Highly efficient xylem transport of arsenite in the arsenic hyperaccumulator *Pteris vittata*

Y. H. Su[1,2], S. P. McGrath[1], Y. G. Zhu[3] and F. J. Zhao[1]

[1]Soil Science Department, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK; [2]Department of Chemistry, Xinjiang University, Tianshan, Urumqi 830046, China; [3]Research Center for Eco-environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

Author for correspondence:
F. J. Zhao
Tel: +44 1582 763133
Fax: +44 1582 760981
Email: Fangjie.Zhao@bbsrc.ac.uk

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Summary

- The hyperaccumulator *Pteris vittata* translocates arsenic (As) from roots to fronds efficiently, but the form of As translocated in xylem and the main location of arsenate reduction have not been resolved.
- Here, *P. vittata* was exposed to 5 μM arsenate or arsenite for 1–24 h, with or without 100 μM phosphate. Arsenic speciation was determined in xylem sap, roots, fronds and nutrient solutions by high-performance liquid chromatography (HPLC) linked to inductively coupled plasma mass spectrometry (ICP-MS).
- The xylem sap As concentration was 18–73 times that in the nutrient solution. In both arsenate- and arsenite-treated plants, arsenite was the predominant species in the xylem sap, accounting for 93–98% of the total As. A portion of arsenate taken up by roots (30–40% of root As) was reduced to arsenite rapidly. The majority (*c.* 80%) of As in fronds was arsenite. Phosphate inhibited arsenate uptake, but not As translocation. More As was translocated to fronds in the arsenite-treated than in the arsenate-treated plants. There was little arsenite efflux from roots to the external solution.
- Roots are the main location of arsenate reduction in *P. vittata*. Arsenite is highly mobile in xylem transport, possibly because of efficient xylem loading, little complexation with thiols in roots, and little efflux to the external medium.

Key words: arsenate, arsenic speciation, arsenite, hyperaccumulation, *Pteris vittata*, xylem sap.

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Introduction

A number of fern species belonging to the order Pteridales, mostly within the genus *Pteris*, are able to hyperaccumulate arsenic (As) in the fronds to very high concentrations (Ma *et al.*, 2001; Visoottiviseth *et al.*, 2002; Zhao *et al.*, 2002; Meharg, 2003; Srivastava *et al.*, 2006; Wang *et al.*, 2007). *Pteris vittata*, the first As-hyperaccumulator identified (Ma *et al.*, 2001), can accumulate several thousand mg As kg^{-1} dry weight in fronds without suffering from As toxicity (Lombi *et al.*, 2002; Tu & Ma, 2002; Caille *et al.*, 2005). Compared with As-nonhyperaccumulating fern species, *P. vittata* has a higher rate of arsenate uptake and a much enhanced translocation of As from roots to fronds (Huang *et al.*, 2004; Poynton *et al.*, 2004; Caille *et al.*, 2005). For example, within 8 h exposure to arsenate, 76% of the As taken up by *P. vittata* was transported to the fronds, whereas in the nonhyperaccumulator *Pteris tremula* the percentage was only 9% (Caille *et al.*, 2005). Similarly, Poynton *et al.* (2004) showed that 74% of the As taken up during 24 h had been translocated to the shoots in the hyperaccumulator *Pteris cretica*, compared with only 8.8% in the nonhyperaccumulator *Nephrolepis exaltata*. It appears that enhanced uptake, translocation and tolerance are three important traits of As hyperaccumulators (McGrath & Zhao, 2003).

Recent studies have shown that arsenate, the predominant form of As in aerobic soils, is taken up by the phosphate transport system in *P. vittata* and *P. cretica* (Wang *et al.*, 2002; Poynton *et al.*, 2004). Following the entry of arsenate into the root cells, some arsenate is reduced to arsenite by arsenate

reductase. A gene encoding an arsenate reductase, *PvACR2*, has been cloned from the gametophyte of *P. vittata* (Ellis *et al.*, 2006). Its expression in the gametophyte is constitutive and unaffected by arsenate exposure. Analysis of As speciation in the roots of both *P. vittata* and *P. cretica* shows that arsenate is the dominant species, while arsenite only accounts for 10–40% of the total As (Zhang *et al.*, 2002; Zhao *et al.*, 2002; Poynton *et al.*, 2004; Pickering *et al.*, 2006). By contrast, the fronds contain mainly inorganic arsenite (70–90% of the total As) (Lombi *et al.*, 2002; Wang *et al.*, 2002; Zhang *et al.*, 2002; Zhao *et al.*, 2002; Webb *et al.*, 2003; Poynton *et al.*, 2004; Pickering *et al.*, 2006). The fact that proportionally more arsenite is found in fronds than in roots has led some authors to suggest that arsenate is reduced mainly in fronds (Zhang *et al.*, 2002; Kertulis *et al.*, 2005). However, the differential pattern of As speciation in roots and fronds may also result from a preferential translocation of arsenite from roots to fronds. In fact, Duan *et al.* (2005) assayed the activity of glutathione-dependent arsenate reductase in roots and fronds of *P. vittata* and found this activity only in the roots, suggesting that arsenate reduction occurs mainly in roots and that arsenite is subsequently translocated to fronds. By contrast, using an X-ray imaging technique to determine As speciation in different tissues of *P. vittata*, Pickering *et al.* (2006) proposed that arsenate is transported through the vascular tissue from the roots to the fronds, where it is reduced to arsenite and stored at high concentrations. Therefore, questions regarding the form of As translocated in xylem and the location of arsenate reduction in *P. vittata* remain unresolved. Identification of As species in the xylem sap will help to resolve this controversial issue, as well as providing an important clue to the mechanism responsible for the efficient translocation, and thus accumulation, of As in *P. vittata*.

The main objective of the present study was to determine As speciation in the xylem sap as well as in root and frond tissues of *P. vittata* supplied with either arsenate or arsenite. An additional objective was to investigate whether arsenite is exuded from roots to the external medium, as has recently been shown in plant species that do not hyperaccumulate As (Xu *et al.*, 2007).

Materials and Methods

Plant growth and As treatments

Spores of *Pteris vittata* L. were germinated as described previously (Wang *et al.*, 2002). At the three–four frond stage, plants were transferred to 1-l vessels (one plant per vessel) containing a modified 1/5-strength Hoagland nutrient solution. The composition of the nutrient solution was: 1.0 mM KNO_3, 1.0 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 0.1 mM KH_2PO_4, 1.0 μM $MnCl_2$, 3 μM H_3BO_3, 1 μM $(NH_4)_6Mo_7O_{24}$, 1 μM $ZnSO_4$, 0.2 μM $CuSO_4$ and 60 μM Fe(III)-ethylenediaminetetra-acetic acid (EDTA). The pH of the nutrient solution was buffered at 6.0 with 2 mM 2-morpholino-ethanesulphonic acid (MES). Plants were grown hydroponically for 4–6 wk before the experiments. The nutrient solution was aerated continuously and renewed once every week. Plants were placed inside a controlled environment growth cabinet under the following conditions: day:night temperature 25 : 22°C, day length 16 h with light intensity 350 μmol m^{-2} s^{-1}, and relative humidity 70%.

In Expt 1, the time-course of As uptake and As speciation in roots and xylem sap was investigated. *Pteris vittata* plants at the seven–eight frond stage were exposed to 5 μM arsenate ($Na_2HAsO_4{\cdot}7H_2O$) for 1, 3, 8 and 24 h. Arsenate was added to the basal nutrient solution containing 100 μM phosphate. At each time-point, fronds of four replicate plants were cut at the base of the stipe (*c.* 1 cm above the roots). The cut surfaces were rinsed with deionized water and blotted dry. Xylem exudates were collected using a micropipette for 1 h after excision. Xylem saps were immediately diluted 500-fold with phosphate-buffered solution (PBS) containing 2 mM NaH_2PO_4 and 0.2 mM Na_2-EDTA (pH 6.0), which was the eluant solution used for As speciation analysis (see following section). Addition of EDTA has been shown to preserve As speciation in groundwater samples, because EDTA complexes transition metals which are catalysts of arsenite oxidation (Bednar *et al.*, 2002). Fronds were rinsed with deionized water, blotted dry, weighed and frozen in liquid nitrogen. Roots were immersed in a 1-l ice-cold desorption solution containing 1 mM K_2HPO_4, 0.5 mm $Ca(NO_3)_2$ and 0.5 mm MES (pH 6.0) for 10 min to remove apoplastic As. Root samples were blotted dry, weighed and frozen in liquid nitrogen.

Expt 2 was designed to investigate As speciation in roots, fronds and xylem sap of *P. vittata* as affected by the form of As supplied (arsenate vs arsenite) and phosphate. An additional objective was to monitor As speciation in the nutrient solution during the 24-h exposure period. Plants at the five–six frond stage were exposed to 5 μM arsenate ($Na_2HAsO_4{\cdot}7H_2O$) or arsenite ($NaAsO_2$), which was added to the nutrient solution containing either 100 μM phosphate (+P) or no phosphate (–P). In the –P treatment, potassium phosphate was replaced with potassium chloride. Each treatment was replicated four times. The exposure to As lasted for 24 h, from 09:00 h (2 h after the light period started) to 09:00 h next day. At 0, 2, 6, and 24 h, 0.5 ml of the nutrient solution was collected from each vessel for the determination of As speciation. At 24 h, xylem exudates were collected as already described. Roots and fronds were then harvested as described in Expt 1.

Arsenic analysis

Roots and fronds were ground to fine powder in a mortar and pestle with liquid nitrogen. Subsamples (*c.* 0.1 g) of the ground materials were extracted with 20 ml of PBS for 1 h under sonication. The extracts were filtered through four

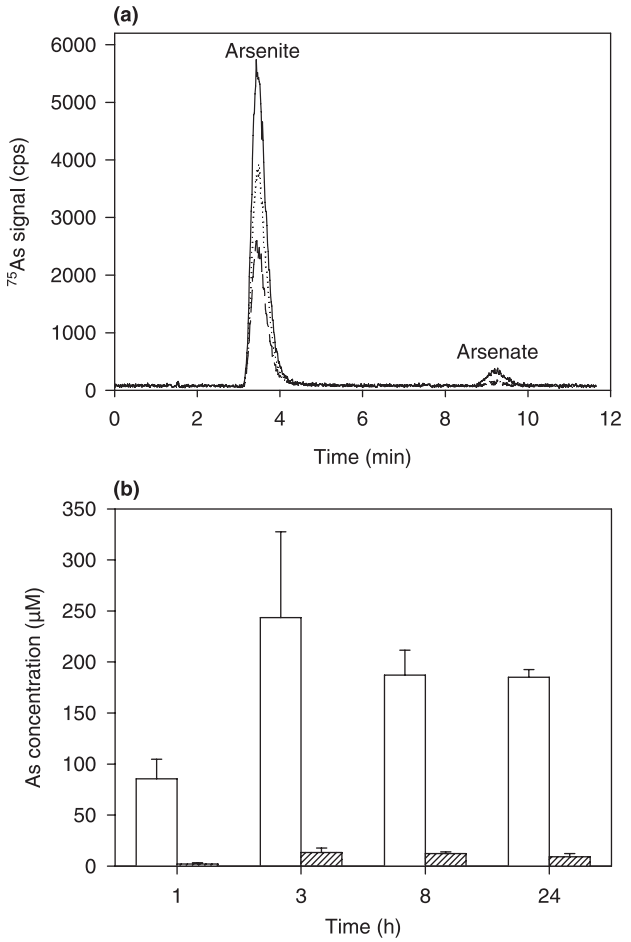

Fig. 1 Arsenic speciation in the xylem sap of *Pteris vittata* in the time-course experiment: (a) high-performance liquid chromatography linked to inductively coupled plasma mass spectrometry (HPLC-ICP-MS) chromatograms for the xylem sap samples collected after 1 (dashed line), 3 (solid line) or 24 (dotted line) h of exposure to 5 μM arsenate (after 500-fold dilution); and (b) concentrations of arsenite (open bars) and arsenate (hatched bars) in the xylem sap samples collected from arsenate-treated plants at different time-points. Data are means ± SE (n = 4). cps, counts per second.

layers of muslin cloth, followed by filtration through 0.45-μm filters before analysis of As speciation.

Arsenic species in the nutrient solutions, xylem sap and plant extracts were determined using high-performance liquid chromatography (HPLC) linked to inductively coupled plasma mass spectrometry (ICP-MS) (Agilent LC1100 series and Agilent ICP-MS 7500ce; Agilent Technologies, Santa Clara, CA, USA) (Xu *et al.*, 2007). Four As species (arsenite, arsenate, dimethyarsinic acid (DMA), and monomethylarsonic acid (MMA)) were separated by an anion-exchange As speciation column (Agilent G3154-65001), fitted with a guard column (Agilent G3154-65002). The PBS solution (2 mM NaH_2PO_4 and 0.2 mM Na_2-EDTA, pH 6.0) was pumped through the column isocratically at 1 ml min^{-1}. The outlet of the separation column was connected to a concentric nebulizer and a water-jacketed cyclonic spray chamber of the ICP-MS. An internal standard containing 500 μg l^{-1} germanium (Ge) was mixed continuously with the post-column eluant. Signals at m/z 75 (As) and 72 (Ge) were collected with a dwell time of 500 ms, and the Ge signal was used to normalize the As signal to correct any drift. Possible polyatomic interference of ArCl on m/z 75 was removed by the Agilent Octopole Reaction System operating in the helium gas mode. Peaks were identified by comparisons with the retention times of standard compounds. Arsenic species in the samples were quantified by external calibration curves with peak areas. Analysis of As species was carried out within 12 h of sample collection or extraction. A preliminary test showed that the PBS solution was effective in preserving As speciation during this period.

Ground plant samples (*c.* 0.1 g fresh weight) were digested in 5 ml of HNO_3/$HClO_4$ (85/15, volume/volume). Total As concentrations in the samples were determined by ICP-MS (Agilent 7500ce) operating in the helium gas mode as already described. Certified reference materials (seaweed IAEA-140/TM and tomato (*Lycopersicon esculentum*) leaves NIST1573a) and blanks were included for quality assurance. Repeated analysis of the two certified reference materials gave 43.6 ± 1.5 μg As g^{-1} for IAEA-140/TM (certified value 44.3 ± 2.1 μg As g^{-1}) and 0.106 ± 0.0057 μg As g^{-1} for NIST1573a (certified value 0.112 ± 0.004 μg As g^{-1}), respectively.

Statistical analysis

One-way or two-way analysis of variance (ANOVA) was performed to test the significance of treatment effects. Where necessary, data were transformed logarithmically before ANOVA to stabilize the variance.

Results

Experiment 1

On average, the fresh weight of fronds and roots was 16.4 ± 1.4 and 22.5 ± 1.7 g per plant, respectively, when plants were harvested after 1–24 h of exposure to As. There was no significant difference in the biomass among different exposure times. Between 30 and 80 μl of xylem exudate was collected from each plant. Although plants were exposed to arsenate in the nutrient solution, arsenite was the predominant species of As in the xylem saps, accounting for 93–98%, with the rest being arsenate (Fig. 1a). No methylated As species were detected in the xylem sap. The relative proportion of arsenite did not change significantly (P = 0.074) during arsenate exposure (Fig. 1b). After only 1 h of exposure to arsenate, large concentrations of As (mainly arsenite) were already detected in the xylem sap; the

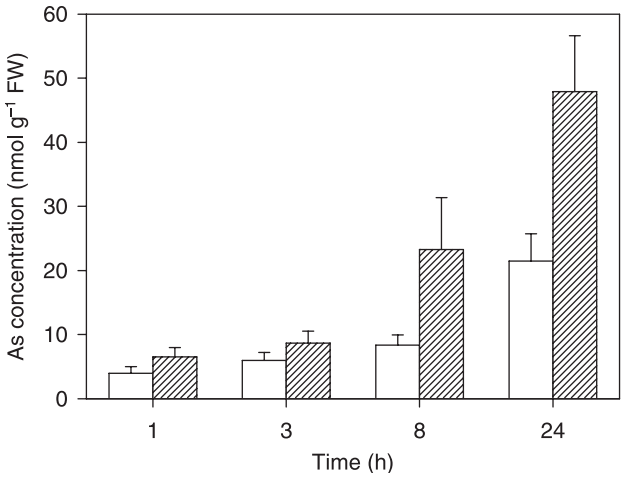

Fig. 2 Concentrations of arsenite (open bars) and arsenate (hatched bars) in the roots of *Pteris vittata* in the time-course experiment. Plants were exposed to 5 μM arsenate. Data are means ± SE (n = 4).

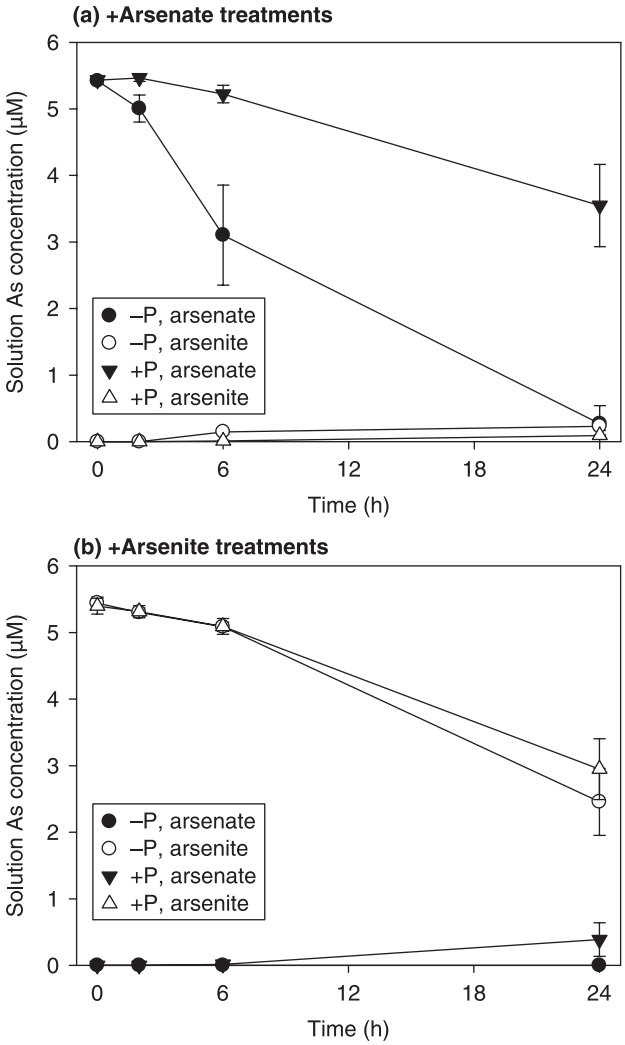

Fig. 3 Arsenic speciation in the nutrient solutions in Expt 2: concentrations of arsenate and arsenite in the +arsenate treatments (a) and the +arsenite treatments (b). Data are means ± SE (n = 4). P, phosphate.

concentration increased further after 3–24 h of exposure (Fig. 1b) and was the greatest at 3 h. Because of a relatively large variation among replicates, the difference in sap arsenite concentration among different time-points was not significant ($P = 0.118$). For the arsenate concentration in xylem sap, the difference among time-points was significant at $P < 0.05$. The sum of arsenite and arsenate in the xylem sap was 18–51 times the initial concentration of arsenate in the nutrient solution.

Speciation of As in roots was determined. The concentrations of both arsenite and arsenate increased with the time of arsenate exposure, with the latter being approximately twofold greater than the former (Fig. 2). Arsenite accounted for 30–41% of the total As extracted in PBS and there were no significant differences among different time-points (Fig. 2). Total As concentration in roots determined by acid digestion was similar to the sum of arsenite and arsenate in the PBS extracts determined by the As speciation method used, with a mean recovery by PBS extraction of 102.2 ± 3.9% (data not shown). The excellent agreement between PBS extraction and acid digestion indicates that the extraction method was effective in recovering As from the root tissues. Arsenic speciation in fronds was not determined in this experiment.

Experiment 2

In this experiment, As speciation in roots, fronds and xylem sap was determined in plants exposed to either arsenate or arsenite for 24 h with or without phosphate. In addition, As speciation in the nutrient solutions was analysed. Plants used in this experiment were smaller than those in the first experiment, fronds being on average 3.2 ± 0.5 g and roots 9.0 ± 0.9 g per plant. There was no significant difference in the biomass among different treatments because of the short exposure time. Between 80 and 300 μl of xylem exudate was collected from each plant.

In the +arsenate treatments, the rate of arsenate depletion in the nutrient solution depended on the phosphate treatment (Fig. 3a). Approximately 95% of the arsenate added to the solution was depleted by 24 h in the –P treatment, compared with only 35% depletion in the +P treatment (Fig. 3a), indicating a strong inhibition by phosphate of arsenate uptake by *P. vittata*. Although the nutrient solutions contained only arsenate initially, small amounts of arsenite were detected in the nutrient solutions after 6 h, and by 24 h arsenite accounted for 1.7 and 4.2% of the total amount of arsenate added in the +P and –P treatments, respectively.

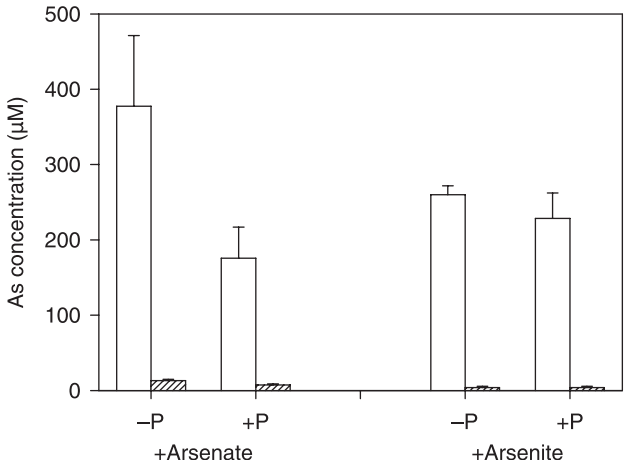

Fig. 4 Concentrations of arsenite (open bars) and arsenate (hatched bars) in the xylem sap of *Pteris vittata* as affected by arsenic (As) species supplied and phosphate (P) treatments in Expt 2. Data are means ± SE (n = 4).

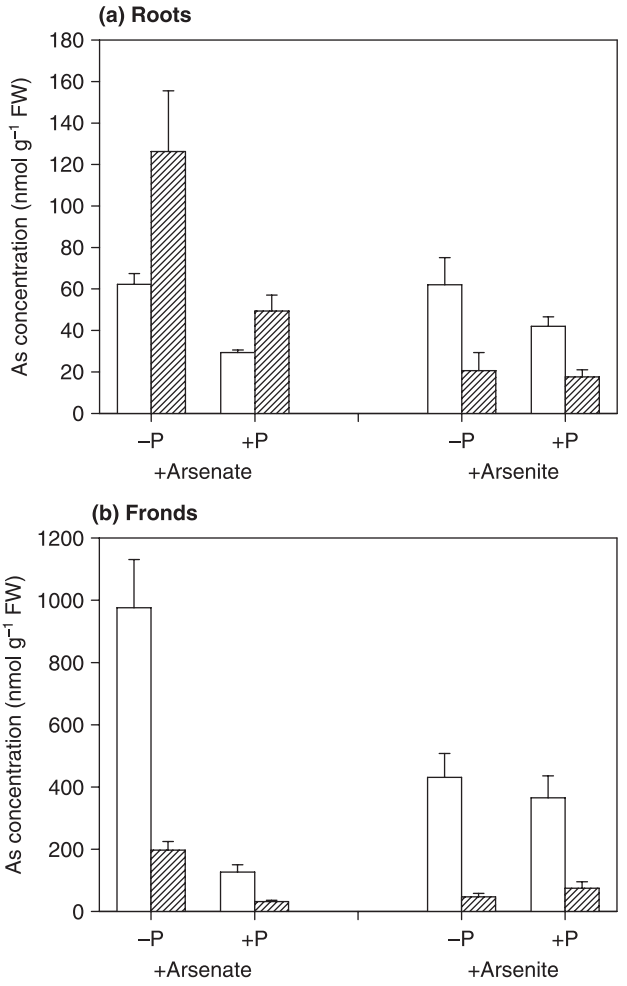

Fig. 5 Concentrations of arsenite (open bars) and arsenate (hatched bars) in the roots (a) and fronds (b) of *Pteris vittata* as affected by arsenic (As) species supplied and phosphate (P) treatments in Expt 2. Data are means ± SE (n = 4).

In the +arsenite treatments, the phosphate treatments had little effect on the depletion of arsenite in the nutrient solutions (Fig. 3b). By 24 h, *c*. 50% of the arsenite added was depleted from the solution. No arsenate was detected in the nutrient solution of the –P treatment, whereas a small amount of arsenate, accounting for 7.1% of the arsenite added, was found in the +P treatment by 24 h.

Regardless the species of As (arsenate or arsenite) supplied to the plants, most of the As in the xylem sap was in the form of arsenite, accounting for on average 95 and 97% of the total As in the sap collected from the arsenate- and arsenite-treated plants, respectively (Fig. 4). Arsenate was only a minor species of As in the xylem sap (3–5%), and no methylated As species were detected. The total concentration of As (sum of arsenite and arsenate) varied from 186 to 394 μM, representing 34–73 times the initial concentration of As added to the nutrient solution. The presence of phosphate decreased the arsenite concentration in the xylem sap significantly ($P < 0.05$; Table 1), with the effect being much more pronounced in the +arsenate treatment (53% inhibition) than in the +arsenite treatment (11% inhibition). For arsenate concentration in xylem sap, a significant ($P < 0.001$) difference was found between the +arsenate and +arsenite treatments, with the former having a 2.2-fold higher concentration than the latter treatment (Fig. 4).

Arsenic speciation in roots was strongly influenced by the species of As supplied to the roots (Fig. 5a, Table 1). In the arsenate-treated plants, arsenate was the dominant species, accounting for 62.5% of the total extractable As in roots, with the remainder being arsenite. By contrast, when arsenite was supplied to the plants, arsenite became the dominant species in roots (73.3% of extractable As). Addition of phosphate to the nutrient solution significantly decreased the concentrations of both arsenate and arsenite in roots, but had no significant effect on the relative proportions of the two As species (Table 1).

Arsenite was the dominant species of As in fronds regardless of whether arsenate or arsenite was supplied to the plants (Fig. 5b). On average, 79 and 86% of extractable As was present as arsenite in the fronds of the arsenate- and arsenite-treated plants, respectively. There was a strong effect of phosphate treatment on the concentrations of both arsenite and arsenate in fronds, as well as a highly significant interaction between phosphate and the As species supplied (Fig. 5b, Table 1). This interaction was observed because phosphate had a strong effect on frond As concentration only in the +arsenate treatment, but not in the +arsenite treatment. After 24 h of exposure, 48 and 69% of the As taken up by plants was distributed to the fronds in the +arsenate and +arsenite treatments, respectively. This difference was highly significant, whereas the phosphate treatment had no significant effect on As distribution to the fronds (Table 1).

Table 1 Significance of treatment effects in Expt 2 (ANOVA *F* values)

Treatment	Xylem sap concentration			Root As concentration			Frond As concentration			% of As translocated to fronds
	Arsenite	Arsenate	% arsenite	Arsenite*	Arsenate*	% arsenite	Arsenite*	Arsenate*	% arsenite	
As species	0.551	0.001	0.001	0.207	< 0.001	< 0.001	0.627	0.095	0.014	< 0.001
Phosphate	0.048	0.064	0.485	< 0.001	0.116	0.73	< 0.001	0.004	0.088	0.284
As × phosphate	0.134	0.077	0.600	0.119	0.151	0.478	< 0.001	< 0.001	0.348	0.737

*Data were transformed logarithmically before ANOVA.
See text for details of Expt 2.
As, arsenic.

As in Expt 1, total As concentrations in roots and fronds determined by acid digestion were in good agreement with the sum of arsenite and arsenate in the PBS extracts determined by HPLC-ICP-MS, with a mean recovery by PBS extraction of 102.4 ± 7.8% (data not shown).

Discussion

The results of the present study showed that arsenate and arsenite were the main form of As in roots and fronds of *P. vittata*, respectively, when plants were supplied with arsenate. Arsenate accounted for 60–70% of the total As in roots, but only *c*. 20% in fronds, with the remainder being present as arsenite. These results are in general agreement with those of previous studies employing different methodologies for As speciation analysis, including HPLC-ICP-MS and X-ray absorption spectrometry (Lombi *et al*., 2002; Wang *et al*., 2002; Zhang *et al*., 2002; Webb *et al*., 2003; Zhao *et al*., 2003; Pickering *et al*., 2006).

Pteris vittata takes up arsenate via the phosphate transport pathway (Wang *et al*., 2002; Poynton *et al*., 2004). This is further confirmed by a large inhibitory effect of phosphate on the depletion of arsenate from the nutrient solution and on As accumulation by the plants observed in the present study (Figs 3, 5). The time-course experiment showed that a portion of arsenate was rapidly reduced to arsenite in roots, although the percentage of arsenite in roots remained relatively constant (30–40%) from 1 to 24 h (Fig. 2). However, until now it has not been resolved whether roots or fronds are the main location of arsenate reduction in *P. vittata*, and what is the main form of As transported from roots to fronds. Our results showed that As was transported in the xylem sap of *P. vittata* predominantly (93–98%) as arsenite, regardless of whether arsenate or arsenite was supplied to the plants (Figs 1, 4). This also means that roots are the main site of arsenate reduction in *P. vittata*, and that, following arsenate reduction, arsenite is preferentially loaded into the xylem. This conclusion is supported by the study of Duan *et al*. (2005), who reported measurable activity of glutathione-dependent arsenate reductase only in the roots of *P. vittata*, and not in the fronds. In Expt 2 of the present study, as well as in the study of Wang *et al*. (2002), proportionally more As was distributed from roots to fronds in the short term (8–24 h) when As was supplied as arsenite than as arsenate. Furthermore, addition of L-buthionine-sulphoximine (BSO) to the nutrient solution was found to inhibit glutathione biosynthesis in the roots of *P. vittata* markedly, leading to decreased arsenate reduction in roots (a smaller proportion of arsenite) and markedly decreased translocation of As to the fronds (by 40–50%) (Zhao *et al*., 2003). These results are consistent with a model in which arsenate is reduced to arsenite in the roots of *P. vittata* first, before being transported to the fronds as arsenite. In both the present study on *P. vittata* and that of Poynton *et al*. (2004) on *P. cretica*, phosphate had no significant effect on As translocation from roots and fronds. Inhibition of As translocation by phosphate might be expected if As were translocated as arsenate. However, phosphate would have no effect if As is translocated as arsenite, as shown in the present study. The fact that the percentage of arsenite in the fronds (*c*. 80%) was smaller than that in the xylem sap (93–98%) suggests that some arsenite may be oxidized to arsenate in the fronds. It is also possible that redox cycling of As may take place in the frond to some extent, although direct evidence for this has yet to be obtained.

The results of the present study do not support the conclusion of Kertulis *et al*. (2005) and Pickering *et al*. (2006) that *P. vittata* transports mainly arsenate from roots to fronds, and that the latter are the main location of arsenate reduction. In the study of Kertulis *et al*. (2005), 'xylem sap' was collected from intact fronds using a Scholander pressure chamber. Whether the sap obtained in this way really represents the xylem sap originating from the root system remains to be tested. One surprising result from the study of Kertulis *et al*. (2005) is that the As concentration in the sap was lower than that in the external solution, which is at variance with the fact that *P. vittata* is extremely efficient at As translocation from roots to shoots (Tu & Ma, 2002; Caille *et al*., 2005). In our study, xylem sap was obtained from the natural root pressure within a short period after stem excision. This is the method that is used most often to obtain xylem sap. Furthermore, the sap samples obtained by Kertulis *et al*. (2005) were stored at −80°C for a unspecified time period before As speciation

analysis. Arsenite is thermodynamically unstable in the oxygenated environment, and it is possible that arsenite is partly oxidized to arsenate during storage. In our study, xylem sap samples were diluted with an EDTA solution, which helps to preserve As speciation (Bednar *et al.*, 2002), followed by immediate analysis of As speciation using HPLC-ICP-MS. The fact that most of the sap As was arsenite in our study indicates that little oxidation occurred, thus giving us confidence in the As speciation data. In the study of Pickering *et al.* (2006), intact fronds were subjected to X-ray absorption spectrometry (XAS) imaging for different As species. They found that arsenate was the dominant species in the central region of the mid-vein of rachis, although the rachis as a whole contained more arsenite (76%) than arsenate (24%). It should be pointed out that the XAS imaging information was for the rachis tissue, not the xylem sap *per se*. Arsenic speciation in the xylem sap may be different from that retained by the vascular bundles.

In a number of As-nonhyperaccumulator species, arsenite was found to be the main species of As in the xylem sap, accounting for *c.* 60% in *Brassica juncea* (Pickering *et al.*, 2000) and sunflower (*Helianthus annuus*; Raab *et al.*, 2005), 87% in cucumber (*Cucumis sativus*; Mihucz *et al.*, 2005) and 90–96% in tomato (*Lycopersicon esculentum*; Xu *et al.*, 2007). Furthermore, Raab *et al.* (2005) showed that arsenite in the xylem sap of sunflower was present in inorganic form, not complexed by thiol compounds. The results suggest that transport of arsenite in xylem is a common feature in both As hyperaccumulators and nonhyperaccumulators.

The translocation of arsenite from roots to fronds was extremely efficient in *P. vittata*, with the As concentration in the xylem sap being 18–73 times greater than that in the external solution. For a comparison, the As concentration in the xylem sap of tomato was only 1.5–10% of the external solution (Xu *et al.*, 2007), *c.* 4% in *B. juncea* (Pickering *et al.*, 2000) and 17.5% in cucumber (Mihucz *et al.*, 2005). Our unpublished data showed a range of 2–50% for several cereal species including barley (*Hordeum vulgare*), maize (*Zea mays*), wheat (*Triticum aestivum*), and rice (*Oryza sativa*). Using the ratio of xylem sap As to external As concentration as an indicator of xylem mobility, *P. vittata* is two to three orders of magnitude higher than the As nonhyperaccumulators already described. Therefore, efficient loading of arsenite to the xylem is likely to be a key step of As hyperaccumulation in *P. vittata*. The second contributing factor to the efficient xylem transport of arsenite may be that very little arsenite in the roots of *P. vittata* is complexed by thiol-containing compounds such as phytochelatins (Zhao *et al.*, 2003; Raab *et al.*, 2004). Complexation of arsenite by thiols is an important mechanism of As detoxification in nonhyperaccumulating plants (Sneller *et al.*, 1999; Schmöger *et al.*, 2000; Raab *et al.*, 2005); complexation may decrease translocation of As from roots to shoots, especially if the arsenite–thiol complexes are subsequently sequestered in the root vacuoles. This interpretation reconciles two pieces of seemingly conflicting evidence regarding the xylem mobility of As: the extremely high mobility of arsenite in *P. vittata* as demonstrated in the present study and the recent report by Dhankher *et al.* (2006) that silencing arsenate reductase in the roots of *Arabidopsis thaliana* markedly increased As accumulation in the shoots. Dhankher *et al.* (2006) proposed that arsenate is the most mobile form of arsenic in the majority of plant species and that arsenite stays sequestered in roots. This model clearly does not apply to *P. vittata*. In *A. thaliana*, and perhaps also other As-nonhyperaccumulating plant species, arsenite may be less mobile than arsenate because of arsenite complexation and sequestration in roots, and possibly also because of the lack of an efficient xylem loading system for arsenite.

The third possible contributing factor to the highly efficient translocation of arsenite in *P. vittata* is the lack of a strong efflux of arsenite from roots cells to the external solution (Fig. 3). Recently, it has been reported that the efflux of arsenite to the external medium, following arsenate reduction in roots, is a prominent process in tomato and rice (Xu *et al.*, 2007). We have found that this is also the case in a range of other plant species including *Arabidopsis thaliana*, *Holcus lanatus*, wheat, barley and maize. Interestingly, *P. vittata* appeared to release very little arsenite to the nutrient solution during the 24-h exposure period. It thus appears that, although *P. vittata* possesses a highly efficient efflux system for arsenite for xylem loading, there is little efflux of arsenite to the external medium.

In conclusion, our study has identified arsenite as the predominant form of As transported in the xylem from roots to fronds of *P. vittata*. This transport is extremely efficient, possibly owing to one or more of the following reasons: efficient reduction of arsenate to arsenite in roots; hyperexpression of an arsenite efflux system toward xylem loading; lack of arsenite complexation by thiol compounds and sequestration in the root vacuoles; and weak efflux of arsenite to the external medium. Unravelling the mechanism responsible for the efficient xylem transport of arsenite in *P. vittata* is imperative to understanding of the As-hyperaccumulation phenotype.

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