XAFS Analysis of Metalloproteins by the DV-Xα Method

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Extended X-ray absorption fine structure (EXAFS) method has been widely applied in metalloprotein structural studies and has the great advantage that non-crystalline sample such as solid and solution can be studied. Bio-related samples are typically "diluted-system" for EXAFS measurements and it is not easy to obtain good-quality data for EXAFS analysis. But some important developments of the EXAFS technique have taken place in recent year. Synchrotron radiation sources with advanced X-ray fluorescence detector technology, *i.e.* multi-element SSD detector, has enabled good-quality EXAFS data to be obtained from diluted-solution protein samples (>1mM). However, some metalloproteins cannot be concentrated to enough for EXAFS measurements. The examples contain membrane-proteins. On the other hand, X-ray absorption near edge structure (XANES) spectrum can be obtained clearly if the sample concentration of the absorbing element is "ultra-diluted" (~0.1mM; a few ppm). Since the spectrum contains electronic and structural information, developments of XANES analysis must be important for structural studies of "ultra-diluted" bio-related samples. In this study, we have performed XANES analysis by a comparison of experimental and theoretical spectra at the K-edge of cupper in the reduced form of azurin. The DV-Xα method was employed to calculate theoretical XANES spectra.

XANES spectra of 1mM azurin frozen-solution (77 K) were collected using 19-element multi-head SSD detector. Protein Data Bank data of azurin (1DZ0) was used for DV-Xα calculation. The model was shown in Figure (inset). The agreement of the theoretical results with experimental data is good enough. The work needs to be continued to develop this XANES analysis of metalloprotein by the DV-Xα method.

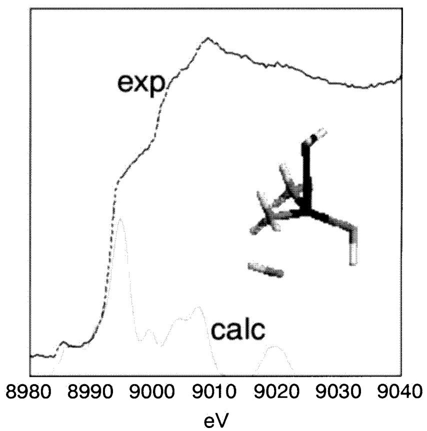

Figure Comparison of experimental and theoretical (DV-Xα method) Cu K-edge XANES spectra of azurin. (Inset : the model for calculation).

Continuous Scanning of the Monochromator using Y1 Axis Speed Feedback

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1. Introduction

SPring-8 standard x-ray monochromator is designed based on a double-crystal monochromator, basically controlled two stepper-motor, one for Bragg angle control (θ-axis), the other for first crystal translation control (y1-axis)[1]. Position of the y1-axis y_1 is a non-linear function of the Bragg angle θ_B,

$$y_1 = \frac{h}{2\sin\theta_B}, \qquad (1)$$

where h is height offset between incident beam and exit beam. To avoid the incident synchrotron radiation beam do not hit the first crystal, i.e., to maintain the y1 position within the some allowed deviation from the ideal position calculated from eq. (1), the control program divides the motion of θ_B into the small steps. Thus the motion of θ_B and y1 axis repeats the start and stop, and it makes rather longer time to change the Bragg angle. This also make it impossible to continuous Bragg angle scanning for XAFS experiments. This scheme was introduced at the beginning of the public opening of the SPring-8 beamlines because of the limitation of pulse motor controllers.

With newly installed VME pulse motor controllers which can change the pulse rate dynamically, it becomes possible to control the y_1 axis speed correspond to θ_B.

2. Off-line Tuning of the Feedback Parameters

A y1 speed control program is written based on the PID (Proportional, Integral and Differential) control. The control parameters were determined by off-line test on a beamline control test bench.

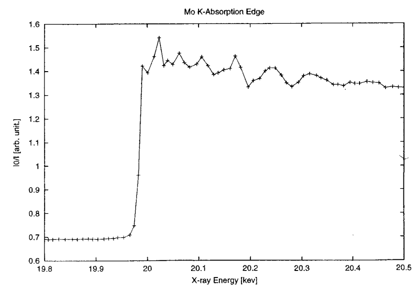
The θ-axis speed was fixed to 500 pps (i.e., 100 arcsec/sec), y1-axis feed back parameters were determined for $5 < \theta_B < 20$ degrees. For $\theta_B < 5$ degrees, the y1-axis speed higher than the mechanical limitation for the condition θ-axis speed = 500pps.

As a result of the off-line tuning, maximum deviation of the y1-axis from the ideal position calculated from eq. (1) is within 100μm, which is small enough to avoid the direct beam do not hit the first crystal.

3. On Beam Experiments

To compensate pitching and yawing of the first crystal motion along the y1-axis, a piezo transducer (PZT) feed forward was introduced together with the y1 axis speed feedback. Before the feedback control experiments, a PZT compensation table was prepared.

The feedback experiments were performed in the θ_B range between 8 degrees and 20 degrees with Si (311) reflection with rotation speed of the θ-axis 500pps and 300pps. A result of the continuous scanning XAFS at Mo absorption edge is displayed in the following figure for 300pps. During the continuous scanning, the x-ray intensity measurement intervals were about 350msec. This rate is not high enough to obtain the detailed spectrum near absorption edge. Improvement of measuring software is required.



[1] M. Yabashi, et. al., "SPring-8 standard x-ray monochromator", Proc. SPIE 3773, 19 July 1999, Denver, Colorado, U.S. (1999) 2.