Developmental Malformations of Tadpoles (*Rana catesbeiana*) from a Coal Fly Ash Basin: Possible Role of Selenium in Disrupting Protein Structure

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Introduction

The biogeochemistry of Se is complex. At low levels, Se is an essential element, yet at higher concentrations, toxicity is often manifest in plants and animals. The essential nature of Se derives from the Se amino acid Se-cysteine, which is the active site in a number of enzymes, including glutathione peroxidase and formate dehydrogenase [1]. By contrast, one proposed mechanism for the toxicity of Se is the indiscriminate substitution of the nonessential Se amino acid, Se-methionine, for methionine during protein synthesis, which subsequently affects protein structure and function. Previous studies have implicated Se in the ecotoxicology of organisms inhabiting wetlands contaminated with coal combustion waste (CCW) [2, 3]. We are currently conducting synchrotron x-ray fluorescence (SXRF), x-ray absorption near-edge structure (XANES), and extended x-ray absorption fine structure (EXAFS) studies in Se-rich regions in organisms exposed to CCW to fully elucidate Se coordination environments [4]. The ability to examine tissues of organisms from different trophic levels *in situ* will allow us to test an emerging hypothesis that the biotransformation of Se into proteinaceous forms controls its biomagnification [5]. Furthermore, we hope to provide specific structural data to confirm the *ex situ* identification of a previously uncharacterized Se-S bridged seleno-amino acid in Se-rich biological tissues [6].

Methods and Materials

Bullfrog larvae (*Rana catesbeiana*) samples were collected from a fly ash settling basin that forms part of the fly ash disposal system of a coal-fired power plant located on the U.S. Department of Energy (DOE) Savannah River Site. Previous studies had shown that bullfrog larvae collected from this site exhibited a higher incidence of oral malformations than animals collected at a reference site. Furthermore, Se had previously been determined to be at relatively high concentrations in the tissue. The bullfrog larvae were allowed to void their gut contents for 24 hours, and the extent of oral malformation was scored as either normal or severe. Mouthparts were sectioned and were kept frozen (–20°C), along with the remainder of the animal, before being shipped to the APS for analysis.

X-ray floresence mapping and μ-XANES and μ-EXAFS were conducted by using the x-ray microprobe at the GeoSoilEnviroCARS sector at the APS. XANES and EXAFS spectra were recorded for Se standards for selenite, selenate, selenocystine and selenomethionine. The tissue samples were allowed to thaw and mounted on Kapton® tape. A small volume of deionized water, followed by a further layer of Kapton tape, was used in an attempt to keep the samples from drying in the beam during EXAFS analysis. However, this attempt to keep the samples hydrated was not successful, leading to poor-quality EXAFS data, presumably because the samples were drying and therefore changing position relative to the beam during data collection.

Results and Discussion

Bullfrog larvae collected from the fly ash settling basins exhibited oral malformations consistent with previous observations. These malformations were manifest as distorted and missing "tooth rows" and distorted labial tissue.

Initial x-ray absorption spectroscopy (XAS) studies of the sectioned mouth parts indicated a correlation between areas of increased Se and Zn concentrations (Figure 1A and 1B). However, while Zn was more localized in the region of the teeth and occurred in both

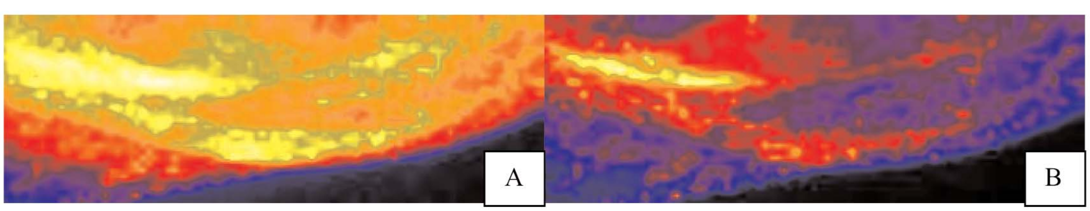

FIG. 1. 2-D elemental map of tadpole mouth section showing correlation between areas of concentration for Se (A) and for Zn (B).

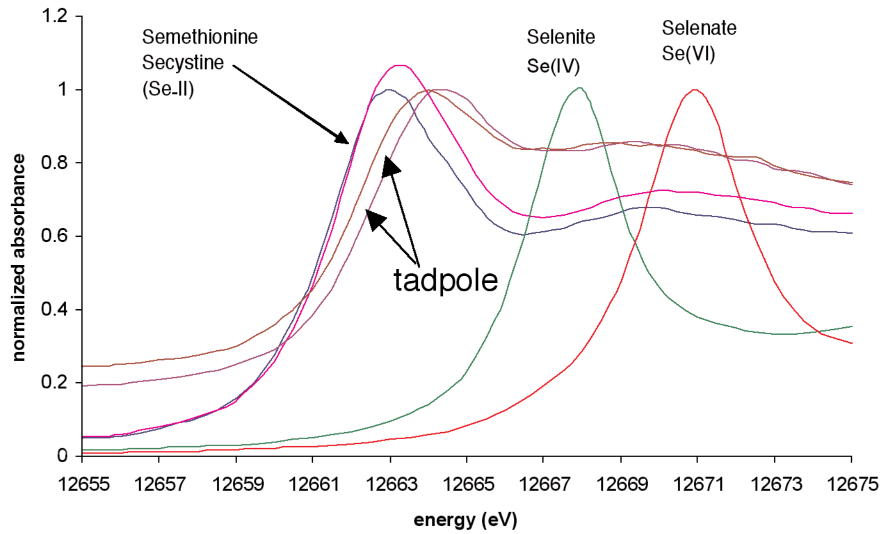

FIG. 2. Xanes spectra for Se inorganic and amino acid standards and tadpole mouth sections.

control and exposed animals, Se was more evenly distributed throughout the soft tissue but occurred only in the CCW-exposed animals. This suggests that Zn is a constituent of proteins that make up the keratinized tooth rows.

Initial XANES analysis confirmed that Se in the sectioned mouth parts occurred in the reduced form [Se(-II)], consistent with its incorporation into organic molecules. The slightly higher edge position for the samples might have been due to surface binding of inorganic Se. Unfortunately, EXAFS analysis did not result in spectra of sufficient quality for data reduction, because of the relatively low concentration of Se in the tissue and possibly because of sample movement and drying during the analysis. The results confirm that Se incorporation into the soft tissue of exposed organisms is widespread, and the oxidation state of Se(-II) is consistent with an organo-Se compound, most likely from Se incorporation into proteins.

Acknowledgments

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