Environmental Pollution 206 (2015) 49–55

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Uptake of antimonite and antimonate by arsenic hyperaccumulator *Pteris vittata*: Effects of chemical analogs and transporter inhibitor

Rujira Tisarum [a, b], Yanshan Chen [a], Xiaoling Dong [b], Jason T. Lessl [a, b], Lena Q. Ma [a, b, *]

[a] *State Key Lab of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Jiangsu 210046, China*
[b] *Soil and Water Science Department, University of Florida, Gainesville, FL 32611, USA*

A R T I C L E I N F O

Article history:
Received 23 March 2015
Received in revised form
19 June 2015
Accepted 23 June 2015
Available online xxx

Keywords:
Antimony
Pteris vittata
Phosphate
Gametophyte
Silicic acid
Glucose

A B S T R A C T

Antimonite (SbIII) is transported into plants via aquaglyceroporin channels but it is unknown in As-hyperaccumulator *Ptreis vittata* (PV). We tested the effects of SbIII analogs (arsenite-AsIII, glycerol, silicic acid-Si, and, glucose), antimonate (SbV) analog (phosphate-P), and aquaglyceroporin transporter inhibitor (silver, Ag) on the uptake of SbIII or SbV by PV gametophytes. PV gametophytes were grown in 20% Hoagland solution containing 65 μM SbIII or SbV and increasing concentrations of analogs at 65−6500 μM for 2 h or 4 h under sterile condition. After exposing to 65 μM Sb for 2 h, PV accumulated 767 mg/kg Sb in SbIII treatment and 419 mg/kg in SbV treatment. SbIII uptake by PV gametophytes was not impacted by glycerol or AsIII nor aquaglyceroporin inhibitor Ag during 2 h exposure. While Si increased SbIII uptake and glucose decreased SbIII uptake by PV gametophytes, the impact disappeared during 4 h exposure. Under P-sufficient condition, P increased SbIII uptake and decreased SbV uptake during 2 h exposure, but the effect again disappeared after 4 h. After being P-starved for 2 weeks, P decreased SbIII with no effect on SbV uptake during 2 h exposure. Our results indicated that: 1) PV gametophytes could serve as an efficient model to study Sb uptake, and 2) unique SbIII uptake by PV may be related to its trait of As hyperaccumulation.

1. Introduction

Antimony (Sb), which is a toxic metalloid and is in the same group as phosphorus (P) and arsenic (As), can cause human diseases such as cancer and respiratory syndromes (Feng et al., 2013). Being chemical analogs, Sb and As share many chemical properties and both exist in 3 and 5 oxidation state forms in the environment. The 3 oxidation state is found under anoxic conditions, whereas the 5 oxidation state occurs in relatively oxic conditions.

Antimony is used in the manufacture of semiconductors, batteries, bullets, and flame retardants (Abin and Hollibaugh, 2013). As the ninth of most mined-metal worldwide, Sb is an emerging element of concern to human health and the environment (Abin and Hollibaugh, 2013; Feng et al., 2013). Crops growing in Sb-contaminated sites are the major source for dietary Sb intake. However, there is limited knowledge about plant Sb uptake and accumulation (Feng et al., 2013). Antimony concentrations in plant tissues are typically 0.01–0.1 mg/kg, with levels of 5–10 mg/kg causing phytotoxicity and growth reduction (Feng et al., 2013; Xue et al., 2014). However, rice exhibits increased Sb tolerance and can accumulate 66 mg/kg Sb in the leaves without showing growth reduction (Feng et al., 2013).

The aquaglyceroporin channels transport glycerol and metalloids including SbIII, AsIII, boric acid, and silicic acid, which are structurally similar to glycerol (Bhattacharjee et al., 2008). With similar neutral charge and a slightly smaller size, it is easier for aquaglyceroporin channels to transport SbIII than glycerol (Table 1). *Arabidopsis thaliana* takes up neutral species of SbIII and AsIII via aquaglyceroporin channels but with different affinities. For example, the transport protein AtNIP5; 1 and AtNIP6; 1 favor uptake of AsIII over SbIII and AtNIP7; 1 favors uptake of SbIII over AsIII (Bhattacharjee et al., 2008). Similarly, in rice plants, AsIII and SbIII are taken up via the same channel and as such SbIII inhibits AsIII uptake. For example, presence of 0.5 mM SbIII reduces AsIII uptake by 50% in rice growing for 20 min in water containing 0.1 mM AsIII (Meharg and Jardine, 2003). Glucose has been reported to share the same pathway with AsIII in yeast (Liu et al., 2004), but it has no impact on AsIII uptake by rice (Meharg and Jardine, 2003).

---

* Corresponding author. State Key Lab of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Jiangsu 210046, China.
E-mail address: lqma@ufl.edu (L.Q. Ma).

http://dx.doi.org/10.1016/j.envpol.2015.06.029
0269-7491/Published by Elsevier Ltd.

Table 1
Molecular size and formula of SbIII and SbV in comparison with their analogs (Mathews et al., 2011; Porquet and Filella, 2007; Tschan et al., 2009).

Molecular structure	Abbreviation	Formula	Diameter (Å)
Antimonate	SbIII	$Sb(OH)_3$	3.05
Glycerol		$C_3H_5(OH)_3$	3.07
Arsenite	AsIII	$As(OH)_3$	2.81
Silicic acid	Si	$Si(OH)_4$	4.38
Boric acid;	B	$B(OH)_3$	2.65
Antimonate	SbV	$Sb(OH)_6^-$	7.36
Arsenate	AsV	$HAsO_4^{2-}$	6.72
Phosphate	P	$H_2PO_4^-$	6.24

Similar to Arabidopsis, SbIII and AsIII uptake by As-hyperaccumulator *Pteris vittata* (PV) sporophytes probably use aquaglyceroporin channels (Kamiya and Fujiwara, 2009; Meharg and Jardine, 2003). However, previous study suggested that PV sporophytes may have a different pathway (Mathews et al., 2011). Mathews et al. (2011) showed SbIII does not impact AsIII uptake by PV sporophytes. In addition, once absorbed, As is rapidly translocated to the fronds, whereas Sb is primarily accumulated in the roots (Mathews et al., 2011; Tisarum et al., 2014). Furthermore, AsIII uptake by PV sporophytes is not inhibited by other neutral species such as silicic acid, boric acid, SbIII, and glycerol (Mathews et al., 2011; Wang et al., 2010), suggesting PV sporophytes might take up AsIII via different aquaglyceroporin channels. Wang et al. (2010) showed that 0.5 mM silicic acid and 0.3 mM boric acid have no impact on PV sporophytes uptake of 15 μM AsIII during 1 d growth in 20% Hoagland solution (HS). Mathews et al. (2011) demonstrated that 1 h uptake of 0.1 mM AsIII in water is not inhibited by 100 mM glycerol or SbIII. They used a 1 h treatment to prevent AsIII oxidation in the media, which may have been too short to demonstrate the competition. Mathews et al. (2011) observed uptake inhibition of 0.1 mM AsIII in PV sporophytes by 0.01 mM $AgNO_3$. However, increasing $AgNO_3$ concentration to 0.1 mM does not show stronger inhibition. They suggested that PV sporophytes take up AsIII via a Ag-sensitive AsIII channel (Mathews et al., 2011).

There are several reports on SbIII uptake via aquaglyceroporins, but to date, no SbV-specific transporters have been identified. Arsenate is known to enter plants via P transporters (Wang et al., 2002), but SbV does not use P transporter (Tschan et al., 2008). In experiments using maize and sunflower, there is no interaction between SbV and P, likely due to their distinct differences in structure and size (Table 1). The structure of P and AsV are tetrahedral, while SbV is octahedral in aqueous solution (Tschan et al., 2008).

There is no evidence of SbV impact on P uptake in PV sporophytes but it has been reported that AsV impacts SbV uptake in PV sporophytes (Müller et al., 2013). Adding 5 mg/kg AsV to PV sporophytes growing in quartz substrate containing 5 mg/kg SbV increased SbV uptake from 49 to 84 mg/kg Sb, but did not enhance Sb translocation to the fronds (Müller et al., 2013). The authors suggested AsV probably changes membrane integrity of the roots, allowing SbV to enter indirectly (Müller et al., 2013). Thus impact of P on Sb uptake by PV was included in this study as it might act like AsV by increasing SbV uptake.

Because the oxidation state of Sb and As are influenced by rhizosphere microflora, in this work, we used sterile PV gametophytes cultured under sterile condition to minimize SbIII oxidation. Gametophytes are known to hyperaccumulate As (Gumaelius et al., 2004), so we expected that PV gametophytes would take up Sb similar to PV sporophytes.

Thus, the goals of this study were to use rapidly-growing uniform PV gametophyte cultures to study SbIII and SbV uptake by PV. First, we evaluated the impact of SbIII and SbV concentrations on growth of PV gametophytes. Using non-toxic Sb uptake doses and under sterile condition, we examined: 1) the effects of SbIII competitors including glycerol, silicic acid, glucose, AsIII, and aquaporin inhibitor silver (Ag) on SbIII uptake by PV gametophytes, and 2) SbV competitors of P on SbIII and SbV uptake by PV gametophytes.

2. Materials and methods

2.1. Gametophyte culture

PV spores of ~20 mg were surface-sterilized in a 2.0-mL tube by mixing with 1.6 mL solution containing 50% (v/v) sodium hypochlorite for 10 min and centrifuged at 16,000 g for 3 min (Beckman Coulter Microfuge® 16 Centrifuge). The spores were washed twice in sterile water and then diluted with 1.6 mL sterile water (Gumaelius et al., 2004). The sterile spore solution was pipetted in 10 μL increments (0.125 mg spore per 10 μL) onto 100 × 15 mm sterile petri dish containing 50% Murashige & Skoog (MS) agar, pH 6 containing 20 g/L sucrose and incubated with a 12 h photoperiod with photo flux of 350 $\mu mol/m^2s$ using cool and warm fluorescent lamps with temperature at 23–28 °C and humidity of 70%. After 8 weeks, spores germinated and formed fully-developed gametophyte clusters, which were used in the experiment (Fig. 1A).

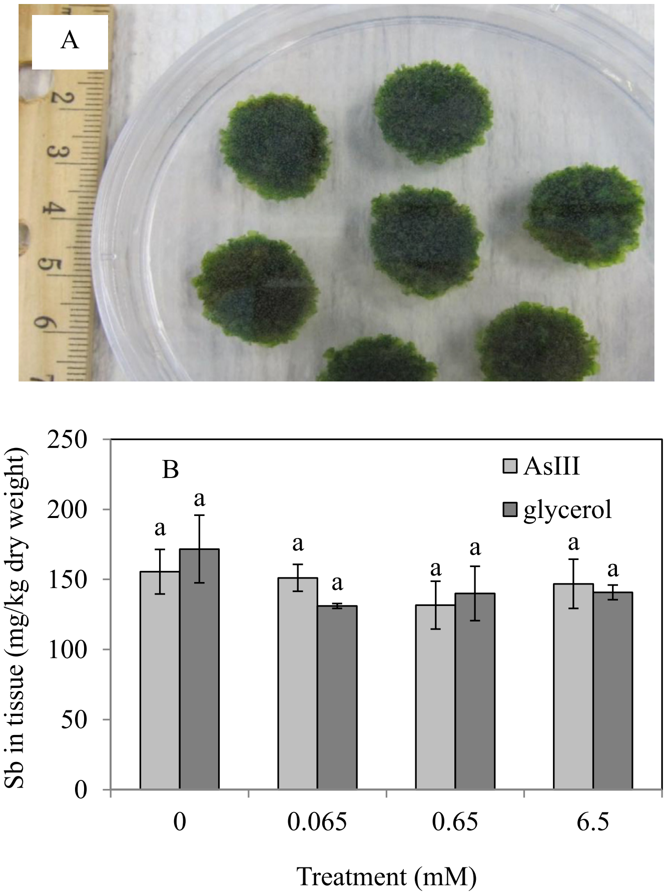

Fig. 1. Eight week old PV gametophyte clusters, each grown from 0.125 mg spore on 50% MS agar (A) and effect of 0.065 to 6.5 mM AsIII and glycerol on Sb concentrations in PV gametophytes after exposure to 65 μM SbIII for 2 h (B). Data represent the mean of three replicates with standard error, and bars with same letters are not significantly different ($p < 0.05$).