stress, but caused directly by As.

γEC synthesis is the rate-limiting factor for the pathway of thiol synthesis (Tripathi et al., 2007). In this study, two putative γEC synthetase genes were isolated (*PvECS1* and *PvECS2*).

Whereas the sequences of these two genes were similar (data not shown), the expression pattern of these genes in response to As exposure was completely different in fronds of *P. vittata* (Fig. 2bc). The expression of *PvECS1* was repressed immediately, but that of *PvECS2* increased greatly after the 2-day treatment (Fig. 2d). Moreover, the expression pattern of *PvECS2* was almost corresponded to the changes in γ EC and GSH concentrations in fronds. Slightly prior to these increases, As accumulation in fronds began to increase (Fig. 2a). Although these genes have not been characterized yet, these different expression patterns imply the contribution of these enzymes to the As-specific induction of thiol synthesis in *P. vittata*.

In conclusion, results in this study strongly suggested that thiol compounds have significant roles in As tolerance mechanism in *P. vittata*. Further detailed examination for elucidating the mechanism of As-induced thiol synthesis will reveal the As tolerance mechanisms in *P. vittata* more closely.

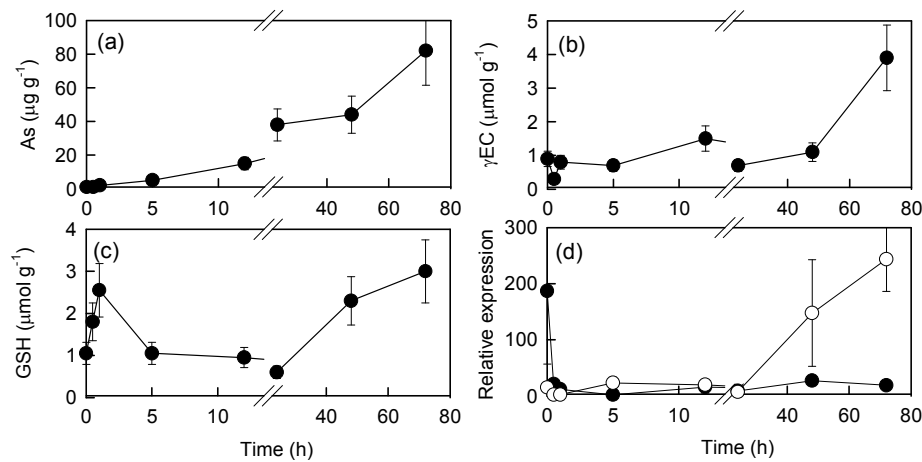


Fig. 2. Changes in concentrations of As, γ EC, and GSH, and relative expression of *PvECS1* and *PvECS2* in fronds of *Pteris vittata*. (a) As; (b) γ EC; (c) GSH; (d) Relative expression of *PvECS1* (●) and *PvECS2* (○). Fronds were sampled at 0, 0.5, 1, 5, 12, 24, 48, and 72 h after the onset of the As exposure. Gene expressions were normalized relative to elongation factor-1b mRNA content.

References

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