

Minireview

Aquaglyceroporins: ancient channels for metalloids

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Abstract

The identification of aquaglyceroporins as uptake channels for arsenic and antimony shows how these toxic elements can enter the food chain, and suggests that food plants could be genetically modified to exclude arsenic while still accumulating boron and silicon.

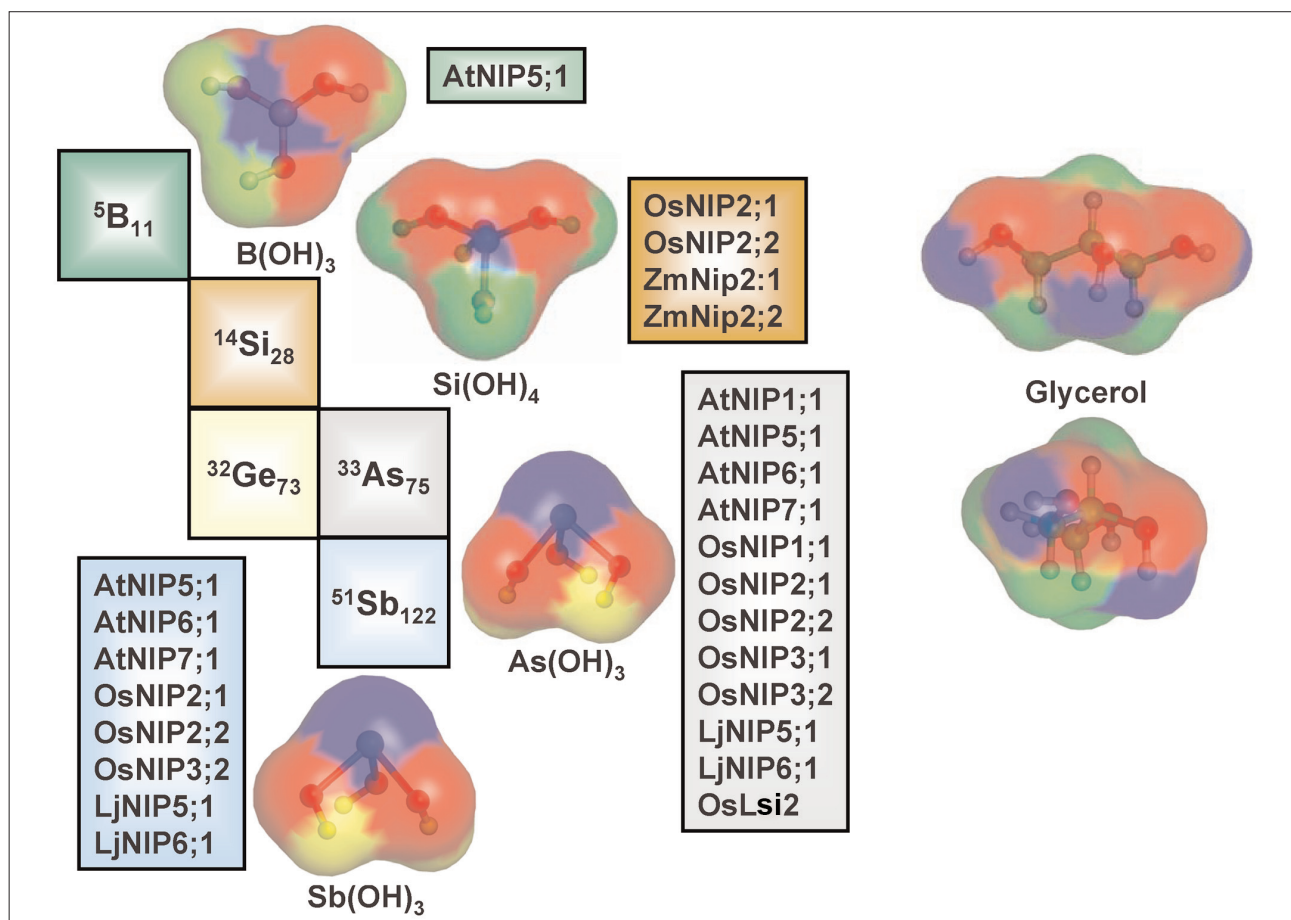
Arsenic, a metalloid, is widely distributed in the Earth's crust and is toxic to all forms of life. Humans can be exposed to arsenic from drinking water that has flowed through arsenic-rich rocks or from crops that have been irrigated with arsenic-contaminated water. Arsenic occurs predominantly in the environment as the pentavalent arsenate (As(V)) and trivalent arsenite (As(III)) forms. Arsenite is more toxic than arsenate and is primarily responsible for the biological effects of arsenic. The toxicity of arsenite is due to its affinity for closely spaced cysteine thiolates; it inactivates enzymes and receptors by binding to active site cysteine residues or by preventing formation of disulfide bonds. Arsenite also leads to the production of reactive oxygen species by binding to reduced glutathione. In addition to the severe health effects of arsenic in drinking water, its accumulation in crops such as rice jeopardizes the safety of our food supply [1]. An understanding of the pathways of arsenic uptake, metabolism, and elimination will help in developing strategies to produce plants that take up essential metalloids while excluding toxic ones.

Aquaglyceroporins and metalloid transport

A little over a decade ago, Sanders *et al.* [2] isolated a mutant of GlpF, the glycerol facilitator of *Escherichia coli*,

that was resistant to antimonite (Sb(III)). Later, Meng *et al.* [3] determined that this mutant also exhibits a 90% reduction in arsenite uptake. Antimony is another metalloid in the same group of the periodic table as arsenic (Figure 1). GlpF is a member of the major intrinsic protein (MIP) or aquaporin superfamily of channels for water and small solutes that is widely expressed in nearly every organism. Aquaporins fall into two broad groups, aquaporins or water-specific channels, and aquaglyceroporins, which conduct water, glycerol and other small, uncharged solutes.

It might seem surprising that a transporter for water and small organic compounds could transport a metalloid. However, analysis of the state of trivalent arsenic in solution shows how this is possible. Although trivalent inorganic arsenic is often referred to as an anion, arsenite, in solution, has a pK_a of 9.2, and it is therefore protonated at physiological pH. Extended X-ray absorption fine structure (EXAFS) analysis has shown that in aqueous solution there are three oxygen ligands 1.78 Å from the arsenic atom; the major species in solution is therefore the neutral hydroxide $As(OH)_3$, which is an inorganic molecular mimic of glycerol [4].

**Figure 1**

Periodic table of the metalloids. Metalloid substrates of aquaglyceroporins are shown both in their position in the periodic table and as molecular models. The size of each metalloid is shown in scale relative to glycerol (right). Atomic coordinates from the Cambridge Structural Database [16] were modeled with InsightII to calculate partial charges (blue, positive; red, negative; green and yellow reflect intermediate charges between blue and red) and plotted with PyMol. Beside each metalloid is listed its transport proteins. Each is a NIP except for Lsi2, which is an ArsB homolog. Species abbreviations: At, *Arabidopsis thaliana*; Lj, *Lotus japonicus*; Os, *Oryza sativa*; Zm, *Zea mays*.

Other aquaglyceroporins were subsequently shown to conduct As(III) and Sb(III). In the yeast *Saccharomyces cerevisiae* the GlpF homolog Fps1p is a glycerol channel involved in osmoregulation. In 2001, Tamás and coworkers [5] showed that disruption of Fps1p confers resistance to both As(OH)₃ and Sb(OH)₃ and that cells expressing a constitutively open form of the Fps1p channel are hypersensitive to both. The human aquaglyceroporins AQP3, AQP7, and AQP9 were subsequently shown to conduct As(OH)₃ [4]. Likewise, the aquaglyceroporin LmAQP1 from the parasitic protozoan *Leishmania* transports As(OH)₃ and Sb(OH)₃ into the parasite [4]; this is important clinically for treatment of the disease it causes, given that the activated form of the antiparasitic drug sodium stibogluconate (Pentostam) is Sb(III).

Plant aquaglyceroporins and metalloid uptake

In several regions of the globe, cultivation in arsenic-rich soil and irrigation with arsenic-contaminated water leads to accumulation of high levels of arsenic in rice, wheat, fruits and vegetables [6], making the role of plant aquaporins in metalloid uptake particularly relevant to human health. Plant aquaporins make up a large and divergent superfamily of proteins, much larger than in animals. The plant homologs are more diverse than the animal ones and do not fall cleanly into the aquaporin and aquaglyceroporin clades, although, as discussed below, some are more similar to aquaglyceroporins in terms of solute selectivity.

On the basis of the aquaporin-encoding genes in the moss *Physcomitrella patens*, the plant aquaporin superfamily has

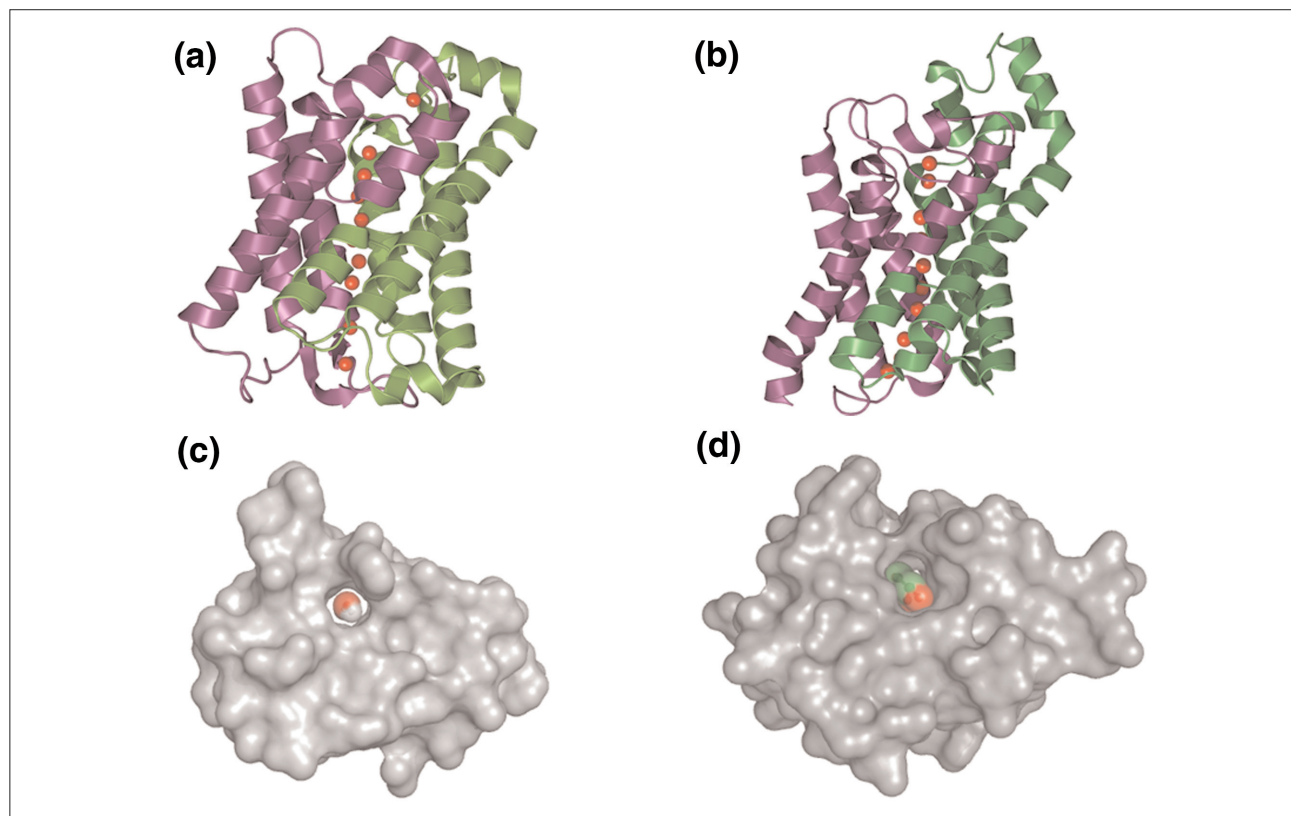


Figure 2

Structures of SoPIP2;1 and GlpF. **(a)** Model of the spinach SoPIP2;1 water channel structure (Protein Data Bank (PDB) entry 1Z98), showing water molecules passing through the channel (red spheres). **(b)** Model of the *E. coli* GlpF glycerol channel (PDB 1LDI) showing water molecules passing through the channel (red spheres). In each structure, the amino-terminal half is in green and the carboxy-terminal half is in purple. **(c,d)** Cross-sectional views of SoPIP2;1 and GlpF, showing the narrowest opening of the pore with a water or glycerol molecule, respectively.

been divided into seven subfamilies as shown in the study by Danielson and Johanson published in *BMC Plant Biology* [7]: plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), nodulin-26-like intrinsic proteins (NIPs), small basic intrinsic proteins (SIPs), GlpF-like intrinsic protein (GIPs), hybrid intrinsic proteins (HIPs), and the uncategorized X intrinsic proteins (XIPs). Two of the clades, PIPs and TIPs, were named after their primary location in the cell. The NIPs are located in the peribacteroid membrane of nitrogen-fixating symbiosomes in root nodules. The fourth clade was named SIPs because the proteins are relatively small like TIPs, but are basic like the PIPs and many of the NIPs but unlike the TIPs. Neither the substrate specificity nor the intracellular localization of SIPs is known. The GIPs are closely related to a subclass of glycerol transporters in bacteria that, in addition to glycerol, are highly permeable to water. GIPs have retained the permeability for glycerol but not for water. The intracellular localization of GIPs is still unknown. The GIPs and HIPs are believed to have been lost during the evolution of vascular

plants (also known as tracheophytes or higher plants), whereas the XIPs, although found in a wide variety of eudicotyledonous plants, are not found in monocots. However, in vascular plants, the remaining subfamilies have expanded and in some cases diversified, resulting in the formation of more specialized groups within these subfamilies [7]. The diverse subfamilies of aquaporins include a wide variety of substrate specificities, subcellular localizations, and modes of regulation.

The crystal structure of the spinach plasma membrane aquaporin SoPIP2;1 has been resolved in both the open and the closed conformations [8]. Although the plant and animal evolutionary lineages separated about 1.6 billion years ago, X-ray structures of aquaporins, such as bovine AQP0, human AQP1, *E. coli* AqpZ and GlpF, spinach SoPIP2;1, and the malarial parasite *Plasmodium falciparum* PfAQP, show that these highly conserved proteins have a superimposable core structure with a characteristic 'hour-glass' fold (Figure 2a,b). Aquaporins have a homotetrameric

arrangement in which each monomer consists of six membrane spanning α -helices, with two membrane-spanning half-helices interacting with each other from opposing sides through two highly conserved Asp-Pro-Ala (NPA) motifs to form a narrow pore across the membrane. Towards the periplasmic side of the membrane, a constriction region about 8 Å from the NPA motifs, termed the aromatic/arginine (ar/R) region, forms a primary selection filter that serves as a main checkpoint for solute permeability (Figure 2c,d).

Not all plant homologs are highly specific for water; like bacterial and plant aquaglyceroporins, many are permeable to a wide range of solutes. For example, PIPs have been reported to facilitate CO₂ diffusion; TIPs facilitate the transport of ammonia and urea; water transport has been reported for the SIP1 subgroup within SIPs; and NIPs are permeable to ammonia, glycerol, lactic acid, urea, formamide and metalloids [7].

It is becoming increasingly clear that NIPs conduct not only As(OH)₃ and Sb(OH)₃ but also other metalloids, including boron, silicon, and probably germanium (Figure 1). These metalloid oxyacids are similar in volume, with compact diameters similar to glycerol in cross-section (although glycerol is longer and more flexible). Whereas electrostatic comparison of As(OH)₃ and Sb(OH)₃ suggests that they are pyramidal molecules with polar and nonpolar faces that resemble glycerol [9], the planar B(OH)₃ molecule and the tetrahedral Si(OH)₄ molecule have a more uniform charge distribution, so the volume of the metalloid compounds is probably more important than their electrostatic surfaces for passage through aquaglyceroporins. Present-day seawater contains approximately 0.4 mM borate and 0.1 mM silicate but only nanomolar amounts of arsenicals and antimonials. This suggests that boron and silicon oxyacids might be physiological substrates of aquaglyceroporins, whereas arsenicals and antimonials might be taken up adventitiously only when present as high-level contaminants. If the concentrations of metalloids were similar in primordial oceans, then it is tempting to speculate that the earliest organisms evolved aquaglyceroporins for uptake of the essential elements boron and silicon rather than for that of organic solutes.

NIPs were first shown to transport the metalloid boron, which is essential for plant cell wall structure and function. Silicon is beneficial for plants and enhances their resistance to pests, diseases, and other stresses. Both are taken up by roots in the form of uncharged boric acid B(OH)₃ and silicic acid Si(OH)₄. In 2006, Takano *et al.* [10] showed that the product of the *Arabidopsis thaliana* gene *NIP5;1*, a member of the NIP clade, conducts boric acid, enabling boron uptake, and it is crucial for plant growth when boron is limited. In the same

year, Ma *et al.* [11] reported that OsNIP2;1 (the gene product of *OsLsi1*) is responsible for silicon accumulation in rice (*Oryza sativa*). Suppression of *OsLsi1* expression by RNA interference resulted in reduced silicon uptake, whereas expression of *Lsi1* in *Xenopus laevis* oocytes resulted in selective uptake of silicon but not glycerol [11]. Two homologs of *OsLsi1* were recently identified in *Zea mays* and shown to conduct Si(OH)₄ when expressed in oocytes [12]. Thus, aquaglyceroporins are responsible for the uptake of B(OH)₃, Si(OH)₄, As(OH)₃, and Sb(OH)₃. (Note that the *O. sativa* *Lsi* mutants were selected for germanium resistance, which suggests that this metalloid is also taken up by OsNIP2;1.)

Because members of the NIP subfamily are the functional equivalents of aquaglyceroporins, it was postulated that, besides boron and silicon, NIPs might also serve as As(III) transporters in plants [13-15]. On the basis of the ar/R regions, the NIP subfamily can be subdivided into three subgroups, NIPI, NIPII, and NIPIII [15], and members from each subgroup were found to conduct As(OH)₃ and Sb(OH)₃. Several NIP isoforms from *A. thaliana*, *Lotus japonicus*, and *O. sativa* were cloned, expressed in a *S. cerevisiae* *Δfps1* mutant that is resistant to As(III) and Sb(III), and analyzed for metalloid sensitivity and transport. Cells of the *Δfps1* strain expressing members of the NIPII subgroup (AtNIP5;1, AtNIP6;1, AtNIP7;1, OsNIP3;2, LjNIP5;1, and LjNIP6;1) and the NIPIII subgroup (OsNIP2;1 and OsNIP2;2) were permeable to As(OH)₃ and Sb(OH)₃, and cells expressing these proteins regained wild-type sensitivity to the trivalent metalloids. Cells expressing members of the NIPI subgroup (AtNIP1;1, AtNIP2;1, and OsNIP1;1) showed no difference in sensitivity to metalloids from that of cells expressing the vector alone (the control). However, *A. thaliana* AtNIP1;1, a member of the NIPI subgroup, was recently shown to conduct As(OH)₃, and disruption of *AtNIP1;1* resulted in tolerance to arsenite but not to arsenate (Takehiro Kamiya and Toru Fujiwara, personal communication). In addition, members of all three NIP subfamilies were shown to conduct As(OH)₃ when expressed in oocytes [15]. The rice aquaglyceroporins OsNIP1;1 (NIPI subgroup), OsNIP3;1 (NIPII subgroup), and OsNIP2;1 and OsNIP2;2 (NIPIII subgroup) showed variable levels of As(OH)₃ transport. However, although OsNIP2;1 and OsNIP2;2 transport both silicic acid and arsenite, OsNIP1;1 and OsNIP3;1 are permeable to arsenite but not to silicic acid. Thus, there seems to be selectivity for metalloids among the various aquaglyceroporins.

Transcellular transport of metalloids

It is not at all clear whether different NIP isoforms are involved in transcellular movement of metalloids from roots to xylem. An ability to allow As(OH)₃ into and out of

cells would be a prerequisite for such movement. The fact that aquaglyceroporins are bidirectional channels that allow both uptake and efflux of $\text{As}(\text{OH})_3$ was first demonstrated in *Sinorhizobium meliloti*, a nitrogen-fixing bacterium, which uses an aquaglyceroporin, AqpS, as a route for detoxification of arsenite generated intracellularly by reduction of arsenate (reviewed in [4]). AqpS is so far the only known example of an aquaglyceroporin with a physiological role in arsenic resistance. With a similar strategy, Bienert *et al.* [13] used an *S. cerevisiae* $\Delta\text{acr3} \Delta\text{fps1} \Delta\text{ycf1}$ mutant, which cannot remove intracellularly generated arsenite, to show that NIP isoforms are bidirectional. Cells of this mutant expressing NIPs showed improved growth on arsenate, indicating that these NIPs were able to remove the intracellularly generated arsenite. Thus, depending on the direction of the concentration gradient, NIP channels catalyze bidirectional movement of metalloids.

The two *Z. mays* aquaglyceroporins, ZmLsi1 (also called ZmNip2;1) and ZmLsi6 (ZmNip2;2), which both transport $\text{Si}(\text{OH})_4$, are localized in different tissues, with ZmLsi1 mainly expressed in roots and ZmLsi6 expressed in leaf sheaths and blades [12]. Importantly, both show polarized intracellular localization. ZmLsi1 is in the plasma membrane of the distal side of root epidermal and hypodermal cells in the seminal and crown roots and also in cortex cells in lateral roots. In contrast, ZmLsi6 shows polar localization on the side facing toward the vessel in leaf sheaths and blades. These results are consistent with transcellular movement of $\text{Si}(\text{OH})_4$; ZmLsi1 catalyzes uptake of $\text{Si}(\text{OH})_4$ into root cells, whereas ZmLsi6 unloads $\text{Si}(\text{OH})_4$ into the xylem. In *O. sativa*, OsLsi1 is also responsible for uptake of $\text{Si}(\text{OH})_4$ [11] and $\text{As}(\text{OH})_3$ [15]. However, the rice transporter responsible for unloading $\text{As}(\text{OH})_3$ into the xylem, OsLsi2, is not an aquaglyceroporin but, rather, is a homolog of a bacterial arsenite efflux permease [15]. OsLsi2 has 18% identity with *E. coli* ArsB, an $\text{As}(\text{OH})_3\text{-H}^+$ antiporter (reviewed in [4]). Thus, aquaglyceroporins are responsible for uptake of $\text{As}(\text{OH})_3$ in both *E. coli* (GlpF) and rice (OsLsi1), and ArsBs catalyze efflux into the medium (EcArsB) or xylem (OsLsi2). In bacteria ArsB functions in arsenite detoxification, whereas OsLsi2 channel activity leads to accumulation of arsenite in rice grains and shoots.

The potential for improving the safety of food crops

Understanding the pathways of arsenic movement will be useful in developing strategies for reducing the arsenic content in food crops such as rice. Methylated arsenicals are also used as herbicides, so the development of arsenic tolerant plants is another potential use for this knowledge. Given that NIPs seem to be the main routes of $\text{As}(\text{OH})_3$ uptake into plants, genetically engineering NIPs that are

permeable to essential nutrients, such as boron and silicon, but not to $\text{As}(\text{III})$, is an obvious next step. The ar/R selectivity filter and other structural features of these channels allow aquaglyceroporins to discriminate between solutes. For example, rice OsNIP2;1 is permeable to $\text{As}(\text{OH})_3$ but not glycerol [11,15]. Although AtNIP5;1 and AtNIP6;1 are more permeable to $\text{As}(\text{OH})_3$ than $\text{Sb}(\text{OH})_3$, the opposite is true for AtNIP7;1, which shows selectivity for $\text{Sb}(\text{OH})_3$ [13]. Thus, engineering NIPs to reduce $\text{As}(\text{OH})_3$ permeability is a plausible approach to the engineering of low arsenic crops. Alternatively, growing crops in soil containing high levels of silicic acid might reduce their arsenic content, as it has been observed that rice seedlings grown in the presence of silicic acid show comparatively lower levels of arsenic accumulation [15]. Finally, selection of rice with natural allelic variations in OsLsi1 and OsLsi2 that favor uptake of silicon over arsenic may result in plants with lower arsenic accumulation. In summary, for engineering low arsenic crops, the old adage, 'nip evil in the bud' could be read here as 'nip evil in the root'. A detailed understanding of the mechanisms of arsenic uptake in plants will lead to strategies for preventing entry of arsenic into the food chain.

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