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XAFS Studies of Transition Metal and Halogen Biomaterials in Invertebrate Tools

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Abstract. A significant fraction of arthropods, as well as some worms and members of other phyla, contain extraordinary amounts of transition metals (Mn, Fe, Cu, Zn) and halogens (Cl, Br, I) in their hardened tools (jaws, claws, fangs, stings, etc.). Bulk Zn EXAFS differs for different kinds of tools, whereas Br appears to occupy a common environment in different tools. Zn binding to histidine is observed for most samples but bulk Zn EXAFS is likely to sample a mixture of Zn coordination environments. Principal Component Analysis (PCA) suggests the presence of three main Zn coordination components. The similar Br EXAFS in widely different tools indicates that Br-phenyl is the dominant form, suggesting the presence of brominated aromatic amino acid-derived organic components in these biomaterials. Calculated Debye-Waller factors are used to simulate EXAFS when model compound data are unavailable.

Keywords: X-ray spectroscopy, invertebrate tools, biomaterials
PACS: 87.64.Fb

INTRODUCTION

There exists a large amount of transition metals and halide elements in many invertebrate tools [1]. What is the biological role of these elements in these tools? How do these elements accumulate during tissue development? What is the chemical nature of these biomaterials? Extensive studies have shown that these metal-halogen biomaterials are not in distinct biomineral phases as found in calcified tissues [1], which can be characterized by diffraction methods. To help answer these questions we have used X-ray absorption spectroscopy to study the chemical form of these elements. We present here the analysis of Zn and Br, two of the major elements present in these tools.

EXPERIMENTAL

We examined air-dried samples of the hard tissue of a variety of invertebrate tools. Here we present data for the mandibular teeth of the ant *Atta cephalotes*, the tips of the tarsal (leg) claws of the crab *Hemigrapsus nudus*, the jaws of the worm *Nereis brandti*, the cheliceral teeth of the scorpion *Vaejovis spinigerus*, the forcipule of a centipede from the family *Cryptopidae*, a tarsal (leg) claw of the pycnogonid *Pycnogonum stearnsi*, mouth spines of the priapulid *Priapulus tuberculatospinosus*, and a fang from the spider *Araneus diadematus*. Br model compounds were from Aldrich, including bromo-L-phenylalanine, dibromo-L-tyrosine and 2-bromohexadecanoic acid.

XAS data were collected on beam line 9-3 of the Stanford Synchrotron Radiation Laboratory (SSRL). Harmonic rejection was achieved by the focusing mirror. All samples were maintained at 10 K during measurement using a liquid helium flow cryostat. We used a 30-element intrinsic Ge solid-state detector and Z–1 fluorescence filter with Soller slits to record the XAS fluorescence signal from samples. The model compounds were measured using transmission mode.

XAS data reduction was carried out to extract EXAFS using the EXAFSPAK program. The theoretical phase and amplitude functions were calculated by FEFF 8.0.

XAS data of model compounds are not always available. To evaluate proposed structural models, we used calculated Debye-Waller factors (DWF) to simulate the EXAFS. To calculate these Debye-Waller factors, force constants for all bond-atom pairs were first calculated using the UFF force field [2], except that the force constants for Zn-N and imidazole ring come from DFT calculations [3].We use the recursion method incorporated into FEFF8 to calculate the DWF

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from the force constants. This method has been proven to be adequate to simulate the EXAFS of relevant models [4]. With calculated DWF and amplitude and phase functions, we can simulate the EXAFS for comparison with measured data. Only E_0 was optimized by EXAFSPAK during these simulations.

PCA was performed using WinXAS. Edge data were used due to the higher signal-to-noise ratio.

RESULTS AND DISCUSSION

Zn Coordination Analysis

All Zn XAS data are compared in Figure 1, which suggests subtle variation in average Zn structures with a range of structural types. PCA was used to evaluate the main components. This analysis suggests the presence of three main structural components.

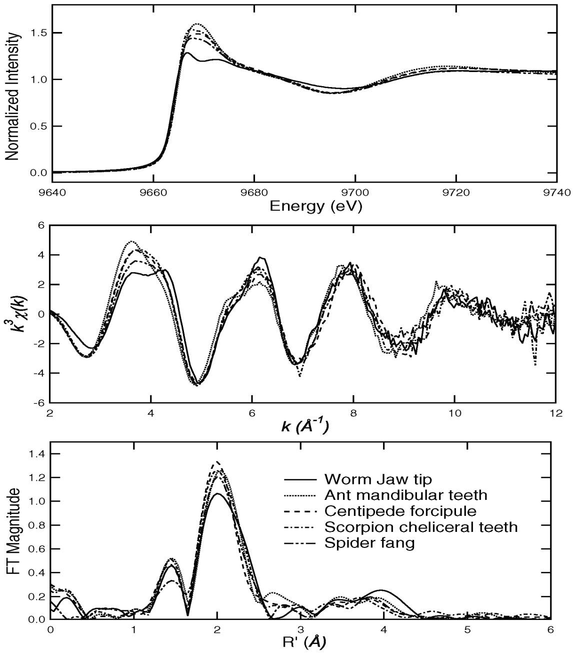

FIGURE 1. Comparison of Zn XAS data of different invertebrate tools. FTs used EXAFS data from $k = 2.0 - 12.0$ Å^{-1}, k^3 weighting, with phase shift correction.

A shoulder or double maximum is observed in the EXAFS at ~4 Å^{-1}, and small "outer-shell" FT peaks exist between 3-4 Å. These features are generally the signature of ligation by histidine residues. These features are especially prominent for the data collected on the *Nereis brandti* (worm) jaw tip. Based on several simulations, two histidine ligands is most likely. Asymmetry in the first-shell FT peak suggested the presence of longer bonds (e.g., Zn-Cl; these specimens also contain a large amount of Cl). However an average of one Cl ligand would cause a splitting in the first-shell FT peak. We constructed and simulated two models, 2Histidine+1Cl+1O and 2Histidine+2O, and mixed these two models in varying ratios to fit the worm jaw tip data (Figure 2).

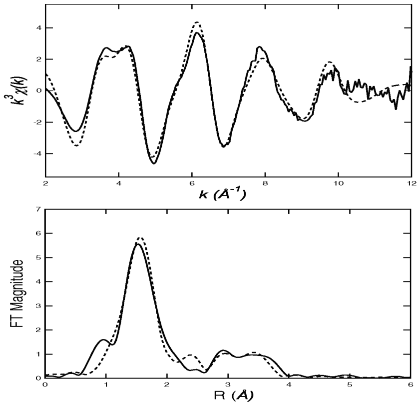

FIGURE 2. Fitting of *Nereis brandti* (worm) jaw data (solid). Simulated data (dashed) were generated for $Zn(His)_2O_2$ (70%) and $Zn(His)_2ClO$ model (30%). No phase correction was applied in the FT.

Furthermore, diverse models have been simulated to test other components. Considering the Zn and Cl stoichiometry in these tools [1], one Zn/Cl mineral, simonkolleite, was simulated but looks dissimilar to our Zn data (not shown). A nanostructured model is also attractive (e.g., ZnO particles), but we do not observe intense scattering from 12 Zn atoms in the 3.2 Å second shell as expected. We are continuing to explore other possible structures, such as a nanostructured particle of Zn hydroxide or Zn-catechol structures, both of which appear possible.

Br Coordination Analysis

In contrast to Zn, Br XAS data display remarkable similarity in diverse samples (Figure 3). The profile, intensity and position of edges are almost identical. The EXAFS also exhibits similar main features, such as the splitting at ~4 and ~8 Å^{-1} and the shoulder at ~5 Å^{-1}. The FT also shows multiple peaks, the position and amplitude of which are quite similar. This suggests similar Br coordination environment in all these samples. No FT peaks are observed at distances expected for Br-metal interactions (ca. 2.4-2.5 Å).

Organic bromine in biology can be roughly divided into aliphatic and aromatic compounds. We simulated the EXAFS for monobromo-phenyl, dibromo-phenyl, bromo-histidine, and bromo-aliphatic. It turned out that the bromo-phenyl models best simulate the observed EXAFS. One of the simulations, based on a simple Br-phenyl model (Br-C = 1.87 Å, C-C = 1.42

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Å), is displayed in Figure 4c. Remarkably, this simulation matches the data quite well. It reproduces the main feature in both EXAFS and FT.

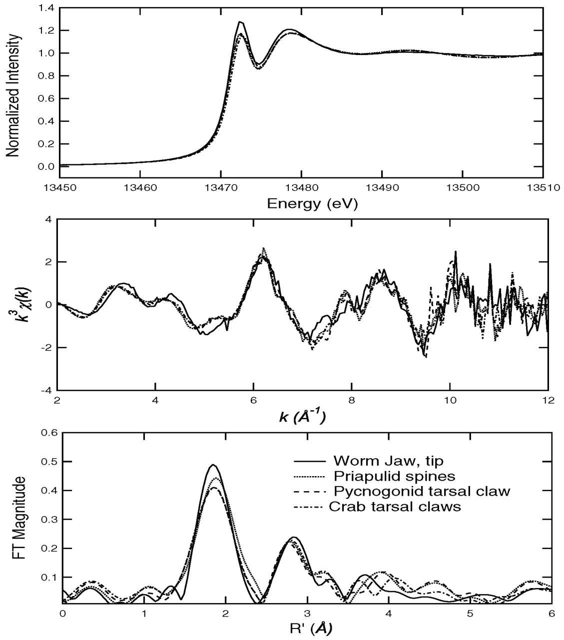

FIGURE 3. Comparison of Br XAS data of different invertebrate tools. FTs used EXAFS data from k = 2.0 – 12.0 Å^{-1}, k^3 weighting, with phase shift correction.

Experimental XAS of bromophenyl compounds confirmed that the monobromo-phenyl model was the best candidate (Figure 4a,b). The experimental data also confirm that our simulation is adequate to mimic the features of the expected models.

As clearly shown in Figure 4a, the Br edge of both bromophenyl model compounds and crab claw exhibit only very slight differences in position, shape, and height. The positions of FT peaks between 1.5 and 5 Å is quite similar among claws and model compounds (Fig. 4, bottom). All of the first-shell FT peaks appear at ~1.88 Å, the Br–C(aromatic) distance. Multiple peaks between 3.5 and 5 Å appear in all of these data, arise from the outer shells of a phenyl ring.

A huge peak appears at ~5.6 Å in dibromotyrosine contributed by the distant Br scatterer (Br–Br distance is close to 5.6 Å). There is also a peak at ~5.6 Å in the crab claw but the amplitude is much lower compared with the peak in dibromotyrosine, so most of the Br is not in a dibromotyrosine–like environment.

In summary, Zn displays different coordination environments, although histidine ligation is believed to exist extensively, while Br is shown to bind to phenyl, suggesting the existence of a brominated aromatic amino acid, possibly bromo-tyrosine. Based on our simulation results, some reference samples are under preparation for Zn site analysis, like nanostructured zinc hydroxide and a zinc-catechol chelate. Microprobe XAS is under way to investigate the subtle structure variation within a given specimen.

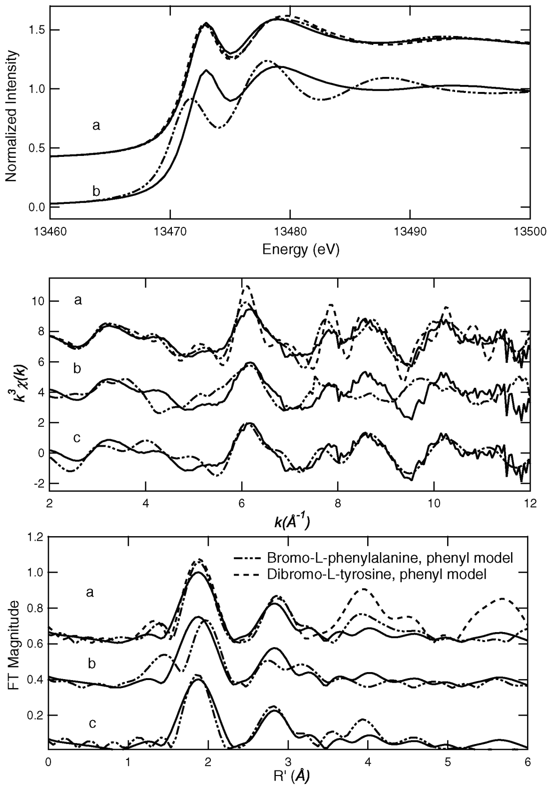

FIGURE 4. Comparison of Br XAS of crab claw (solid) with bromophenyl compounds (a), aliphatic compound (b) and the simulated phenyl model (c).

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