Analysis of Synchrotron X-ray based Absorption Data of Fe & Zn atoms in Consumer Product Tissue Samples

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Abstract—The iron and zinc environments in selected tissue samples have been studied with EXAFS at Brookhaven Synchrotron Light Source and the scientific process including data analysis has been used to show community college pre-engineering students about hand-on experience in student research projects. The EXAFS data collection was done using the Fe and Zn K-edges. The selected tissue samples were from consumer products which included yeast, vegetable and meat. The Zn bond length in fresh yeast samples was found to be about 25 pm shorter than reconstituted yeast samples, whereas about 10 pm difference was found in chicken breast versus the drumstick with shorter Zn bond length. The Fe bond length in chayote seed center seed outer layer samples was found to be about 8 pm shorter as compared to the chayote skin tissue samples. The extension to the development of functional synchrotron imaging for tissue engineering application based on spectroscopic technique is discussed.

Keywords— EXAFS; Zn metalloprotein; Fe metalloprotein; bond length; local environment

I. INTRODUCTION

Community college pre-engineering students need counseling on which career path such as electrical engineering, chemical engineering, environmental engineering, biomedical engineering, etc. Hands-on experience gained in doing a research project in a laboratory and presenting the results in conferences would enhance motivation and improve retention. Our community college, Queensborough Community College QCC, is about an hour drive from Brookhaven Synchrotron Light Source where its extended x-ray absorption fine structure EXAFS facility has been designed to include undergraduate student conducting research projects, after passing the various required safety protocols. Dedicated beam time allocation is controlled by those public and private institutions that pay directly to support the facility and free beam time allocation is usually done according to project ratings with minimal required cost for traveling and lodging that would fit comfortably within the research budget of a community college. Fourier transform between frequency and time as complimentary variables has been a standard topic in signal analysis courses where data in large frequency range would be needed to investigate small time scale information. An EXAFS scan usually would cover a range of scattered wave vector (wave vector \(k \sim 2\pi/\text{wavelength}\)) that corresponds to an energy range of about 800 to 1,000 eV beyond the absorption edge. In fact, since no bond length would be expected to be less than 150 pm in tissue samples, a range of a few hundred eV beyond the absorption edge could give bond length information in the final stage of Fourier transform but data up to 1,000 eV in the same scan would be needed for the initial stage of EXAFS data processing. Brookhaven National Lab maintains the synchrotron beam and the data analysis via the Fourier transform of the EXAFS data could be done on campus over a semester in duration. As Fourier transform is a fundamental analysis tool in engineering, this EXAFS student research project has been gaining acceptance by our pre-engineering students.

Zinc and iron are important in metalloproteins that are involved in biological pathways with zinc metalloprotein being also implicated in neuro pathways [1]. Synchrotron X-ray based absorption spectroscopic technique has been used to investigate the local environments of arsenic in an arsenic hyperaccumulator, Cretan brake [2]. It was reported that As-O has bond length values from 170 pm to 180 pm and As-S has bond length value of about 225 pm. Another report for Zn in tobacco roots also shows that Zn-O bond length values have more variation (196 to 207 pm) as compared to Zn-S in cysteine at 235 pm [3]. At the tissue type level, copper resistant and sensitive mature leaf samples was reported to exhibit an apparent peak shift of about 10 pm in the Fourier transform graph in a project that used Cu K-edge beam time of up to a month for the weak EXAFS signal [4]. In general it is not easy for EXAFS to delineate ZnO/N. However, zinc to histidine bonding is readily detectable as arising from the multiple scattering in the His ring such that the Zn-His bonding would show up as Fourier transform peaks at about 290 pm and 360 pm in stem tissue samples when compared to the negligible signals in leaf tissue samples [5]. Interestingly,
atmospheric aerosol containing iron has been identified as alpha-Fe₂O₃ due to the observed Fe-O bond length value at 195 pm in EXAFS data [6]. Shorter Fe-O bond length of 182 pm in a cytochrome P450 study using the Advanced Photon Source at Argonne National Lab, USA have been reported. [7]. They also listed the range of other reported Fe-O EXAFS bond lengths with a span from 164 to 193 pm, Fe-N EXAFS bond lengths with a span from 199 to 203 pm, Fe-S EXAFS bond lengths with a span from 221 to 248 pm in Fe metalloproteins. Recently the chemical form of Zn in the leaf tissue samples has been reported using Zn K-edge EXAFS spectroscopy at European Synchrotron Radiation Facility, France [8]. It was reported that Zn-O and Zn-C interatomic distances in the studied leaf samples span the range of 203 to 207 pm and 292 to 298 pm respectively.

Zn metalloprotein pathways are important in growth processes and Fe metalloprotein pathways are important in metabolism. The project aims to study the bond length variability of Zn and Fe in consumer product tissue samples for the understanding of the dynamic length scale in protein folding.

II. MATERIALS AND METHODS

The EXAFS data were collected at beam line X10C of National Synchrotron Light Source at Brookhaven national Laboratory BNL. Rhodium coated cylindrical mirror is used to focus the beam on the sample. A double crystal Si(220) monochromator was used for energy selection. Ion chambers were used to measure beam intensity before the hutch, IO (before sample), and transmission intensity. A 7-element Si drift detector was used to measure fluorescence intensity. Piezoelectric driver using A/C feedback system locks the beam. Beam size on the sample was 10 mm x 2 mm. Beamline and data collection was controlled by microvax II computer running on VMS. The tissue samples were obtained from consumer sources, the calibration samples (Zn and Fe foils, Fe₂O₃, zinc oxalate, and ZnS) used for calibration were purchased from Sigma Aldrich. The EXAFS data analysis for the extraction of the standard k-cubed weighted Fourier Transform was done with the new Windows based WIN-XAS software, and the traditional FDG software, as released by Dr. Marten L. denBoer. The FORTRAN based FDG software has several output stages so such that a student can track the signal normalization, background subtraction, EXAFS scattering data extraction in wave vector space, and Fourier transform for first peak identification, beyond 100 pm, as bond length value with their favorite tools such as MAPLES, Matlab, Visual Basic in Excel environment, etc., as learned from the STEM courses. The convenient WIN-XAS lacks education value in a community college setting but is essential for keeping students’ confidence that the traditional FORTRAN based software is still functional in WINDOWS environment. The details of ligand model fitting using electron scattering theory would not be suitable at the level of community college pre-engineering students. For the k-cubed weighted Fourier transform of EXAFS data, the bond length values of 195 pm in Fe₂O₃ [4] and 207 pm in zinc oxalate [3] have been used for phase shift calibration.

III. RESULTS OF DATA ANALYSIS

A set of raw EXAFS Zn-edge signals for fresh yeast and reconstituted yeast samples is shown in Figure 1. The k-cubed weighted Fourier Transform results are shown in Figure 2. The fresh yeast culture has smaller bond length value as compared to the reconstituted yeast culture by about 25 pm.

![Figure 1: Raw EXAFS Zn-edge signal (y-axis arbitrary unit) versus energy (y-axis eV) of fresh yeast sample (lower) and reconstituted yeast sample from dry powder (upper).](image1)

![Figure 2: The k-cubed weighted Fourier Transform of Figure-1 EXAFS Zn-edge data (y-axis with arbitrary unit) versus distance (x-axis with 100 pm unit). The left curve corresponds to fresh yeast sample and the right curve corresponds to reconstituted yeast sample from dry powder.](image2)

Similarly, the k-cubed weighted Fourier Transform results of a set of raw EXAFS Zn-edge signals for chicken breast and drumstick tissue samples are shown in Figure 3. The drumstick samples show shorter bond length value of about 10 pm shorter than the breast samples.
A set of raw EXAFS Fe-edge signals for chayote skin and seed is shown in Figure 4. The k-cubed weighted Fourier Transform results are shown in Figure 5. The seed tissue sample has shown smaller bond length value as compared to the skin by about 8 pm using the FDG software. The WINXAS software gave a larger separation of about 12 pm. Since FDG software has been used traditionally and that it would be better to be conservative in the shift calculation, the 8 pm separation result is shown in Figure 5. The slight discrepancy could be due to the weak signal from the chayote skin tissue. The His ring multiple scattering effect are also visible as peaks around 300 pm and 360 pm.

Figure 3: The k-cubed weighted Fourier Transform of EXAFS Zn-edge data (y-axis with arbitrary unit) versus distance (x-axis with 100 pm unit). The left curve corresponds to chicken drumstick tissue sample and the right curve corresponds to breast tissue sample from the same chicken.

Figure 4: Raw EXAFS Fe-edge signal (y-axis arbitrary unit) versus energy (y-axis eV) of chayote seed tissue sample (upper curve) and chayote skin tissue sample (lower curve).

Figure 5: The k-cubed weighted Fourier Transform of Figure-4 EXAFS Fe-edge data (y-axis with arbitrary unit) versus distance (x-axis with 100 pm unit). The left curve corresponds to chayote seed tissue sample and the right curve corresponds to chayote skin outer layer tissue sample from the same chayote.

IV. DISCUSSION

Besides providing hands-on experience for students, the EXAFS project also offers an opportunity for the students to appreciate the concept of optimization beyond numerical calculation with software packages. The Brookhaven beam time is a finite allocation in a specific duration and planning is crucial to the data collection efficiency. The beamline has been off-line in the allocated time slots and real time decision is necessary to optimize which samples are to be measured with Zn or Fe edge signal. Data recollection could be six or nine months away when a decision does not yield fruitful data. For a graduate student spending several years on his/her PhD program, such delay would be easily accommodated. However a community college student usually only would have one or two semesters left when he/she is ready to do research project, the optimization process carries an added level of difficulty as compared to the case of a graduate student.

A trend has emerged where the bond length values are different according to the functionality of the various tissue types. Fresh yeast usually is more potent than reconstituted yeast from dry powder and shorter bond length would correspond to a more tightly packed protein configuration in comparison. The EXAFS technique would be a feasible tool to probe less than optimum protein configuration in cell aging research concerning Zn pathways. The chicken drumstick tissue is able to support higher on-demand metabolism with shorter Zn bond length as compared to breast tissue. The Fe in the chayote seed is expected to include a contribution from cytochrome P450 and a short bond length value of about 186 pm deduced from our EXAFS data is consistent with the Reference 7 findings. It appears that EXAFS is an effective tool to explore tightly packed metalloprotein configuration for the understanding of Zn and Fe pathways in tissue comparison.
The next generation synchrotron technology with two to three order of magnitude brighter beamlines in Brookhaven around 2016 will open up the reality of functional synchrotron imaging. For example lactoferrin in ear wax (cerumen) and used in immunotherapies, etc would be favorable EXAFS samples for direct functionality probe [9, 10], or using EXAFS data as calibration for optical probe [11]. Our EXAFS work done in Brookhaven using the current synchrotron technology shows that ear wax sample of about 0.5-mm in size is sufficient for the Fe-edge signal detection. One of our earlier Photonics West Conference Proceedings report on Synchrotron based X-ray absorption of ear wax shows that Zn local environment would be quantifiable with correlation to optical diffusion parameters [12]. The ear wax Zn metalloprotein signal is expected to come from bacteria and skin cells, given that Zn metalloprotein had not been detected in the secreted antimicrobial proteins in ear wax so far. The Zn and Fe local environment information would be helpful to separate the bacterial iron contribution from ear wax antimicrobial lactoferrin in future EXAFS Fe-edge studies. The high throughput X-ray absorption data collection with Brookhaven X3B beamline on expressed microbial metalloproteins would be valuable as well [13]. Recent advances of NMR Spectroscopy application on blood samples of over 17,000 individuals revealed that citrate is among one of the 4 biomarkers for mortality in an All-Cause Mortality study [14]. The citrate as a chelator for zinc metalloprotein has been cited as an underlying explanation that increased citrate circulation could serve as an effective mortality biomarker. Synchrotron based X-ray absorption with bright source suitable for high volume scanning could be used to provide further evidence that Zn chelation abnormality is a key biomarker in future all-cause mortality studies. Furthermore, the proposed automated black-box approach for biological EXAFS data analysis would be most suitable for the development of functional synchrotron imaging for tissue engineering applications [15].

V. CONCLUSIONS

The project studied the EXAFS of tissue samples using Zn and Fe K-edges. Shorter bond length values were found for samples with tissues that are higher metabolism rates in comparison. Future studies could include the use of the EXAFS technique to track metalloprotein folding mechanism in engineered tissue samples.

ACKNOWLEDGMENT

We thank Brookhaven staff for their hospitality and Dr. Marten L. denBoer for making his FDG software available for our use. The project was partially supported by several CUNY PSC grants.

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