Plant Science 238 (2015) 212–227

Contents lists available at ScienceDirect

Plant Science

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

Review

# Metalloido-porins: Essentiality of Nodulin 26-like intrinsic proteins in metalloid transport

Benjamin Pommerrenig, Till Arvid Diehn, Gerd Patrick Bienert*

*Metalloid Transport Group, Department of Physiology and Cell Biology, Leibniz Institute of Plant Genetics and Crop Plant Research, Corrensstrasse 3, D-06466 Gatersleben, Germany*

ARTICLE INFO

*Article history:*
Received 27 January 2015
Received in revised form 30 May 2015
Accepted 1 June 2015
Available online 8 June 2015

*Keywords:*
Nodulin 26-like intrinsic protein
Metalloid transport
Aquaporin
Boron
Silicon
Arsenic

ABSTRACT

Metalloids are a group of physiologically important elements ranging from the essential to the highly toxic. Arsenic, antimony, germanium, and tellurium are highly toxic to plants themselves and to consumers of metalloid-contaminated plants. Boron, silicon, and selenium fulfill essential or beneficial functions in plants. However, when present at high concentrations, boron and selenium cause toxicity symptoms that are detrimental to plant fitness and yield. Consequently, all plants require efficient membrane transport systems to control the uptake and extrusion of metalloids into or out of the plant and their distribution within the plant body. Several Nodulin 26-like intrinsic proteins (NIPs) that belong to the aquaporin plant water channel protein family facilitate the diffusion of uncharged metalloid species.

Genetic, physiological, and molecular evidence is that NIPs from primitive to higher plants not only transport all environmentally important metalloids, but that these proteins have a major role in the uptake, translocation, and extrusion of metalloids in plants. As most of the metalloid-permeable NIP aquaporins are impermeable or are poorly permeable to water, these NIP channel proteins should be considered as physiologically essential metalloido-porins.

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* Corresponding author. Tel.: +49 39482 5385.
*E-mail addresses:* pommerrenig@ipk-gatersleben.de (B. Pommerrenig), diehn@ipk-gatersleben.de (T.A. Diehn), bienert@ipk-gatersleben.de (G.P. Bienert).

http://dx.doi.org/10.1016/j.plantsci.2015.06.002

## 1. Introduction

### 1.1. Metalloids

Metalloids are defined as compounds that possess physical and chemical properties that are intermediate between metals and nonmetals. A rigorous assignment of the associated elements constituting the group of metalloids is very difficult. Arsenic (As), antimony (Sb), boron (B), germanium (Ge), silicon (Si), and tellurium (Te) are commonly considered as metalloids (Table 1). Certain other elements, such as selenium (Se), are sometimes added to the list of metalloids. Metalloids are generally present in the soil solution as either negatively charged ions or undissociated (uncharged) molecules depending on the pH and the redox potential of their environment (Fig. 1). It is likely that the only Si species that is available to organisms is orthosilicic acid ($H_4SiO_4$), which is uncharged at physiological pH ranges. Similar to Si, the most prominent and bio-available species of Ge in the soil solution is the uncharged germanic acid ($H_2GeO_3$/$H_4GeO_4$). As B does not undergo oxidation–reduction reactions at physiological conditions, its only bio-available forms are boric acid ($H_3BO_3$) and borate ($B(OH)_4{}^-$) which occur in a pH-dependent equilibrium. Due to the $pK_{a1}$ value of 9.25 for boric acid (Fig. 1), the uncharged $H_3BO_3$ molecule quantitatively dominates over $B(OH)_4{}^-$ in physiological conditions. In comparison to B, the chemistry and speciation of As, Sb, and Se is more complex. The pH and redox potential are key factors controlling the oxidation and dissociation states of these elements, and thereby their availability and transport in the environment. Silicon, As, Sb, and Ge species can be chemically and/or biologically methylated yielding organic metalloid compounds. Plants have to deal with several chemical species of the same element depending on the chemical environment in which the metalloid occurs (Fig. 1). The speciation of the metalloid is determined either by the chemical environment of the soil solution or of the cell saps which might vary among plant organs or tissues, or even between the different compartments within a cell. Due to the high $pK_a$ values (above 9, see Fig. 1) of most environmentally important metalloid acids, these metalloid species occur in soils or organisms dominantly as uncharged molecules.

While metalloids vary in their chemical properties such as atomic number and valence electrons, the chemical structures of their acids is highly similar (Table 1). Metalloids affect living organisms in different ways. Arsenic, Sb, Ge, and Te are highly toxic to consumers of metalloid-contaminated plants and to plants themselves, unless they are sequestered in vacuoles or complexed. In contrast, B, Si, and Se fulfill essential or beneficial functions in plants. When present at high concentrations, B and Se are also toxic to plants, and exposure causes a range of toxicity symptoms that are detrimental to fitness and yield. Consequently, metalloid homeostasis must be carefully regulated in plants. Evidently, the regulation of transport of these compounds into, out of, and within the plant represents a crucial control lever to adapt the plant metabolism to different levels of metalloids.

The transport of various metalloid species is regulated by active and/or passive transport mechanisms in plants [1,2]. The active and passive transport mechanisms are controlled by different transporter protein families, which together most efficiently regulate the uptake, translocation, and extrusion of various metalloid species (e.g. B, Si, and As) [1–3]. Knowledge about metalloid transport mechanisms on the molecular level was mainly gained in the last 10 years. Before then, it was assumed that the transmembrane transport of uncharged metalloid species, which include the biologically most important ones, was merely determined by the passive diffusion across the lipid bilayer, and not by proteins. Bit by bit, it was demonstrated that Nodulin 26-like intrinsic channel proteins (NIPs), which exhibit strict pore selectivity for uncharged molecules, are essential for the transport of environmentally important metalloids in plants. In the following, we highlight, synthesize, and extrapolate the current knowledge on the crucial functions of NIPs dominating the transport regulation of undissociated metalloid species in the plant kingdom.

### 1.2. Nodulin 26-like intrinsic proteins

NIP proteins belong to the major intrinsic proteins (MIPs), which form a family of essential membrane channel proteins facilitating the diffusion of water and small uncharged solutes in all domains of life [1]. MIPs are typical members of diffusion facilitater proteins. The process in which the flow of molecules across cell membranes is facilitated by special types of proteins is called facilitated diffusion (Fig. 2). Facilitated diffusion (protein-mediated) and simple diffusion (non-protein-mediated) are responsible for passive transport processes in biological systems [4]. The structure of MIP channels is highly conserved, although the amino acid sequences of the proteins differ substantially. MIPs form tetramers (Fig. 3A). Each monomer is composed of six transmembrane-spanning helices (TMHs) with five connecting loops (loops A–E) and two cytoplasmic termini (Fig. 3B). MIPs form a narrow path (ca. 0.2–0.5 nm in diameter) across various cellular membranes allowing the passage of just a single continuous file of substrate water molecules. The cavity forms the so-called aromatic/arginine (ar/R) selective filter toward the luminal side of the membrane. This filter is constructed of four amino acids, which are crucial for the substrate selectivity of MIPs [5].

GmNOD26 was the first described plant MIP [6]. Nodulins (abbreviated NODs) are proteins, which are involved in the symbiotic processes between legumes and rhizobia. Nodulin genes show a specific expression pattern in nodules, a specialized tissue in which the fixation of molecular nitrogen occurs. GmNOD26 is the major proteinaceous membrane constituent of soybean nodules representing 10–15% of the total membrane protein [6–8]. It belongs to the plant NIP family for which it became the eponym. GmNOD26 was the first plant MIP to be investigated on a biochemical level [6]. This was 3 years after the first biochemical description and identification of an MIP, from bovine lens fibers [9]. GmNOD26 was initially suggested to be permeable to malate as its phosphorylation status correlated closely with malate uptake across the symbiosome membrane [10]. While a functional evidence for a permeability to malate remains to be shown, GmNOD26 was found to be a functional water channel in 1997 [11], and in 2010 it was shown to facilitate the diffusion of ammonia when reconstituted