Imaging of selenium in plants using tapered metal monocapillary optics

Ingrid J. Pickering,[a]* Gregory Hirsch,[b] Roger C. Prince,[c] Eileen Yu Sneeden,[a] David E. Salt[d] and Graham N. George[a]

[a]Stanford Synchrotron Radiation Laboratory, SLAC, 2575 Sand Hill Road, MS 69, Menlo Park, CA 94025, USA, [b]Hirsch Scientific, 1277 Linda Mar Center Suite 128, Pacifica, CA 94044, USA, [c]ExxonMobil Research and Engineering Co., Annandale, NJ 08801, USA, and [d]Department of Horticulture and Land Architecture, Purdue University, West Lafayette, IN 47907, USA. E-mail: pickering@ssrl.slac.stanford.edu

Tapered metal monocapillary optics provide a potential alternative to conventional methods of producing small X-ray beams. This paper presents the initial results of chemically specific imaging using such devices. Cellular resolution of organic selenium is obtained in a longitudinal section of mature *Astragalus bisulcatus*, a selenium hyperaccumulating plant. This work demonstrates the utility of metal monocapillary optics for imaging dilute levels of target elements in biological tissues.

Keywords: monocapillary optics; chemically specific imaging; selenium; hyperaccumulators; *Astragalus bisulcatus*.

1. Introduction

Selenium, an essential trace element in eukaryotes (Hatfield, 2001), is toxic at higher levels. Hyperaccumulating plants are able to actively and specifically accumulate high levels of the element into their tissues (Brooks, 1998); *Astragalus bisulcatus* hyperaccumulates more than 0.6% of its shoot dry biomass as Se from inorganic selenium naturally occurring in soil (Trelease *et al.*, 1960). We have previously used chemically specific imaging with an apertured 100×100 µm beam to show how selenium is partitioned between selenate and organic selenium in the leaves and stems (Pickering *et al.*, 2000). Here we use a smaller beam, produced by a novel tapered metal monocapillary optic, to investigate the localization and chemical form of selenium on a cellular scale within the plant structures.

Metal monocapillary optics provide a potential alternative to conventional methods of producing small X-ray beams (Hirsch, 1998, 2000, 2003) for hard X-ray imaging and micro X-ray absorption applications (Bertsch & Hunter, 2001). Compared with zone plates, capillary optics have the advantage of not requiring adjustment along the beam direction on changing energy, which allows images at multiple energies to be perfectly spatially aligned. Metal monocapillary optics allow higher photon-energy operation than glass monocapillaries in a shorter length, because of the dense metal's higher critical angle for total external reflection. Metal monocapillaries are also a cheaper alternative to Kirkpatrick–Biaz mirrors. A shortcoming is that the very short focal length requires the end to be very close to the sample, which makes it difficult to measure the intensity incident on the sample. Nevertheless, this problem can be circumvented by measuring a background in the form of a blank pixel at the end of each raster.

In this paper we present the initial results of using a metal monocapillary optic with a 5 µm exit aperture to probe the localization and speciation of selenium at cellular resolution in a sectioned sample from a mature *A. bisulcatus* plant.

2. Materials and methods

A paraboloidally tapered metal monocapillary optic with a gold interior was manufactured and characterized as described elsewhere (Hirsch, 1998, 2000, 2003). Experiments were carried out on the structural molecular biology wiggler beamline 9-3 of the Stanford Synchrotron Radiation Laboratory (SSRL), with a double-crystal Si(220) monochromator, an upstream vertically collimating Rh-coated mirror for the rejection of harmonics and a downstream refocusing Rh-coated mirror. Intensity was measured upstream of the optic and downstream of the sample using N_2-filled ion chambers. Motorized slits were mounted upstream of the tapered metal capillary optic to reduce the incident beam size. The optic itself was mounted on a custom motorized orientation stage.

Mature *A. bisulcatus* plants were cultivated according to the method of Pickering *et al.* (2000). A section of mature leaflet branch was made using a microtome, mounted on adhesive Mylar tape, and covered with polypropylene to minimize dehydration. Optical micrographs were collected, and the sample was mounted on an *xy* raster stage within 0.1 mm of the output of the capillary and normal to the beam. Se $K\alpha$ fluorescence was monitored with a Canberra 30-element germanium detector, upstream of the sample, at approximately 55° to the incident beam.

Data were collected using the *SCAN_CONTROL* software, which was developed at SSRL for these experiments. This software consists of two separate programs that run concurrently – an XWindows graphical user interface or GUI (Fig. 1) and a data acquisition program. The latter is a relatively small and robust program that communicates with the GUI using shared memory, thus allowing the real-time display and analysis of the data while the experiment is in progress. The use of two discrete programs in this way means that if the GUI crashes the collector will continue, and the GUI will simply be relaunched with no loss of data. During a typical experiment the sample abscissa is scanned at each X-ray energy for each point in the ordinate scan, so that the maximum completeness of the data at any given time is ensured. A variety of optional capabilities are available, such as automated optimization of the hutch table position at the end of every raster. The GUI program has data manipulation and display capabilities, such as adjustable false-color displays and the ability to arithmetically combine images.

3. Results and discussion

Astragalus bisulcatus has pinnately compound leaves comprising several pairs of leaflets growing from either side of the stem-like rachis (the rachis itself branches from the main stem; Raven *et al.*, 1992). Fig. 2(*a*) is a low-magnification optical micrograph of a 100 µm-thick longitudinal section through a stem-like rachis. Fig. 2(*a*) shows a pair of nodes where the leaflets branch off on small stalk-like petiolules. A higher-magnification optical picture of one of the nodes (Fig. 2*b*) reveals several details, including the vascular bundles that are visible as bright vertical lines running from bottom to top. The helical structure of the xylem vessels can be seen. The vascular bundles are seen to diverge, with a smaller bundle, the leaflet trace, forming an extension to the leaflet.

Chemically specific imaging was carried out on the same region of plant tissue, and the results are shown in Fig. 2(*c*) for organic selenium measured at 12660.7 eV. The map shows that the higher selenium concentrations occur in the cortex, those layers of cells that lie

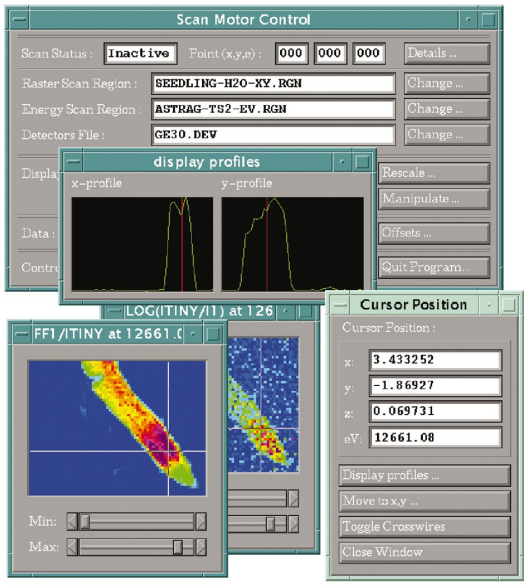

Figure 1
Example windows from the data collection and analysis software.

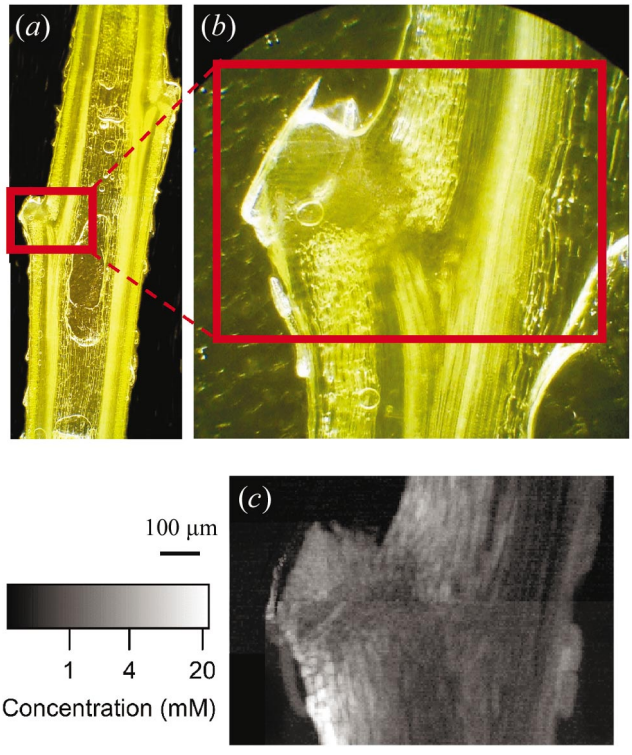

Figure 2
Longitudinal section of a mature leaf branch of *Astragalus bisulcatus*, imaged with optical microscopy and using a metal monocapillary optic. The step size is 5 µm in both dimensions, and the specimen is approximately 100 µm thick. Concentration is derived as previously described (Pickering *et al.*, 2000) *via* a concentration standard. (*a*) and (*b*) are optical micrographs; (*c*) is a map of organic selenium.

outside the vascular bundle, with the highest concentration close to the epidermis. This result is consistent with our previous observations of the highest concentrations in the periphery of such structures (Pickering *et al.*, 2000). There is an indication that the selenium is sequestered predominantly within, rather than outside of, these cells. Some selenium concentration is observed in the trichomes (hairs), although to a lesser extent than was observed for cadmium in *Brassica juncea* (Salt *et al.*, 1995).

The concentration in the vascular bundles is considerably lower than in the surrounding cells. However, periodic structure in the sub-1 m*M* level coincides with the location of the xylem vessels. The extremely fine nature of these (10–15 µm across) suggests that the selenium is associated with fibres in the xylem bundle. A map at the energy sensitive to selenate (12667.0 eV) was also collected, but no significant presence of selenate was detected. This result is consistent with the low level of selenate in the medium (5 µ*M*) and with our previous observations of predominantly organic selenium in this region of the plant (Pickering *et al.*, 2000).

Excellent spatial resolution is achieved with the metal monocapillary optic, as shown in particular by the periodic structures in the xylem vessels, which are only some 10–15 µm across. The sensitivity to low levels of selenium is also demonstrated by these structures, which show a detection level of better than 1 m*M* (equivalent to 1.5×10^9 atoms per pixel). In a subsequent paper (Pickering *et al.*, 2003), we will present chemically specific images of seedlings of *A. bisulcatus* germinated in the presence and absence of selenate.

In summary, tapered metal monocapillary optics have been successfully used to quantitatively image and speciate selenium at dilute physiological levels in living tissues of plants. Excellent spatial resolution has been achieved. Future applications of these optics will include studies at low X-ray energies (*e.g.* the sulfur *K*-edge) and at higher spatial resolutions (*e.g.* 1 µm).

SSRL is supported by the US DOE OBES and the Structural Molecular Biology program by the DOE OBER and NIH NCRR BTP. Software development was supported in part by NIH grant No. GM57375. GH was partially supported by DOE grant No. SBIR DE-FG03-96ER82185. DES was supported in part by NIH grant No. IR41CA80444-01. We gratefully acknowledge the support of the SSRL staff.

References

Bertsch, P. M. & Hunter, D. B. (2001). *Chem. Rev.* **101**, 1809–1842.
Brooks, R. R. (1998). Editor. *Plants that Hyperaccumulate Heavy Metals: Their Role in Phytoremediation, Microbiology, Archeology, Mineral Exploration and Phytomining.* Wallingford: CAB.
Hatfield, D. L. (2001). Editor. *Selenium: Its Molecular Biology and Role in Human Health.* Hingham: Kluwer.
Hirsch, G. (1998). Tapered Capillary Optics. US Patent 5 772 903.
Hirsch, G. (2000). *Synchrotron Radiation Instrumentation, Eleventh US National Conference* (*AIP Proceedings* 521), edited by P. Pianetta, J. Arthur & S. Brennan, pp. 253–257. Melville, New York: AIP.
Hirsch, G. (2003). *X-ray Spectrom.* In the press.
Pickering, I. J., Prince, R. C., Harris, H. H., Nichol, H., Hirsch, G., Durkin, D. R., Burroughs, A., Salt, D. E. & George, G. N. (2003). In preparation.
Pickering, I. J., Prince, R. C., Salt, D. E. & George, G. N. (2000). *Proc. Natl Acad. Sci.* **97**, 10717–10722.
Raven, P. H., Evert, R. F. & Eichorn, S. E. (1992). *Biology of Plants*, 5th ed. New York: Worth.
Salt, D. E., Prince, R. C., Pickering, I. J. & Raskin, I. (1995). *Plant Physiol.* **109**, 1427–1433.
Trelease, S. F., Di Somma, A. A. & Jacobs, A. L. (1960). *Science*, **132**, 618.

 Received 30 August 2002 • Accepted 7 February 2003