Competition for Metal-binding Ligands in the Zn/Cd Hyperaccumulator *Thlaspi caerulescens*

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Introduction

The study of heavy metal homeostasis in plants has benefited from the availability of x-ray absorption fine structure (XAFS). Plant researchers have used XAFS to determine the coordination of Cr, Mn, Se, Ni, and Zn in plant tissues [1-8], yet few have applied this technique to examine the ligand coordination of Cd in plants [2, 5]. Those few studies have primarily focused on phytochelatins (PCs), common non-peptide-binding proteins synthesized by many plants upon exposure to Cd. PCs, however, do not represent the primary Cd ligand in metalliferous (metal-loving) plants like *Silene vulgaris* and *Thlaspi caerulescens* [9-12]. Time on the GSECARS beamline in March 2002 was used to investigate the *in situ* coordination of Cd in different populations of *T. caerulescens*. While this experiment provides important fundamental information concerning the localization and coordination of Cd in plant tissues of both normal and metalliferous plants, its true novelty lies in the comparison between populations. The majority of studies with metal-tolerant or hyperaccumulators compare a single population of the unique plant to the "normal" plant to identify the predominant differences in metal homeostasis [2, 9-17]. A more useful approach is to compare different populations of the unique plant, including populations that range from moderate hyperaccumulators to highly efficient hyperaccumulators. By using different populations of the same hyperaccumulator that have demonstrated differences in their ability to hyperaccumulate Cd [16], the relationship between individual aspects of hyperaccumulation can be addressed, allowing for a subsequent physiological and biochemical dissection of the trait.

Physiological studies with *T. caerulescens* have shown that when exposed to elevated concentrations of Cd and Zn, this plant hyperaccumulates both elements [16, 18, 19]. The published information on the *in situ* Zn coordination [2, 7] and the predicted ligand coordination for Cd potentially involve the same ligands: organic acids. This has been reiterated by computer speciation modeling [20]. When these elements are present together in plants, there may be competition between the metals for organic ligands. If one competes more effectively, then *in situ* coordination of the other metal may shift accordingly. The goal of the measurements described here was to examine reciprocal effects of Cd and Zn on the *in situ* coordination of these metals in *T. caerulescens* populations. This should provide useful information about the dynamics of metal coordination in metalliferous plants, the contribution of ligand coordination to hyperaccumulation, and population-level variation in this trait.

Methods and Materials

Given the low tissue concentrations encountered in the March 2002 beam run, plants were exposed to Cd or Cd+Zn for 21 d to try to increase the signal strength obtained from the samples. Preparation of samples for XAS analysis followed procedures described previously [2, 7]. Briefly, plant tissues for analysis were ground under liquid nitrogen and immediately packed into sample holders for analysis. A new sample holder was constructed for use to facilitate sample cooling in a new Peltier cell that had been obtained (Fig. 1). This new system was preserved in later studies because of the efficient transmission of cold throughout the sample, insuring that it remained frozen at all times. Extended x-ray absorption fine structure (EXAFS) was performed on these samples on the bending magnet under the direction of M. Newville. Because the Kα-edge for Cd occurs at 26.69 keV, samples were scanned over a range from 26.55 to 28.55 keV. The Canberra Ge detector was used during this run. A Lytle detector was used later for samples (cell wall preps) with high Cd concentrations. Samples were maintained at –40°C by a Peltier cooling cell supplied by J. Cross. Samples were mounted at a 45° angle incident to the beam, with the detector placed perpendicular to the beam. Teflon® holders containing the tissue samples were placed directly within the Peltier cell. All samples were covered by a layer of Kapton® film to facilitate XAS.

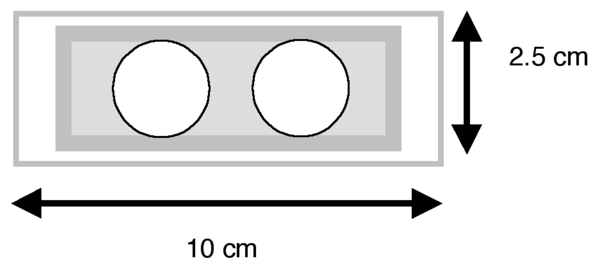

FIG. 1. Schematic diagram of the sample "slides" developed for XAS analysis of plant tissue samples. Dimensions are not drawn to scale.

Results and Discussion

Scans of the plant tissues indicated that the modifications to the plant growth and treatment protocols were insufficient to achieve the desired signal strength. The larger plants that were utilized, despite being hyperaccumulators, distributed Cd evenly across leaf tissues at a low concentration. For the available plants, future experiments will require longer periods of exposure to achieve sufficient Cd concentrations for the desired analyses. The decision was also made to use atomic absorption spectroscopy (AAS) to determine the Cd content of tissues prior to XAS analysis to insure that this problem is not encountered during future beam runs. The new sample scheme, however, was shown to be acceptable, since the solid standards analyzed showed clear spectra.

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