

Complexation of Cd in seeds of Cd-Hyperaccumulating *Thlaspi praecox* and metabolism of Cd ligands during seed germination

Vogel-Mikuš K., I. Arčon^{2,3}, A. Kodre^{2,4}, P. Kump²

¹Biotechnical Faculty, University of Ljubljana, Večna pot 111, 1000 Ljubljana, Slovenia.

²Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia

³University of Nova Gorica, Vipavska 13, 5000, Nova Gorica, Slovenia

⁴Faculty of mathematics and physics, University of Ljubljana, Jadranska 19, 1000 Ljubljana, Slovenia

Key words: EXAFS, metal hyperaccumulation, cadmium, phytoremediation, phytate, thiols

Abstract

The cadmium hyperaccumulator *Thlaspi praecox* Wulfen (Brassicaceae) can accumulate unusually high amounts of Cd ($>1,000 \mu\text{g g}^{-1}$ dry weight) in its seeds without drastically affecting seed viability and germination. As embryonic tissues are the most sensitive to Cd toxicity, the aim of this study was to investigate the Cd coordination and ligand environment in seeds and germinated seedlings of field-collected *T. praecox* by Cd K-edge extended X-ray absorption fine structure (EXAFS). In intact seeds almost two thirds of the Cd ligands were thiol groups (Cd-S-C-). In addition, there was coordination to phosphate groups via bridging oxygens (Cd-O-P-), as for phytate. During germination the proportion of Cd phytate ligands decreased. The number of oxygen (Cd-O-C) ligands, probably belonging to free carboxyl and hydroxyl groups, increased during imbibition, germination and the shedding phase, however in the mature seedlings, when the cotyledons became photosynthetically active, there was an increase in binding of Cd to strong sulphur ligands, especially in embryonic axis, where 70% of ligands belonged to thiol groups. Our results showed that Cd-ligand complexes are degraded during germination and mobilized to the embryonic axis. In the developing embryonic axis the most important detoxification mechanism is binding of Cd to strong sulphur ligands, while in cotyledons binding of Cd to free carboxylic and hydroxylic groups of organic acids and cell wall components also play a role in Cd detoxification.

Introduction

The efficient cellular sequestration and immobilization mechanisms in roots and shoots with the consequent decrease of phloem loading with metals in leaves, provide lower metal concentrations in the seeds of metal-tolerant ecotypes than in any other plant part (Vogel-Mikuš et al., 2007, 2008). However, in Cd-hyperaccumulating *Thlaspi praecox* Cd can to a great extent accumulate in seed embryonic tissues (up to $1,350 \mu\text{g g}^{-1}$ DW), without drastically affecting seed viability or germination (Vogel-Mikuš et al., 2007). Although Cd often accumulates in seeds of edible plants at concentrations that exceed the maximum dietary critical value of $0.3 \mu\text{g g}^{-1}$ DW, such as soybean, wheat, rice and flaxseed, there is very little information available on Cd-binding components in seeds and there is no information at present available on the metabolism of Cd ligands during seed germination. The aim of the present study was therefore to determine the chemical coordination and ligand environment of Cd in the seeds and germinated seedlings of the Cd-hyperaccumulator *T. praecox* collected in the plant's natural metal-polluted environment using the non-destructive synchrotron-based technique Cd K-edge extended X-ray absorption fine structure (EXAFS).

Materials and methods

Seeds of *T. praecox* were collected at a highly Cd-, Zn- and Pb-contaminated area in Slovenia (Vogel-Mikuš et al. 2007). For Cd K-edge EXAFS analysis, the seeds were germinated in twice-distilled water for 21 days.

Seedlings were collected in four phases: imbibition, germination, shedding of the testa and mature seedlings (when cotyledons became photosynthetically active), frozen in liquid nitrogen and freeze-dried for two days. XAS measurements were carried out at beamline C of HASYLAB at DESY in transmission and fluorescence detection mode. The beamline was equipped with a Si 311 two-crystal monochromator with about 2 eV resolution at Cd K-edge (26 711 eV). Higher harmonics were eliminated by detuning the monochromator crystals to 50% of the rocking curve maximum, using the beam-stabilization feedback control. Cadmium concentrations in seeds and germinated seedlings were determined using energy-dispersive X-ray fluorescence spectrometry (Vogel-Mikuš et al. 2010).

Results

Seeds and germinated seedlings exhibited extremely high Cd concentrations (Table 1). The increase in Cd concentrations during germination was a consequence of a concentration effect due to degradation and dissimulation of stored food reserves such as lipids and starch.

Sulphur and oxygen atoms were identified in the first coordination shell in all samples, with Cd-S distances in the range of 2.49 Å (as for thiols) and Cd-O distances in the range of 2.17 Å. In the second shell, there was coordination to carbon atoms at a distance of 2.87 Å and phosphorus atoms at a distance of 3.27 Å, indicating that part of the Cd was bound to phytate (Cd-O-P-).

During germination and early development of young seedlings, the number of phytate (Cd-O-P-) and thiol (Cd-S-C) ligands was decreasing, while a number of oxygen ligands (Cd-O-C-) was increasing (Fig. 1), until the seedlings reached their mature stage. At this mature stage there was an increase in the proportion of S-ligands and a subsequent decrease in oxygen (Cd-O-C) ligands (Fig. 1). Analysis of separate cotyledons (with 2400 ± 200 mg Cd/kg) and embryonic axis consisting of a developing root (with 1300 ± 100 mg Cd/ kg), showed that in the embryonic axis 70% of Cd ligands were S-ligands. In cotyledons, only 45% of the ligands belonged to the thiol groups, while the rest were oxygen ligands. A small proportion of Cd in cotyledons remained bound to phytate, while in the embryonic axis phytate ligands were not detected.

Table 1. Cd concentrations in *T. praecox* seeds and germinating seedlings (mean \pm se).

	Conc. Cd (mg/ kg)
Intact seeds	1030 \pm 70
Imbibition	1080 \pm 70
Germination	1100 \pm 85
Shedding of the testa	1300 \pm 120
Mature seedling	1600 \pm 150

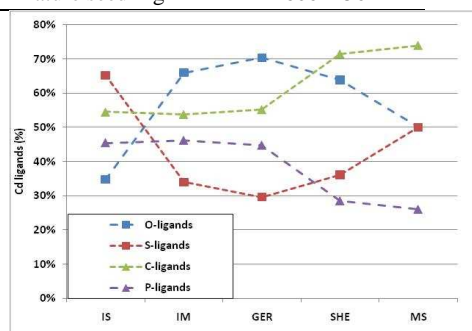


Figure 1. Relationship between Cd ligands in different phases of germination of *T. praecox* seeds (IS- intact seeds, IM – imbibition, GER-germination, SHE – shedding, MS- mature seedlings).

Discussion

EXAFS analysis of intact seeds showed that sulphur in the first shell with carbon in the second shell (Cd-S-C-thiol groups) represents the majority of Cd ligands. The remaining Cd ligands were formed by oxygen in the first shell and with phosphorus or carbon in the second shell (Cd-O-P- and Cd-O-C coordination), implying a bond to phytate and free carboxyl and hydroxyl groups which can be found in cell wall compounds and organic acids (Vogel-Mikuš et al. 2010).

With development and growth of the seedlings, the proportions of Cd phytate ligands decreased, which is in line with phytate degradation and release of the mineral nutrients bound to phytate during seed germination. In addition, the results indicate that during germination sulphur compounds stored in cotyledons were degrading,

releasing the stored Cd. The released Cd was then probably bound to non-phytate oxygen ligands (carboxylic and hydroxylic groups), since their proportion increased during early germination phases. In the rapidly growing embryonic axis, 70% of Cd ligands were thiol groups, indicating that Cd ions that were released from phytate and other compounds during germination and mobilised from cotyledons to embryonic axis were then bound to strong S-ligands in order to protect the developing seedlings from Cd ion toxicity. In photosynthetically active cotyledons the majority of Cd remained bound to oxygen ligands suggesting that binding to the cell wall compounds and possibly vacuolar sequestration may play important roles in detoxification and protection of photosynthetically active tissues at this stage of development.

Conclusions

The presence of a high proportion of sulphur ligands in *T. praecox* seeds indicates that in embryonic tissues the prevailing Cd detoxification mechanisms is synthesis of strong S-ligands (Vogel-Mikuš et al. 2010). During germination, Cd is released from phytate and sulphur compounds. In the embryonic axis, Cd mobilized from cotyledons is then bound mainly to strong sulphur ligands, while in cotyledons binding to sulphur and oxygen ligands are more or less equally represented.

Acknowledgements

This work was supported by the Slovenian Research Agency, and by DESY and the European Community's Seventh Framework Programme (FP7/2007-2013) ELISA under grant agreement n° 226716." Access to SR facilities of HASYLAB (project II-20080058 EC) and support and expert advice on beamline operation of Adam Webb, Roman Chernikov and Edmund Welter of HASYLAB is acknowledged.

References

- Vogel-Mikuš, K., P. Pongrac, P. Kump, M. Nečemer, J. Simčič, P. Pelicon, M. Budnar, B. Povh, and M. Regvar. 2007. Localisation and quantification of elements within seeds of the Cd/Zn hyperaccumulator *Thlaspi praecox* by micro-PIXE. *Environ. Pollut.* 147:50–59.
- Vogel-Mikuš, K., I. Arčon, and A. Kodre. 2010. Complexation of cadmium in seeds and vegetative tissues of the cadmium hyperaccumulator *Thlaspi praecox* as studied by X-ray absorption spectroscopy. *Pl. Soil* 331: 439-451.
- Vogel-Mikuš, K., J. Simčič, P. Pelicon, M. Budnar, P. Kump, M. Nečemer, J. Mesjasz-Przybyłowicz, W-J. Przybyłowicz, and M. Regvar. 2008. Comparison of essential and non-essential element distribution in leaves of the Cd/Zn hyperaccumulator *Thlaspi praecox* as revealed by micro-PIXE. *Plant Cell Environ.* 31:1484–1496.