Speciation of Mercury in Tissues: Pilot Study Final Report

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Purpose of this Pilot Study
The purpose of this pilot study was to determine detection limits of XAS for mercury in tissues using the best available x-ray technology at the BioCAT undulator beamline at the Advanced Photon Source, and to assess the extent to which speciation of mixtures can be assessed by Principal Components Analysis and related methods. These goals were achieved, and the results suggest some better alternative ways to address the scientific problem. Further studies appear to be warranted.

Samples
The original plan was to study tissue samples of marine mammals and fish procured by Dr. Jon Talbott at WMRC. Unfortunately many of the expected samples were not available. However, good substitutes became available fortuitously through Dr. Karyn Bischoff, a veterinary toxicologist who took a position in the same department as the PI. Through her contacts we were able to procure appropriate alternative tissue samples from aquatic birds, and these because the principal focus. These tissue samples demonstrated that it is indeed possible to obtain useful data on tissue samples from birds at normal environmental exposures.

Experimental measurements
Cormorant livers were harvested by Laura Quinn, Director of the Florida Keys Wild Bird Center, Taverneir, Florida, on Key Largo. The birds were collected in the vicinity of the southern tip of Florida and in the Keys, and were submitted the Center for medical treatment and rehabilitation. The birds whose livers were used in this study were either dead on arrival at the center, or died during treatment. Dr. Karyn Bischoff procured the livers and secured appropriate federal permits.

Experiments were carried out in May/June 2000 and November 2000 on the BioCAT undulator beamline. A third attempt to acquire data in March 2001 was unsuccessful because of unexpected monochromator problems. The silicon (111) double crystal monochromator was used with the x-ray mirror adjusted to reject harmonics. BioCAT’s multilayer array analyzer was used to reject scattered background photons from the
sample, and two 12.5 cm x 12.5 cm fast plastic scintillator/photomultiplier detectors were used to collect the x-rays transmitted by the analyzer.

Samples of liver tissue approximately 3mm by 15mm were dissected from cormorant livers and placed in Copper sample cells and frozen in a displex closed cycle helium refrigerator at the beamline. Experiments were also carried out at room temperature with little change in spectra. Interestingly, measurable radiation damage effects, which are a fairly serious problem for purified metalloproteins solutions, occurred much less rapidly in the liver samples.
Figure 1: BioCAT beamline, sector 18, Advanced Photon Source

Figure 2: BioCAT’s Multilayer analyzer
Results

The first experiment was carried out in May/June of 2000, with encouraging results. Although the average concentration in cormorant livers was very low, we found that the mercury content in the liver was very heterogeneous, with “hot spots” in the sample that had much larger mercury concentrations than the background level. The beam size was approximately 100 microns by 100 microns, and the hot spots were smaller in size than that. The sample thickness was approximately 2 mm. The apparent concentration then depended on the beam size and location on the sample.

Figures 3-5 show that analyzable data on the mercury edge were obtainable, although improved data quality would be needed to examine subtle details about coordination. Selenium edge data were also acquired, and although they were of lower quality, it is apparent that it is feasible to obtain data of sufficient quality for quantitative analysis, particularly if a microfocus beam were used.

The data were reduced to Fourier transform level by standard methods: averaging, background subtraction, and Fourier transforms, shown in figures 3-5. The prominent peak in Fig 5 indicates that the first coordination shell signal is clearly observed above the noise level. The apparent distance in the Fourier transform should not be taken at face value – it is shifted downward by phase shifts and interference effects.

The second coordination shell however is basically invisible in these data; it is highly disordered and not analyzable with the present data quality. The lack of prominent second shell confirms that the Hg is not in an ordered mineral phase, and more likely is in a disordered amorphous phase or mixture of phases, and partial cancellations between S and Se scattering may occur.

Selenium edge spectra were also successfully acquired, but their data quality was not sufficient to be of much use for quantitative analysis. It is clear however that there is no fundamental problem acquiring data on the selenium edge in tissues, particularly if alternative approaches described below are used.
Figure 3: Averaged Liver Tissue Spectra (first experiment May/June 2000). Two independent partial sums of 36 five minute scans, and their average, are shown.
K (Å⁻¹)

Figure 4: Background subtracted EXAFS (total sum and both partial sums)
Figure 5:

\( K^2 \) weighted Fourier Transforms (total sum and partial sums) of the data.
Subsequent measurements

Subsequent experiments were less successful. Despite attempts to locate hot spots in the liver tissue by scanning the beam over the sample, only low concentrations of mercury were found. However, comparison of the ratio of the fluorescence to the scattered background with a standard 0.01% Thimerosal solution measured under the experimental conditions indicates the average tissue concentration in the liver was approximately 20 micromolar. Extensive signal averaging was done (Figure 6) but the data quality was still insufficient to support very detailed analysis. Although the performance of the multilayer analyzer in this case was improved relative to the first experiment, the low concentration still resulted in too much background transmitted through the analyzer. Continuing improvements are being made to the analyzers however.

![Figure 6: Signal averaged Hg data from experiment Nov 2000. It is a sum of 65, 5-minute scans.](image-url)
Theoretical Calculations
The FEFF8 program was used for ab initio calculation of x-ray absorption edges of Hg for Cinnebar (HgS) and Hg tetrahedrally coordinated and linearly two-fold coordinated with S and Se. The relative lack of order in the higher shells as shown in the fourier transform, and the relative lack of high frequency fine structure in the near-edge region both indicate that the local average coordination in the tissue is not like that in the model minerals such as HgS. The theoretical edge spectra show the average coordination most resembles a low-coordinate Hg species.

Figure 7: FEFF8 theoretical calculations of Cinnebar and two-fold linear Hg-S site. The mineral has considerable high frequency structure in the near edge spectrum due to non-nearest neighbor atoms. Such structure is not observed in the tissues.
Figure 8: FEFF8 theoretical calculations of Cinnebar and tetrahedral Hg-S site. The mineral has considerable high frequency structure in the near edge spectrum due to non-nearest neighbor atoms. Such structure is not observed in the tissues.
Figure 7: FEFF8 theoretical EXAFS calculations of Cinnebar. The mineral has considerable high frequency structure in the EXAFS due to non-nearest neighbor atoms. Such structure is not observed in the tissues.
Utility of Singular Value Decomposition, and Principal Components Analysis

Programs were written and tested to implement each of these approaches, which rely on modeling the average coordination environment as a linear combination of the spectra of related model complexes. Two other approaches (nonlinear fitting, and linear programming with positivity constraints) were implemented as well.

Each of these approaches turned out to be irrelevant for this system however, because the amorphous nature of the immediate coordination environment that is apparent in the fourier transforms, and the near edge spectra indicates that the Hg site in the tissue cannot be modeled by a linear combination of the spectra of various solid state standard compounds such as HgS (Cinnebar). Fitting the data results in poor fits and unphysical (e.g. negative) coefficients.

However, if an appropriate alternative basis set of solutions can be constructed the approach may still have merit. Alternative analytical approaches seem more promising in this system, however. In particular, an alternative method we have recently developed seems very promising: direct reconstruction of radial distribution functions for C, S, and Se using a technique called Friedman Regularization (Khelashvili and Bunker, in preparation 2001). Quantitative modeling using theoretical FEFF8 calculations appears to be feasible, as suggested by our initial results, some of which are presented here.

Conclusions and future opportunities

The observations make perfect sense when considered in light of electron microscopic studies described in Nigro and Leonzio [Marco Nigro, Claudio Leonzio, Marine Ecology Progress Series, vol 135, p137-143, 1996 (Inter-research)]. These authors found that micron scale granules of Hg, Se, and S are formed in localized regions near the portal vein, but not elsewhere. The local concentration within these precipitates is high. Presumably these granules near the portal vein correspond to the hot spots observed in the first experiment.

This observation suggests two lines of future development. It is feasible and not difficult to focus undulator beams down to micron scale with lower but substantial flux, using synthetic structures called fresnel zone plates. BioCAT presently has such a device, which we intend to employ for microfocus XAFS experiments. Since the local
concentration in the tissue Hg/Se/S granules is high, it should be possible to more precisely characterize the composition of the granules. The granules could be located either by raster scanning the sample stage to maximize fluorescence, or offline by means of visible or electron microscopy.

To study the low concentration Hg that is distributed through the rest of the tissue will require better analyzer performance. Fortunately this is being pursued along two lines: higher performance multilayer analyzers are under development, and even more exciting, we have recently found theoretically that bent laue analyzers (Karanfil, Chapman, and Bunker 2001) of high performance and low cost can be constructed in the desired energy range. The predicted solid angle is excellent, and the reduced energy bandwidth will reduce the amount of elastic scatter. These calculations give us optimism that much lower concentrations are feasible than we have achieved to date.

Figure 8: X-ray Fresnel zone plates can focus APS undulator x-ray beams to micron size with bend-magnet type fluxes (10^11 photons/sec). The logarithmic spiral bent laue crystal analyzer offers better background rejection than present versions of the multilayer analyzer.