Silicon (2020) 12:2641–2645
https://doi.org/10.1007/s12633-019-00360-w

ORIGINAL PAPER

How is silicic acid transported in plants?

Christopher Exley[1] · Gea Guerriero[2] · Xabier Lopez[3]

Received: 29 August 2019 / Accepted: 15 December 2019 / Published online: 3 January 2020

Abstract
Plants accumulate silicon in their tissues as amorphous silica. The form of silicon taken up by plants is silicic acid, a neutral molecule that passes through membrane channels with water. After seminal work on rice identified an aquaporin that appeared to mediate the passage of silicic acid, several papers followed and classified similar channels (referred to as "transporters") in a number of plant species. These channels have been described as essential for silicon uptake and specific for the metalloid. Herein, we critically review the published data on the characterisation of one channel in particular, *Lsi1*, and identify possible caveats in results and limitations in methods used. Our analysis does not support the suggestion that the identified channels are specific for silicic acid. Computational analyses of the size of the *Lsi1* pore additionally suggest that it may not play a significant role in mediating the movement of silicic acid *in planta*. We suggest that to avoid further confusion, channels currently implicated in the transport of silicic acid *in planta* are not referred to as silicon-specific transporters. Future research including the use of molecular dynamics simulations will enable the unequivocal identification of channels involved in silicon transport in plants.

Keywords Silicic acid · Silica · Plants · Aquaporin · Oocytes

1 Introduction

Silicon is biologically available in soil solution as neutral, monomeric silicic acid. The vehicle for its uptake and distribution in plants is water. Silicic acid is a substantially larger molecule than water [1, 2] (Fig. 1). However, like water, hydroxyl groups dominate its chemistry and while it has no known organic chemistry and extremely limited inorganic chemistry, it is capable of hydrogen bonding [3]. In following water via the symplastic route, silicic acid encounters enumerable water channels, such as aquaporins, and these size-restrictive pores will either allow or deny the passage of the larger silicic acid molecule [4]. Ostensibly, silicic acid has access to all areas in all plants, but in practice the myriad different water channels that characterise plant species limits the movement of silicic acid in some, while allowing it in others.

✉ Christopher Exley
c.exley@keele.ac.uk

Gea Guerriero
gea.guerriero@list.lu

Xabier Lopez
xabier.lopez@ehu.es

[1] The Birchall Centre, Lennard-Jones Laboratories, Keele University, Keele, Staffordshire, UK

[2] Environmental Research and Innovation Department, Luxembourg Institute of Science and Technology, Esch/Alzette, Luxembourg, Luxembourg

[3] Kimika Fakultatea, Euskal Herriko Unibertsitatea (UPV/EHU), and Donostia International Physics Center (DIPC), P.K. 1072, 20080 Donostia, Euskadi, Spain

2 Mechanism of Transport of Silicic Acid

In a landmark paper, published in Nature in 2006, Ma *et al.* described a putative silicon transporter in rice [5]. They identified a gene, *Lsi1* which codes for an aquaporin-like transmembrane protein that was permeable to silicic acid. The research showed that suppression of *Lsi1* in a rice cultivar reduced the deposition of biogenic silica in shoots over 12 h, while expression of *Lsi1* in *Xenopus* oocytes increased their silicon content relative to water-injected oocytes over 30 min. The authors acknowledged that the form of silicon entering either the root of rice or the cytoplasm of oocytes was silicic acid [3]. The authors concluded that *Lsi1* is a transporter for silicon in rice roots.

We have re-examined the evidence in [5] that directly supports *Lsi1* as a specific transporter for silicon in rice. In

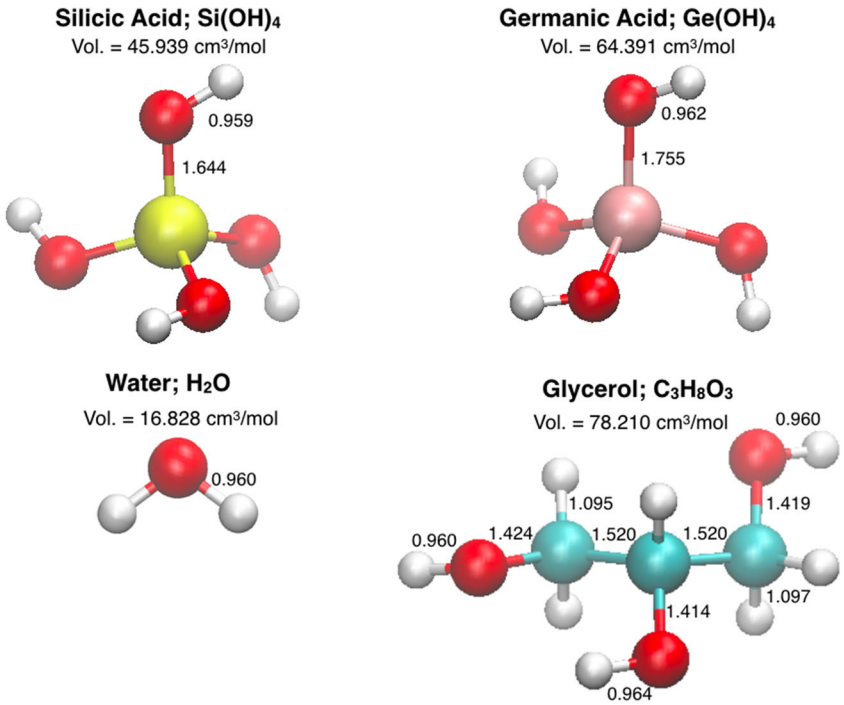

Fig. 1 Diagram showing optimized structures for silicic acid, germanic acid, water and glycerol. These structures were obtained using Density Functional Theory with the wB97XD functional [32] and with the 6–31++G(d,p) basis set in the case of water, silicic acid, and glycerol, and with the def2SVP [33] basis set for germanic acid. Calculations were done using the Gaussian16 programme

particular, we have scrutinised their use of kinetic assays using oocytes and the application of ^{68}Ge in estimating the intracellular concentration of silicic acid in oocytes. These assays have since become *de rigueur* in silicon transport research.

There are significant reliability issues associated with the use of oocyte assays in determining the uptake of silicic acid. The volume of an oocyte is approximately 1 μL [6, 7] and using the data presented in Fig. 4c,d [5] it can be calculated that the concentration of silicon inside oocytes never approaches the respective concentration in exposure media. For example, when the silicic acid content of the exposure medium is 2.0 mM the concentration of silicon in oocytes as indicated in Fig. 4 is slightly above 1.0 mM. Following just 30 min exposure to external media (no explanation is given for such a short period of exposure) the concentration of silicic acid in acid hydrolysates of just 10 oocytes was measured using the classic molybdenum blue assay [8]. Those who have used this method to measure silicic acid will question its applicability to a procedure involving such low (μL) volumes [9] and acknowledge that without the requisite method blanks, justifiably question the rigour of the data generated. The method referred to [8] has never been validated for such an application and one would have expected the requisite quality assurance data supporting its use to be presented either in [5] or in a complementary publication. The data presented in Fig. 4c,d should be considered at best as estimates and if accepted, show the approach to equilibrium across oocyte membranes over just 30 min. Arguably, the data show the passive diffusion of silicic acid into oocytes down a concentration gradient. They should not have been interpreted as active transport of silicic acid against a concentration gradient.

Whether the data also show that injection of *Lsi1* cDNA increased the intraoocyte content of silicic acid remains equivocal without appropriate controls. The injection of water into control oocytes cannot mimic the incorporation of transmembrane proteins in oocyte membranes. Although the authors demonstrated that the aquaporin coded by *Lsi1* localized to the membrane of root cells in rice, they did not provide direct evidence of its incorporation into oocyte membranes. This is essential, as is information on the density of incorporated channels and an appropriate control would be injection of cRNA coding for another similar transmembrane channel (ideally the mutant *Lsi1*) not implicated in the movement of silicic acid.

The provenance of data reported in Fig. 4d [5] is questionable. The data were not obtained from measuring the silicic acid content of oocyte hydrolysates, but from using ^{68}Ge as an analogue for silicic acid. The method is not described anywhere in the paper other than an unusual mention that oocytes were washed several times with a silicon-free solution prior to measurement of ^{68}Ge by scintillation counting. The significance of the silicon-free solution when ^{68}Ge and not silicon, is being measured is confusing. The reference cited by the authors to support the use of ^{68}Ge to measure the silicic acid content of oocytes was presumably in error [10], since it is completely inappropriate (see later).

If the data presented in Fig. 4c,d are accepted as robust, then another interpretation is that they show that silicic acid entered oocytes down a concentration gradient by passive diffusion and, in the timeframe of the assay, that its concentration never approached equilibrium with the silicic acid content of the exposure medium. In addition, contrary to what the authors report, the observation that the expression of *Lsi1* did not affect the oocyte content of the significantly larger glycerol molecule (Fig. 1), or that glycerol did not competitively influence the movement of silicic acid into oocytes, should not have been interpreted as that *Lsi1* encodes an aquaporin that is specific for silicon. An alternative interpretation of these data is that while the glycerol molecule, the volume of which is 70% greater than silicic acid (Fig. 1), is too large for effective diffusion through this specific water channel (encoded by *Lsi1*), it does not block the movement of the smaller and more labile silicic acid molecule. Glycerol is additionally significantly more hydrophobic than silicic acid and this will influence its passage through aquaporin channels [11]. Recent evidence continues to question an exclusive role for *Lsi1* in the movement of silicic acid *in planta*. An orthologue of *Lsi1* has been identified in *Cannabis sativa*, a plant not usually classified as a silica accumulator, that deposits silica in trichomes and in cell walls of xylem and bast fibers [12]. Modelling of the channel predicts a maximum pore size (bottleneck) of 1.77 Å, significantly smaller than that of silicic acid, the maximum radius of which was recently computed to be 4 Å [1] (Fig. 1). Although proteins *in vivo* do show flexibility and may thus accommodate the passage of bulkier molecules, the difference between 4 Å and 1.77 Å is more than double. With this new evidence, it is difficult to rationalise a significant and specific role for *Lsi1* and its orthologs in the movement of silicic acid in rice or any other plant. However, shorter side chains in amino acids lining the ar/R constriction site, similar to that observed for *Lsi1*, may allow a larger pore [2], though such awaits definitive experimental confirmation.

3 An Unhelpful Dogma?

In publications subsequent to [5], right up to the present day, the results of this seminal work have possibly been misconstrued not only in exaggerating a role for *Lsi1* in the movement of silicic acid *in planta*, but in claiming that certain other water channels (including mammalian) are specific for silicon and that these channels are silicon transporters [13–25]. The latter term is inconsistent with pores that do not bind silicic acid and mediate the passive diffusion of neutral silicic acid down a concentration gradient. Despite the importance of these publications in identifying putative channels for the passive movement of silicic acid in different plant species, they have consistently referred to them as "transporters". Without exception, in every publication since [5], where oocytes were used to investigate the movement of silicic acid in *planta*, the intraoocyte concentration of silicic acid (and sometimes ^{68}Ge) never approached the silicic acid (or ^{68}Ge) content of respective exposure media. The evidence, direct or indirect, that any water channel is specific for silicon transport in plants remains equivocal.

4 ^{68}Ge as an unproven tool

Finally, many papers purporting to demonstrate silicic acid transport in plants using oocytes have used ^{68}Ge as an analogue for silicon. It is worth examining the appropriateness of this method. It is true that at low concentrations (<10 μM) and below pH 9 the hydrolysis chemistry of germanium predicts that $Ge(OH)_4$ will be the predominant soluble form [26]. However, $Ge(OH)_4$ is a significantly larger molecule than $Si(OH)_4$, its volume is 40% greater (Fig. 1) and so, similarly to glycerol, its rate of passive diffusion into oocytes will be significantly slower than for silicon. This difference in molecular size and weight may help to explain why the concentration of ^{68}Ge in oocytes measured in these studies is always significantly lower than the ^{68}Ge concentration of exposure media (e.g. [18]). These studies are demonstrating slow, passive diffusion of $Ge(OH)_4$ down a concentration gradient from exposure medium to oocyte. Additionally, if one follows the citation trail to establish the science behind the use of ^{68}Ge as an analogue for silicic acid in silicon transport research in plants, you find one publication purporting to demonstrate its applicability [27]. However, detailed scrutiny of this paper, while revealing excellent research on the uptake of germanium in plants, does not provide critical data to support the contention that ^{68}Ge is an effective analogue for silicic acid in silicon plant transport studies. The data presented in Table 1 of the paper are the basis given by the author for the applicability of ^{68}Ge as an analogue for uptake of silicic acid. The data show that the molar ratio of ^{68}Ge to Si are constant between exposure media and plant tissues. However, all of the plants used in the uptake experiment would have accumulated some silicon, deposited as biogenic silica, prior to their use in these experiments and yet, the molar ratio of ^{68}Ge to Si *in planta* is described by the authors as being unchanged. In addition, it is unusual that all data describing ratios of ^{68}Ge to Si are similar, regardless of whether the plant is a known silica accumulator or not. The silica accumulators, rice and barley, should have a much higher silica baseline than plants such as cucumber and tomato. Attempts to reconcile these issues with the first author were unsuccessful due to original data no longer being available (personal communications by email). Another paper cited with respect to ^{68}Ge as a suitable analogue for silicic acid (for example [5]) actually concerns its use in establishing adsorption equilibria in lake sediments [10]. While, for example Ma *et al.* [5] cited this paper as the

method they used to measure ^{68}Ge in oocytes, there is nothing in this paper applicable to silicon transport in plants. What we do know about ^{68}Ge is that as $^{68}Ge(OH)_4$ it can diffuse through water channels that are permeable to silicic acid. It has not yet been demonstrated that ^{68}Ge is unequivocally an analogue for the diffusion of silicic acid through these same channels.

5 In Summary and Future Research

The seminal study by Ma *et al.* [5] identifying a putative role for *Lsi1* in the transport of silicic acid has in subsequent papers over many years been used to suggest that *Lsi1* and other water channels involved in the movement of silicic acid *in planta* have specificity for silicic acid and that they are silicon transporters. The use of the term "transporter" is arguably confusing, because the biochemical definition of a transporter is a protein that binds to the transported solute [28] and as of today there is no evidence for binding of silicic acid by any water channel [4]. Although we can agree on the term "transport", as the identified channels transport (or transfer) silicic acid across the membrane, they are not transporters according to biochemical nomenclature and ideally to avoid further confusion should be referred to as channels.

Many channels will be involved in the transport of silicic acid in plants and future studies will identify those with specific permeability to silicic acid. It will be helpful to apply, systematically, computational analyses to estimate the pore size of water channels putatively implicated in silicon transport in plants. Future research incorporating molecular dynamics simulations will help to predict the permeability of proposed channels for silicic acid. The movement of silicic acid throughout plant tissue and its subsequent deposition as biogenic silica has recently been elaborated upon in a number of studies [4, 29–31]. Future research is required to address other important physiological factors, such as the role of root cell walls (porosity, composition) in the movement of silicic acid via the apoplastic pathway and the role of plasmodesmata (with associated callose) in its symplastic movement. It is time to apply Occam's razor to what we actually know about the biology of silicification. Only then can we take this important and exciting field of study forward.

Acknowledgements XL was supported by Grants PGC2018-099321-B-I00 from the Ministry of Science, Research and Universities, and Grant IT1254-19 from the Basque Government.

References

1. Exley C, Guerriero G, Lopez X (2019) Silicic acid: The omniscient molecule. Sci Total Environ 665:432–437. https://doi.org/10.1016/j.scitotenv.2019.02.197
2. Wu B, Beitz E (2007) Aquaporins with selectivity for unconventional permeants. Cell Mol Life Sci CMLS 64:2413–2421. https://doi.org/10.1007/s00018-007-7163-2
3. Exley C (1998) Silicon in life:A bioinorganic solution to bioorganic essentiality. J Inorg Biochem 69:139–144. https://doi.org/10.1016/S0162-0134(97)10010-1
4. Exley C (2015) A possible mechanism of biological silicification in plants. Front Plant Sci 6:853. https://doi.org/10.3389/fpls.2015.00853
5. Ma JF, Tamai K, Yamaji N et al (2006) A silicon transporter in rice. Nature 440:688–691. https://doi.org/10.1038/nature04590
6. Kelly SM, Butler JP, Macklem PT (1995) Control of cell volume in oocytes and eggs from *Xenopus laevis*. Comp Biochem Physiol A Physiol 111:681–691. https://doi.org/10.1016/0300-9629(95)00046-A
7. Rasar MA, Hammes SR (2006) The physiology of the *Xenopus laevis* ovary. Methods Mol Biol Clifton NJ 322:17–30. https://doi.org/10.1007/978-1-59745-000-3_2
8. Feng Ma J, Higashitani A, Sato K, Takeda K (2003) Genotypic variation in silicon concentration of barley grain. Plant Soil 249:383–387. https://doi.org/10.1023/A:1022842421926
9. Belton DJ, Deschaume O, Patwardhan SV, Perry CC (2010) A solution study of silica condensation and speciation with relevance to in vitro investigations of biosilicification. J Phys Chem B 114:9947–9955. https://doi.org/10.1021/jp101347q
10. Tallberg P, Koski-Vähälä J, Hartikainen H (2002) Germanium-68 as a tracer for silicon fluxes in freshwater sediment. Water Res 36:956–962
11. Hub JS, de Groot BL (2008) Mechanism of selectivity in aquaporins and aquaglyceroporins. Proc Natl Acad Sci 105:1198–1203. https://doi.org/10.1073/pnas.0707662104
12. Guerriero G, Deshmukh R, Sonah H et al (2019) Identification of the aquaporin gene family in *Cannabis sativa* and evidence for the accumulation of silicon in its tissues. Plant Sci 287:110167. https://doi.org/10.1016/j.plantsci.2019.110167
13. Ma JF, Yamaji N, Mitani N et al (2007) An efflux transporter of silicon in rice. Nature 448:209–212. https://doi.org/10.1038/nature05964
14. Mitani N, Yamaji N, Ma JF (2008) Characterization of substrate specificity of a rice silicon transporter, Lsi1. Pflugers Arch 456:679–686. https://doi.org/10.1007/s00424-007-0408-y
15. Mitani N, Chiba Y, Yamaji N, Ma JF (2009) Identification and Characterization of Maize and Barley Lsi2-Like Silicon Efflux Transporters Reveals a Distinct Silicon Uptake System from That in Rice. Plant Cell 21:2133–2142. https://doi.org/10.1105/tpc.109.067884
16. Chiba Y, Mitani N, Yamaji N, Ma JF (2009) HvLsi1 is a silicon influx transporter in barley. Plant J Cell Mol Biol 57:810–818. https://doi.org/10.1111/j.1365-313X.2008.03728.x
17. Mitani-Ueno N, Yamaji N, Ma JF (2011) Silicon efflux transporters isolated from two pumpkin cultivars contrasting in Si uptake. Plant Signal Behav 6:991–994

18. Mitani-Ueno N, Ogai H, Yamaji N, Ma JF (2014) Physiological and molecular characterization of Si uptake in wild rice species. Physiol Plant 151:200–207. https://doi.org/10.1111/ppl.12125
19. Grégoire C, Rémus-Borel W, Vivancos J et al (2012) Discovery of a multigene family of aquaporin silicon transporters in the primitive plant *Equisetum arvense*. Plant J Cell Mol Biol 72:320–330. https://doi.org/10.1111/j.1365-313X.2012.05082.x
20. Deshmukh RK, Vivancos J, Guérin V et al (2013) Identification and functional characterization of silicon transporters in soybean using comparative genomics of major intrinsic proteins in *Arabidopsis* and rice. Plant Mol Biol 83:303–315. https://doi.org/10.1007/s11103-013-0087-3
21. Deshmukh RK, Vivancos J, Ramakrishnan G et al (2015) A precise spacing between the NPA domains of aquaporins is essential for silicon permeability in plants. Plant J Cell Mol Biol 83:489–500. https://doi.org/10.1111/tpj.12904
22. Vivancos J, Deshmukh R, Grégoire C et al (2016) Identification and characterization of silicon efflux transporters in horsetail (*Equisetum arvense*). J Plant Physiol 200:82–89. https://doi.org/10.1016/j.jplph.2016.06.011
23. Sun H, Guo J, Duan Y et al (2017) Isolation and functional characterization of CsLsi1, a silicon transporter gene in *Cucumis sativus*. Physiol Plant 159:201–214. https://doi.org/10.1111/ppl.12515
24. Sun H, Duan Y, Qi X et al (2018) Isolation and functional characterization of CsLsi2, a cucumber silicon efflux transporter gene. Ann Bot 122:641–648. https://doi.org/10.1093/aob/mcy103
25. Ratcliffe S, Jugdaohsingh R, Vivancos J et al (2017) Identification of a mammalian silicon transporter. Am J Phys Cell Phys 312: C550–C561. https://doi.org/10.1152/ajpcell.00219.2015
26. Baes CF, Mesmer RS (1977) The Hydrolysis of Cations. John Wiley & Sons, New York, London, Sydney, Toronto 1976. 489 Seiten, Preis: £ 18.60. Berichte Bunsenges Für Phys Chem 81: 245–246. https://doi.org/10.1002/bbpc.19770810252
27. Nikolic M, Nikolic N, Liang Y et al (2007) Germanium-68 as an adequate tracer for silicon transport in plants. Characterization of silicon uptake in different crop species. Plant Physiol 143:495–503. https://doi.org/10.1104/pp.106.090845
28. Alberts B, Johnson A, Lewis J, et al (2002) Principles of Membrane Transport. Mol Biol Cell 4th Ed
29. Brugiére T, Exley C (2017) Callose-associated silica deposition in *Arabidopsis*. J Trace Elem Med Biol Organ Soc Miner Trace Elem GMS 39:86–90. https://doi.org/10.1016/j.jtemb.2016.08.005
30. Guerriero G, Law C, Stokes I et al (2018) Rough and tough. How does silicic acid protect horsetail from fungal infection? J Trace Elem Med Biol Organ Soc Miner Trace Elem GMS 47:45–52. https://doi.org/10.1016/j.jtemb.2018.01.015
31. Guerriero G, Stokes I, Exley C (2018) Is callose required for silicification in plants? Biol Lett 14. https://doi.org/10.1098/rsbl.2018.0338
32. Chai J-D, Head-Gordon M (2008) Systematic optimization of long-range corrected hybrid density functionals. J Chem Phys 128: 084106. https://doi.org/10.1063/1.2834918
33. Weigend F, Ahlrichs R (2005) Balanced basis sets of split valence, triple zeta valence and quadruple zeta valence quality for H to Rn: Design and assessment of accuracy. Phys Chem Chem Phys 7: 3297–3305. https://doi.org/10.1039/b508541a