Uptake and translocation of arsenite by *Pteris vittata* L.: Effects of glycerol, antimonite and silver

Shiny Mathews a, Bala Rathinasabapathi b, Lena Q. Ma a,⁎

a Soil and Water Science Department, University of Florida, Gainesville, FL 32611, USA
b Horticultural Sciences Department, University of Florida, Gainesville, FL 32611, USA

**A R T I C L E   I N F O**

Article history:
Received 14 February 2011
Received in revised form 2 August 2011
Accepted 10 August 2011

Keywords:
Arsenate
Arsenite
Uptake
Aquaglyceroporins
Glycerol
Antimonite
Silver nitrate

**A B S T R A C T**

Arsinite uptake in living cells is through aquaglyceroporin transporters, but it is unknown in arsenic-hyperaccumulator *Pteris vittata*. We investigated the effects of AsIII analogs glycerol and antimonite (SbIII) at 0–100 mM and aquaporin inhibitor AgNO3 at 0–0.1 mM on the uptake of 0.1 mM AsIII or AsV by *P. vittata* over 1–2 h. Glycerol or SbIII didn’t impact AsIII or AsV uptake by *P. vittata* (p < 0.05), with As concentrations in the fronds and roots being 4.4–6.3 and 3.9–6.2 mg/kg. However, 0.01 mM AgNO3 reduced As concentrations in the fronds and roots by 64% and 58%. Hence, AsIII uptake in *P. vittata* might be via an aquaporin transporter different from glycerol and SbIII transporters. Further AsIII analogs and aquaporin inhibitor had no impact on AsV uptake, AsIII and AsV were likely taken up by different transporters in *P. vittata*. Our results imply a different AsIII transporter in *P. vittata* from other plants.

**1. Introduction**

Arsenic is ubiquitous in the environment, which mainly exists as inorganic forms including arsenate (AsV) and arsenite (AsIII). Arsenic-hyperaccumulator *Pteris vittata* (Chinese brake fern) is capable of taking up both AsV and AsIII (Ma et al., 2001; Kertulis et al., 2005). The mechanisms of plant arsenic uptake depend on arsenic species as they are structurally and chemically different.

Arsenate ions behave as oxyanions in solution, i.e., HAsO2 3− and H2AsO4 − at pH 5–7 (Jeon et al., 2009), which are similar to phosphate, HPO4 2− and H2PO4 − (Teo et al., 2009). As chemical analogs, they compete for entry through the phosphate transport system. This has been observed in *Escherichia coli* (Willsky and Malamy, 1980), yeast (Yompokdee et al., 1996), and plants including barley (Lee, 1988), wheat (Zhu et al., 2006), rice (Meharg, 2004), *Holcus lanatus* (Meharg and Macnair, 1991), and *Brassica napus* (Quaghebeur and Rengel, 2005). Studies also indicate that AsV uptake into *P. vittata* is by the phosphate transporter (Tu and Ma, 2003; Wang et al., 2002). Arsenite (H3AsO3), on the other hand, is present as a neutral species at pH < 9.2 (ODay, 2006) and is transported via aquaglyceroporins in *E. coli* (Meng et al., 2004), yeast (Robert et al., 2001), *Arabidopsis thaliana* (Isayenkov and Maathuis, 2008), rice (Zhao et al., 2010) and human cells (Liu et al., 2002). Hence a similar mechanism of AsIII uptake is expected in *P. vittata*.

There are several AsIII analogs competing with AsIII during its uptake into living cells. Antimonite (SbIII) is one such analog, which is chemically and structurally similar to AsIII. The pH values of SbIII and AsIII are 11.8 and 9.2, respectively, and hence both exist as neutral solutes [Sb(OH)3 and As(OH)3] in the environment (Meng et al., 2004) and may compete for plant uptake. A similarity also exists among AsIII, SbIII and a specific conformation of glycerol, C2H5(OH)3, where the 3 hydroxyl groups occupy nearly the same positions as in As(OH)3 and Sb(OH)3 with similar charge distribution and volume (Porquet and Filella, 2007). Though the molecular diameters of As(OH)3 (2.56–2.81 Å), Sb(OH)3 (2.76–3.05 Å), and C2H5(OH)3 (2.68–3.07 Å) are similar, As(OH)3 is the smallest and C2H5(OH)3 is the largest (Porquet and Filella, 2007).

Though is not essential for organisms SbIII is taken up by living cells through the same channel as glycerol and AsIII. The glycerol facilitator in *E. coli* (GlpF) takes up SbIII and a lack of this transporter in mutants makes them resistant to both SbIII and AsIII (Sanders et al., 1997). Similarly, the glycerol facilitator in yeast (sfp1) takes up both SbIII and AsIII (Wysocki et al., 2001). Mammalian aquaglyceroporins AQP7 and AQP9 permeate both SbIII and AsIII (Liu et al., 2002). The small diameter of As(OH)3 and Sb(OH)3 is an additional advantage for transit through the narrowest region of the glycerol transporter channel (Bhattacharjee
et al., 2008). Hence it can be predicted that a glycerol facilitator that transports glycerol may also be responsible for AsIII and SbIII uptake in *P. vittata* and the presence of SbIII or glycerol in the substrate may inhibit AsIII uptake by *P. vittata*.

The function of these aquaporins can be inhibited by chemicals that interfere with the permeation of water and neutral solutes. Aquaporin inhibitors include mercury, silver, gold, copper, chloroform, tetraethylammonium salts and acetazolamide compounds (Haddoub et al., 2009). Silver is a potent inhibitor of aquaporins due to their interaction with sulfhydryl groups of cysteine near the conserved NPA motif, which blocks the constriction regions of the channel (Niemietz and Tyerman, 2002). Compared to the widely conserved NPA motif, which blocks the constriction regions of the channel (Niemietz and Tyerman, 2002), silver interacts with sulfhydryl groups of cysteine near the conserved NPA motif, which blocks the constriction regions of the channel (Niemietz and Tyerman, 2002). Therefore, it can be predicted that a glycerol facilitator that transports glycerol may also be responsible for AsIII and SbIII uptake in *P. vittata* by investigating 1) the competitive effects of glycerol and SbIII, and 2) the inhibiting effect of AgNO₃ on AsIII and AsV uptake by *P. vittata*. 

2. Materials and methods

2.1. Experimental setup

*P. vittata* ferns from Milestone Agriculture Inc. (Apoka FL, USA) were used. They were 4 months of age, 15–18 cm in height, and uniform in size. The ferns were acclimatized hydroponically in a 0.2-strength Hoagland solution with pH adjusted to 5.7 with 1 mM KOH-MES [2-(N-morpholino) ethanesulfonic acid] buffer for 2 weeks. During the experiments, the ferns were maintained with constant aeration, photon flux of 350 μmol m⁻² s⁻¹ using cool and warm white fluorescent lamps with temperature maintained at 23–28 °C and relative humidity of 70%.

After acclimatization in 0.2-strength Hoagland solution, the ferns were acclimatized in a solution of 0.5 mM MES and 0.5 mM CaCl₂ (pH 5.7) for 1 day. Following this they were transferred to opaque containers containing 1 L of solutions spiked with 0.1 mM AsV (Na₂H₂AsO₄ 7H₂O) or 0.1 mM AsIII (Na₂AsO₃) (Sigma, St. Louis, MO). The arsenic was provided in denitized water in all experiments to minimize P competition for AsV.

2.2. Preliminary experiment

An experiment was conducted to study the time dependency of AsIII and AsV uptake into *P. vittata*. They were grown in 1 L solution of 0.1 mM AsIII or AsV for 1, 2, 4, 6 and 24 h. The root samples were analyzed to understand the short term influx of AsIII into *P. vittata*. The water samples were analyzed to understand AsIII stability in the media in the presence of *P. vittata*. The data were used to decide the time required for the uptake competition and inhibition experiments.

2.3. Competition and inhibition experiment

Glycerol [C₃H₅(OH)₃] and SbIII (potassium antimonyl tartrate) at concentrations 0, 0.1, 1.0, and 100 mM were used to compete with 0.1 mM AsIII or AsV. The inhibition studies used silver nitrate (AgNO₃) at concentrations 0, 0.001, 0.01 and 0.1 mM against 0.1 mM AsIII or AsV. For the inhibition study *P. vittata* were first treated with Ag for 1 h before addition of AsIII or AsV to ensure prior inhibition of the aquaporina. All experiments were performed in triplicates with distilled water, digested with concentrated HNO₃ (1:1, v/v), and followed by 30% H₂O₂ (USEPA Method 3050A).

 Arsenic concentrations in the growth media and plant tissues were determined by a graphite furnace atomic absorption spectrophotometer (Varian 240Z, Walnut Creek, CA). In addition, standard reference materials from the National Institute of Science and Technology (Gaithersburg, MD) and appropriate reagent blanks, internal standards and spikes were used as quality checks and were within 100 ± 20% of the expected values. The Sb concentration in the media and plant samples were analyzed using inductively coupled plasma-atomic emission spectrometry (PerkinElmer 5300DV, Waltham, MA).

![Fig. 1](image.png)

**Fig. 1.** Arsenic concentrations in the media i) total As and AsIII concentrations in AsIII treatment, and ii) total As concentration in AsV treatment, and b) total As concentrations in the root of *P. vittata* after exposing to 0.1 mM AsIII or AsV for 1, 2, 4, 6, and 24 h. Lower case letters indicate significant difference in total As concentrations in AsIII treatment and upper case letters for AsV treatment (p < 0.05). The bars indicate standard error of triplicate. Total As = AsV + AsIII.

3. Results and discussion

The effects of AsIII analogs (glycerol and SbIII) at 0, 0.1, 1.0 and 100 mM and aquaporin inhibitor (AgNO₃) at 0, 0.001, 0.01 and 0.1 mM on arsenic uptake by *P. vittata* after exposing to 0.1 mM AsIII or AsV for 1 h were investigated. Arsenic speciation in the growth media and plant biomass was determined. Since arsenic is predominantly present as AsIII and AsV in *P. vittata*, arsenic speciation data were expressed as total As (AsIII + AsV) and AsIII.

A preliminary experiment determined arsenic stability in the growth media and compared AsIII and AsV uptake by *P. vittata*. In the presence of *P. vittata*, AsIII in the media was stable within 1 h, after which it was gradually transformed to AsV. In comparison, AsV was stable beyond 24 h (Fig. 1a). The data were important to understand As stability in the media during the treatment time as different As species are taken up by different transporters systems.
in plants. The influx of both AsIII and AsV into *P. vittata* roots was linear for up to 8 h (Fig. 1b). To minimize AsIII oxidation, 1 h was used for further experiments. During this period, the difference in AsIII and AsV uptake by *P. vittata* was minimal (Fig. 1b).

### 3.1. Effects of glycerol and SbIII on AsIII uptake by *P. vittata*

AsIII uptake in rice (Meharg and Jardine, 2003) and Arabidopsis (Kamiya et al., 2009) is via aquaporins. Aquaporins can either be water channels (aquaporins) or glycerol transporters (aquaglyceroporins) and are known to allow the passage of water, glycerol, boric acid, silicic acid and metalloids such as AsIII and SbIII. The major difference between aquaporins and aquaglyceroporins are in constriction regions of the channel. The narrow constriction in water-specific aquaporins have a diameter of 2.8 Å similar to that of a water molecule and aquaglyceroporins have a diameter of 3.4 Å similar to that of a glycerol molecule (Beitz et al., 2004).

Our study showed no significant difference (*p* < 0.05) in AsIII uptake by *P. vittata* as the arsenic concentrations in the roots and fronds treated with different concentrations of glycerol (Fig. 2b, c) or SbIII (Fig. 4b, c) were similar. The analysis of the growth media in the presence of different concentrations of glycerol (Fig. 3b, c) and SbIII (data not shown) were similar to the AsIII treatment in the presence of glycerol (0.1–1000 mM) or SbIII (0.1–0.5 mM). Glycerol competes with AsIII for uptake in rice similar to that observed in yeast (Wysocki et al., 2001). However, this was not observed in *P. vittata*.

The total arsenic concentrations in the fronds with the glycerol treatment (4.6–6.3 mg/kg As; Fig. 2c) were slightly greater than those in the SbIII treatment (4.4–5.7 mg/kg As; Fig. 4c). The root arsenic concentrations were 3.8–6.2 mg/kg (Fig. 2b) and 3.9–4.2 mg/kg (Fig. 4b) in the glycerol and SbIII treatments. The slightly increased As uptake in the presence of glycerol may attribute to the positive impact it had on microbes. Plant growth promoting rhizobacteria are known to enhance metal accumulation by enhancing plant growth or sequestering metal ions inside the cell walls (Khan et al., 2009). Glycerol in the media can act as a carbon source for the microbes promoting As accumulation by *P. vittata*. On the other hand, SbIII is known to inhibit microbial activity in soil (An and Kim, 2009), reducing As uptake in SbIII treatment compared to glycerol. Even though the experiment lasted only 1 h, the arsenic in the fronds was predominantly AsIII ranging from 78 to 96% in both glycerol and SbIII treatments whereas the roots contained almost all AsV (Figs. 2b and 3b).

In addition to As, total Sb concentrations in *P. vittata* were analyzed. There was an increase in Sb concentrations in the fronds and roots with an increase in Sb concentration in the media (Fig. 5). The Sb concentrations in the roots (45–574 mg kg−1) were much greater than those in the fronds (3.2–13 mg kg−1), which is consistent with Müller et al. (2009). The fact that *P. vittata* accumulated up to 5742 mg kg−1 Sb in the roots without impacting AsIII uptake indicated that SbIII and AsIII were probably taken up by different transporters in *P. vittata*.

Compared to SbIII, B uptake by *P. vittata* was much less effective. After exposing to 0.3 mM boric acid for 1 d, B concentrations in the roots and fronds are 20–22 mg kg−1 and 2.5–6.2 mg kg−1 (Wang et al., 2010). In comparison, after exposing to 0.1 mM SbIII...
for 1 h, the root and frond concentration was 45 and 3.2 mg kg\(^{-1}\) (Fig. 5a). However, Si uptake by \textit{P. vittata} is different from SbIII and B. Exposing 0.5 mM boric acid for 1 d has no effect on Si concentrations in \textit{P. vittata}, which are 1.4–1.6 g kg\(^{-1}\) in the fronds and 1.4–1.8 g kg\(^{-1}\) in the roots (Wang et al., 2010). Though no Si is provided in the Hoagland solution, \textit{P. vittata} takes up substantial amount, most likely from the soil media before being transferred to Hoagland solution.

Our data also indicated that \textit{P. vittata} was effective in taking up SbIII by the roots, but was ineffective in translocating SbIII from the roots to fronds during the 1 h experiment. Though both AsIII and SbIII were present as neutral species, AsIII over SbIII was preferentially translocated by \textit{P. vittata}. For example, the highest Sb translocation factor (TF; ratio of Sb in the fronds to roots) in \textit{P. vittata} was 0.07 (Fig. 5). In comparison, the highest As TF in \textit{P. vittata} treated with AsIII and SbIII was 2.4 (Fig. 4). This indicates, upon uptake, \textit{P. vittata} translocated 71% As to the fronds compared to only 7% Sb. Similar results are obtained for B, with 13–28% of the B being translocated to the fronds (Wang et al., 2010). However, \textit{P. vittata} is effective in translocating Si, with similar distribution in the roots and fronds (Wang et al., 2010).

The results clearly showed that though AsIII and SbIII are analogs, their uptake and translocation mechanisms in \textit{P. vittata} were different. Competition experiments using AsIII analogs silicic acid and boric acid also showed no impact on AsIII uptake by \textit{P. vittata} (Wang et al., 2010). However, silicic acid \([\text{Si(OH)}_4]\) has a molecular diameter of 4.38 Å, which is larger than that of As(OH)\(_3\) (4.11 Å; Ma et al., 2008) and the ar/R region of the aquaglyceroporin, and hence silicic acid may not be an efficient competitor of AsIII uptake. Boric acid (2.65 Å; Greenwood, 1973), on the other hand, was taken up by both passive diffusion through lipid bilayers and active channel mediated uptake (Dordas et al., 2002), and hence may not be solely taken up by an aquaporin transporter. In a study on rice, a dose dependent reduction in AsIII uptake was observed when similar treatments using glycerol and SbIII was performed on excised roots, indicating that AsIII was taken up by glycerol transporters in rice (Meharg and Jardine, 2002), was used to further shed light on the mechanisms of AsIII uptake in \textit{P. vittata}. A hexose permease pathway has been proposed for AsIII uptake in yeast (Liu et al., 2004) and a glucose permease pathway in mammals (Liu et al., 2006). Our results provide a base to search for alternate and novel AsIII transporters in \textit{P. vittata}.

### 3.2. Effects of AgNO\(_3\) on AsIII uptake by \textit{P. vittata}

Silver, an aquaglyceroporin inhibitor (Niemietz and Tyerman, 2002), was used to further shed light on the mechanisms of AsIII uptake by \textit{P. vittata}. Hg is commonly used as inhibitor of aquaporin activity but at higher concentrations it may be phytotoxic to plants or non-specific. For example, the plasma membrane aquaporin in \textit{Nicotiana tabacum} is Hg insensitive (Biela et al., 1999). Due to its phytotoxicity, reduction in AsIII uptake may result from phytotoxic effect. Moreover, a previous study indicates that 0.01 mM Hg had no impact on AsIII uptake by \textit{P. vittata} when treated with 0.015 mM AsIII for 2 d (Wang et al., 2010).

Unlike Hg, the presence of Ag significantly reduced As uptake by \textit{P. vittata} (Fig. 6). As the Ag concentrations increased from 0 to 0.001 and to 0.01 mM, the arsenic concentrations in the roots decreased from 3.8 to 2.5 and to 1.4 mg kg\(^{-1}\), and those in the fronds from 5.1 to 5.0 and to 3.0 mg kg\(^{-1}\) (Fig. 6b, c). The impact was most pronounced at 0.01 mM Ag, with As reduction being 64% in the roots and 58% in the fronds compared to the control. To confirm the impact of Ag at 0.01 mM, the experiment was repeated for 2 h,
which showed similar results. At 0.01 mM Ag, arsenic concentrations in the fronds and roots were reduced by 63% and 48% compared to the control (Fig. 7). Similar to this study, a significant decrease in As accumulation was observed when P. vittata was treated with 10 and 100 μM Ag for 15 d (Nagarajan and Ebbs, 2007).

Compared to AsIII uptake by P. vittata, the impact of Ag on AsV uptake was much limited. Regardless of the Ag concentrations used, Ag had little impact on arsenic concentrations in the fronds, ranging from 4.9 to 5.2 mg kg⁻¹ (data not shown). However, with Ag concentrations increasing from 0 to 0.001–0.01 mM, the arsenic concentrations in the roots decreased from 3.8 to 3.5 and 2.7 mg kg⁻¹, however, they were statistically insignificant (data not shown).

3.3. AsIII oxidation in the media and in P. vittata

To minimize AsIII oxidation, we used 1 h in our experiment. Based on the preliminary data, little AsIII oxidation was observed in the growth media within 1 h (Fig. 1a). However, limited AsIII oxidation occurred in the glycerol and AgNO₃ treatments. For example, 1.7–8.9% AsIII was oxidized in the presence of glycerol and 4–13% AsIII was oxidized in the presence of AgNO₃. With AgNO₃ concentrations increasing from 0.001 to 0.01 and to 0.1 mM, the percentage of AsIII decreased from 96, to 92 and to 87% though total As remained constant (Fig. 6a). This means some of the As was taken up by P. vittata as AsV instead of AsIII.

In addition to the limited oxidation of AsIII in the growth media (Fig. 2a), AsIII can be oxidized to AsV on the root surface, which are rich in microbes (Mathews et al., 2010). The oxidized AsV may be taken up by the plant immediately and would not contribute to AsV concentration in the media. This means though AsV was not detected in the media, some of the As was taken up as AsV by P. vittata, which may follow the phosphate transporter pathway and therefore would not be inhibited by glycerol or SbIII.

This hypothesis was supported by arsenic speciation data in the roots, which included the rhizomes. Though only 1.7–13% AsV was present in the growth media (Figs. 2a and 6a), only AsV was present in the roots in the glycerol (Fig. 2b) and Ag (Fig. 6b) treatments. In the case of SbIII, little AsV was detected in the media (Fig. 4a), yet only AsV was present in the roots (Fig. 4b). We suspect that part of the AsV in the roots was taken up by phosphate transporters in P. vittata. In contrast to the roots, most of the As in the fronds was present as AsIII, ranging from 69 to 96% in the presence of AsIII and glycerol (Fig. 3c). Even in the AsV and glycerol treatment, 81–85% of the As in the fronds was present as AsIII (Fig. 3c). Similar data were observed in the AsIII-SbIII and AsV-SbIII treatments, with 72–98% (Fig. 4c) and 82–95% arsenic (data not shown) being present as AsIII in the fronds. Regardless AsIII or AsV was provided in the media, AsV dominated in the roots whereas AsIII dominated in the fronds of P. vittata. The high AsIII concentrations in the fronds may have resulted from both faster translocation of AsIII from the roots to the fronds (Su et al., 2008) and further reduction of AsIII to AsV in the roots. Further study is needed to confirm these hypotheses.

Unlike AsIII uptake in microbes, humans and plants (Sanders et al., 1997; Wysocki et al., 2001; Liu et al., 2002 and Bhattacharjee et al., 2008), AsIII uptake in P. vittata was not significantly affected by glycerol or SbIII. AsIII, which is a neutral solute similar to boric acid, may be taken up by both passive diffusion through lipid bilayers and active channel mediated uptake (Horst et al., 2002) and hence

Fig. 6. Effect of AgNO₃ on arsenic concentrations in the a) growth media, b) roots and c) fronds of P. vittata after exposing to 0.1 mM AsIII for 1 h. Different lower case letters indicate significant difference in AsIII concentration and different upper case letters in total As concentration (p < 0.05). The bars indicate standard error of triplicate. Total As = AsV + AsIII.

Fig. 7. Effect of 0.01 mM AgNO₃ on arsenic concentrations in the fronds and roots of P. vittata after exposing to 0.1 mM AsIII (a) and AsV (b) for 2 h. Different lower case letters indicate significant difference in AsIII concentration and different upper case letters in total As concentration (p < 0.05). The bars indicate standard error of triplicate. Total As = AsV + AsIII.
may not be solely taken up by an aquaporin transporter. Thus, together with other studies on AsIII uptake in *P. vittata* (Wang et al., 2010), our results implicate the operation of a silver-sensitive AsIII uptake system in *P. vittata* where unusual aquaglyceroporins or other novel transporter proteins may participate.

**Acknowledgments**

This research was supported in part by University of Florida IFAS Innovation Fund.

**References**


