Life Science

Selenium Acquisition by *Arabidopsis* Plants

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Introduction

Selenium is an essential micronutrient for all animals but is toxic at amounts only 50 times higher than those required for good health. In some areas of the world, including parts of Canada, the level of selenium in the soil is too high to allow grazing by livestock. In other parts of the world the soil selenium is so low that plants do not acquire enough selenium to satisfy the dietary requirements of people and livestock. An improved understanding of how plants take-up, transport and metabolise selenium will promote the development of crop plants with improved selenium acquisition and contribute to eliminating health problems arising from selenium deficiency. It will also allow the development of fodder crops that take-up less selenium and thereby increase the area of usable grazing land.

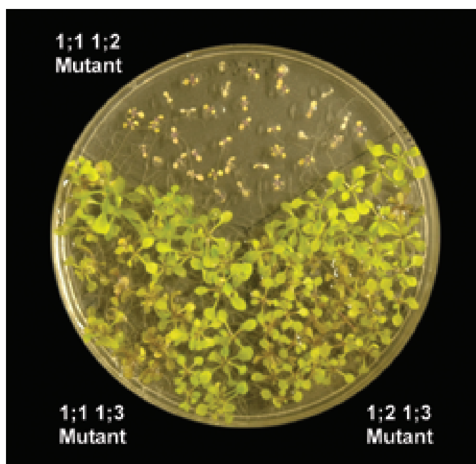

Figure 1: The *Sultr* 1;1, *Sultr* 1;2 double mutant dies from sulphur starvation on minimal medium. Seedlings of three *Arabidopsis* double mutant lines have been grown for two weeks on solid minimal medium with 50 *μM* sulphate as the sole source of sulphur. All three double mutants grow well on media supplemented with 500 *μM* sulphate (data not shown).

Selenium is the element below sulphur in the periodic table and selenate (a chemical analogue of sulphate) is the most common source of biologically accessible environmental selenium. In microbes, external selenate is acquired and metabolised by the proteins of the sulphate assimilation pathway to produce seleno-cysteine. In plants, the uptake and transport of selenate is poorly defined. Sulphate-proton symporter *(SULTR)* proteins are key elements in sulphate transport and we have used lines of the model plant *Arabidopsis thaliana (Arabidopsis)* carrying mutations in multiple *SULTR* genes to establish that *SULTR 1;1* and *SULTR 1;2* are the two genes responsible for the initial uptake of sulphate from the environment (Figure 1). This has recently been established independently by Yoshimoto et al. [1]. Using a combination of genetically defined plant lines and synchrotron-based selenium *K*-edge X-ray absorption spectroscopy, [2] it has been possible to establish the role of sulphate transporters in selenium uptake in *Arabidopsis.*

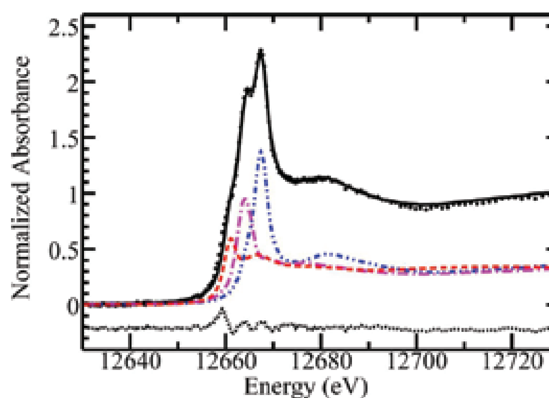

Figure 2: Selenium K-edge spectrum from wild-type *Arabidopsis* plants challenged with 500μM selenite. Data points, ● fit, ● selenate, ● selenite, ● selenomethionine, • residual

Experimental

Arabidopsis lines carrying T-DNA gene-knockout insertions in *SULTR 1;1* (At4g08620: Salk_093256.47.15.x), *SULTR 1;2* (At1g78000: SM_3_30254) and *SULTR 1;3* (At1g22150: Salk_018910.28.00.x) were obtained from the Arabidopsis Biological Resource Centre [3,4]. Plants fixed for mutations in multiple *SULTR* genes were generated by crossing and recurrent inbreeding with molecular selection. When measuring selenium uptake, wild-type Arabidopsis and plants homozygous for the *SULTR 1;1* and *SULTR 1;2* mutations were grown hydroponically in minimal media with 500 *μM* (micromolar) sulphate as sole sulphur source for two weeks and then sulphur starved for two days prior to a one hour treatment with minimal media where sulphate had been replaced by either 50 *μM* selenate, or 500 *μM* selenate, or 500 *μM* selenite.

The Se *K*-edge spectra of plant samples were measured on the HXMA beamline using Si(220) monochromator crystals and a Rh-coated mirror stripe. Spectra on the dilute plant material and aqueous standards were collected in fluorescence using a 32-element germanium detector and samples were held at approximately 10 K using a liquid helium cryostat system to minimize photodamage. The edge step was used to estimate the total selenium and quantitative analysis of the relative contributions of different selenium species was accomplished by least-squares curve-fitting spectra in terms of selenate, selenite, and selenomethionine according to established methods. [2]

Treatment	Genotype[a]	Tissue	Total Selenium (µM)	SeO_4^{2-}(%)	SeO_3^{2-}(%)	Se-R (%)
50 µM Selenate	wt	Roots	479	96	0	0
		Shoots	17	94	0	6
	dm	Roots	10	94	0	8
		Shoots	6	80	0	18
500 µM Selenate	wt	Roots	520	98	0	0
		Shoots	40	99	0	0
	dm	Roots	48	100	0	0
		Shoots	60	100	0	0
500 µM Selenite	wt	Roots	171	54	33	11
		Shoots	9	17	63	20
	dm	Roots	122	4	92	3
		Shoots	12	0	102	0

Footnote: a wt, wild-type; dm, sultr 1;1, sultr 1;2 double mutant.

Table 1: Concentration and chemical speciation of selenium in samples from plants challenged with selenate or selenite.

The analysis of selenate and selenite concentration standards demonstrated a linear response to selenium in the range from 5 µM to 2 mM (5 µM = 400 ppb). It was also possible to determine the chemical speciation of selenium across this range of concentrations in biological samples. Figure 2 shows a typical spectrum and its analysis.

Discussion

Table 1 presents selected data from the analysis of plant samples. Some of the interesting observations that can be drawn from these data are:

• The high-affinity sulphate transporters *SULTR1;1* and *SULTR1;2* constitute the major pathway for the acquisition of selenate. With the 50 µM treatment, the root selenate concentration of the double mutant was only 2% that of the wild-type.

• *SULTR1;1* and *SULTR1;2* play little if any role in the initial uptake of selenite. With the 500 µM selenite treatment, the root selenium concentration of the double mutant was almost the same as (71%) that of the wild-type, whereas, with the 500 µM selenate treatment the root selenium concentration of the double mutant was only 9% that of the wild-type.

• Somewhat surprisingly, 54% of the selenite acquired by the wild-type was oxidized to selenate (500 µM selenite treatment wild-type roots) whereas only 4% was oxidized in the corresponding sample from the double mutant.

• The total accumulated selenium levels in the wild-type samples under all three treatments demonstrate that the rate of selenate/selenite acquisition by the roots is faster than the rate of selenium translocation from the roots to the leaves (shoots). Selenium concentrations in the shoots are only approximately 5% of those in the roots after one hour.

Conclusion

Selenium *K*-edge X-ray absorption spectroscopy is an excellent way to measure selenium and determine its chemical speciation in biological samples. This initial experiment suggests that high-affinity sulphate-proton symporter proteins are responsible for the acquisition of external selenate by plants and that the initial uptake of selenite occurs via a different mechanism. In future, it will be possible to control the uptake of selenate by plants by modifying the specificity of the sulphate-proton symporter proteins with respect to sulphate and selenate.

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