XAS Speciation of Arsenic in a Hyperaccumulating Fern

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Introduction

Arsenic contamination in drinking water has become a problem that has received increasing attention in recent years. The National Research Council [1] and U.S. Environmental Protection Agency [2] have recommended that the maximum contaminant level standard for drinking water in the United States be lowered from the current value of 50 $\mu g\ L^{-1}$. Elevated levels of arsenic can be present in the environment as a result of mineral weathering and dissolution [3], geothermal activity [4, 5] and numerous anthropogenic sources, including mine wastes [6], coal fly ash [7], and arsenical pesticides [8].

Arsenic is a redox-sensitive trace element whose toxicity, bioavailibility, and mobility vary depending on its speciation and oxidation state. Arsenate (H_3AsO_4) is frequently found under oxic conditions, whereas arsenite (H_3AsO_3) and arsenic sulfides are commonly found in anoxic conditions [9]. In general, arsenite is considered the more toxic and bioavailable form of arsenic in the environment [10]. Thus, determining the oxidation state and coordination of arsenic is important in assessing its fate and impact in the environment.

An attractive strategy for the attenuation of arsenic in soils is phytoaccumulation. This employs growing tolerant plants in order to accumulate the toxic species in the biomass of the plant, which can later be harvested and removed. However, in order to use phytoremediation effectively and efficiently, it is important to obtain a detailed understanding of the biochemical pathways and mechanisms that operate to translocate these species from the soil to the shoots and leaves. Thus the chemical environment of arsenic inside the plant must be studied. To this end, x-ray absorption spectroscopy (XAS) provides an ideal probe for investigating the coordination environment of As by a direct method.

Methods and Materials

Leaves and shoots from *Pteris vittata* (brake fern) [11] were examined by using x-ray spectroscopy. Leaves were harvested and sealed between layers of Kapton® tape. The encapsulated samples were then stored in liquid nitrogen to ensure minimizing any chemical effects from storage until they were placed in the beam. X-ray absorption measurements were performed at the APS on the Dupont-Northwestern-Dow Collaborative Access Team (DND-CAT) bending magnet beamline. All the spectra were acquired by using a continuous scan mode (CS-XAS) to improve the signal-to-noise ratio and determine experimental errors. A Si(111) piezo-driven double-crystal monochromator was used to vary the energy of the x-ray photons produced by the bending magnet from 200 eV below the absorption K edge of As (11868 eV) to 1000 eV above it. The monochromator was kept detuned at all energies to approximately 50% of the maximum intensity to minimize harmonic interferences. The incident intensity I_0 and transmitted intensity I_T were measured by appropriately positioned ionization chambers. The fluorescence signal I_F was measured with a Stern-Heald detector (EXAFS Co.) [12] that was filled with xenon. The outputs of the fluorescent detector and of the two ionization chambers were connected to individual Stanford Research System SRS 570 current amplifiers that were continuously sampled at 12.5 kHz by a 16-bit analog-to-digital converter. Data were collected while the monochromator was continuously slewed between the initial and final energies. Nine successive scans were recorded for each sample at a rate of 120 s per scan.

The spectroscopic data were analyzed by using a series of codes that were developed specifically for the interpretation of the formatted data structure as described by Webb [13]. The original data were binned and averaged over 0.5-eV increments in the edge region and over 0.05 $Å^{-1}$ in the EXAFS region. The experimental errors for each channel were calculated by using the standard deviations over each bin of data and individual signal statistics. To improve the signal-to-noise ratio of an experiment, the multiple scans of each sample were averaged. By using these values, transmitted and fluorescent x-ray absorption signals, μ_T and μ_F, were determined together with their associated experimental errors. The data were then normalized by following conventional procedures, and the EXAFS signal was extracted after background removal by using the AUTOBK routine [14]. To carry over the calculation of the errors during these steps, a pseudo-Monte Carlo method was performed by using a Gaussian distribution with a 2σ standard deviation to sample the errors. The simulation consisted of n = 1000 trials, which was determined to be a reasonable number of repetitions without affecting the numerical results.

Results

The brake fern (*Pteris vittata*) was discovered in previous work to be an extremely effective hyperaccumulator of arsenic [11]. The fern leaves accumulated As at a concentration up to 126 times that of the soil and reached concentrations in excess of 20,000 parts per million (ppm) dry weight. In this study, CS-XAS was used to examine the coordination environment in various parts of the fern. CS-XAS was employed to ensure that the high photon flux of the beam did not change the oxidation state of arsenic during the measurements. In each case, the sample from the first scan was identical to the last, indicating that the beam had not affected the speciation of As.

Figure 1 shows XANES data from ferns with an As concentration of about 1,000 ppm. Leaves examined were immature and mature fronds, half-dried and fully dried. In actively growing plants, the arsenic present exists primarily as As(III). As the plants dry, a distinct shift in the absorption edge can be seen, representing a change in oxidation state from As(III) to As(V). The same trend is also noticed in the plant stems. When compared with arsenic standards, the spectrum of the arsenic in the leaves resembles most closely the spectrum of the aqueous arsenite ion (AsO_3^-). Other As(III) compounds, such as sodium salts and oxides, have a distinctly smaller white line at the edge. EXAFS fitting results show that the As present in the leaves of active ferns is dominated by threefold oxygen coordination. This supports the XANES data that the As is present as the arsenite ion.

Fern leaves that exhibited higher (about 10,000 ppm dry weight) arsenic concentrations were also examined. The XANES of these samples showed again the dominance of As(III) in the leaves of the plant. However, fitting the XANES edge to a linear combination of arsenic standards indicates the presence of arsenic sulfides. The EXAFS spectrum of the high-concentration sample was examined further to explore the apparent sulfur coordination. The Fourier transform of the data (Fig. 2) shows that there is a distinct second coordination shell in addition to the first shell of oxygen. A theoretical fit of the data from using *ab initio* calculations from FEFF8 [15] confirms that the shell is composed of sulfur.

Discussion

The data presented in previous work showed that arsenic is stored preferentially in the leaves of brake fern fronds [11]. The x-ray absorption spectroscopy results indicate that arsenic in the fern leaves is present primarily as aqueous arsenite ion As(III), which is consistent with data obtained by using high-performance liquid chromatography coupled with hydride generation atomic fluorescence spectrometry [16].

For a typical soil, most of the As is provided to the plant as As(V), which requires a reduction step that most likely occurs within the plant after arsenic uptake. The arsenate in the soils is most likely taken up through an analog of the phosphate uptake system, although further investigations are required in order to confirm this. Arsenic in the stems of the fronds shows that most of the arsenic at this point has already been reduced, showing only a small fraction of detectable arsenate by x-ray spectroscopy. This again suggests that the initial reduction of arsenic occurs immediately after uptake and before transport into the leaf biomass.

Since the thermodynamically stable oxidation state of arsenic under oxic conditions is As(V) [9], the brake fern must continually supply reductants in order to maintain arsenic in the observed As(III) state. This is supported by the observation that once the fronds are harvested and allowed to slowly dry out, the arsenic slowly oxidizes back to As(V). This suggests that there is an active

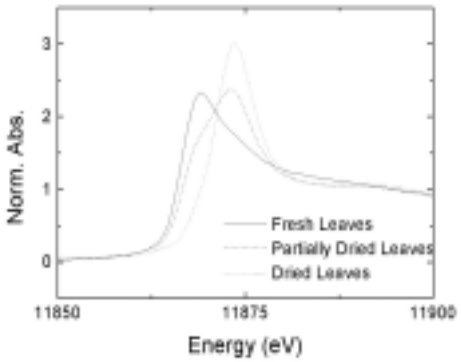

FIG. 1. Arsenic K-edge XANES data of Pteris vittata leaves at various stages of drying. The shift in oxidation state from As(III) to As(V) can be seen as the aging process progresses. Arsenic concentration is approximately 1000 ppm.

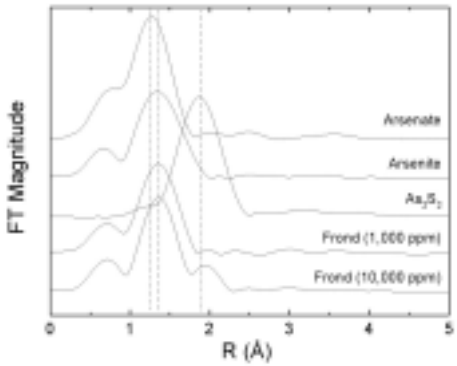

FIG. 2. Radial distribution functions, uncorrected for phase shifts, for fern leaves and arsenic standards. Arsenic concentration in leaves is approximately 10,000 ppm.

mechanism in the brake fern, linked to its metabolic activity that reduces arsenic. Such arsenic reduction has been observed in many bacterial assemblages in both aerobic and anaerobic settings [17-20] as well as in higher plants, such as the Indian mustard (*Brassica juncea*) [21].

At higher arsenic concentrations in the fern leaves, As displays a significant degree of coordination by sulfur atoms. Since the x-ray absorption data provide information about the average coordination of arsenic in the sample illuminated by the beam, it is difficult to extract precise information about a pure compound in a mixture. At best, we can suggest that As is present as a mixture of arsenite-oxygen and arsenite-sulfur coordinations. Since As(III) has a strong affinity for thiols, it is likely that a thiol group is responsible for the observed arsenite-sulfur coordination. Previous works have shown that As(III) is complexed entirely by thiols in an As(III)-tris-glutathione compound in the Indian mustard [21] and by phytochelatins in *Holcua lanatus* [22]. These thiol compounds are strong candidates for being involved in the reduction pathway of arsenic, as hypothesized in Ref. 21. Similar thiols have been shown to reduce As(V) to As(III) in aqueous solutions [23, 24]. In the case of the brake fern, the complex appears to be either a transient or an intermediate species in comparison to the dominant aqueous species. At high total arsenic concentrations, however, the As(III)-thiol complex concentration increases in order to keep maintaining As at its reduced oxidation state and contributes significantly to the x-ray absorption spectrum of the whole plant.

A major difference between the brake fern and the observations in the Indian mustard is that the thiol-coordinated arsenic compound is the minority species in the case of the fern. Another difference between brake fern and other arsenic sequestering plants is that As(III) is the predominant species (>90%) in brake fern, whereas <50% of arsenic is present as As(III) in the latter [25]. In all other cases of As sequestration in plants, the brake fern shows a higher affinity for the accumulation of As in the plant leaves. This hyperaccumulation of As into the aboveground plant material, as opposed to root material, makes the brake fern an ideal candidate for phytoremediation. Further study is needed to determine if these speciation differences are indeed responsible for the observed differences in accumulation behavior.

Acknowledgments

This work was performed at the DND-CAT Synchrotron Research Center located at sector 5 of the APS. DND-CAT is supported by E.I. DuPont de Nemours & Co., The Dow Chemical Company, the U.S. National Science Foundation through Grant DMR-9304725, and the State of Illinois through the U.S. Department of Commerce and the Illinois Board of Higher Education, Higher Education Cooperation Act Grant No. IBHE-HECA NWU 96. Use of the APS was supported by the U.S. Department of Energy, Office of Energy, Office of Basic Energy Sciences, under Contract No. W-31-102-ENG-38.

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This article was taken in part from S. M. Webb, J. F. Gaillard, L. Q. Ma, and C. Tu,, "Arsenic speciation in a hyper-accumulating fern using CS-XAS," Abstr. Pap. Am. Chem. S. **222**, 60-ENVR, Part 1 (2001).

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