



	<b>Experiment title:</b> The role of copper in cyclic tetrapeptides	<b>Experiment number:</b> CH-2576
<b>Beamline:</b> ID26	<b>Date of experiment:</b> from: 07-Feb-08 to: 10-Feb-08	<b>Date of report:</b> 29-Feb-08
<b>Shifts:</b> 9	<b>Local contact(s):</b> Sigrid G. Eeckhout	<i>Received at ESRF:</i>
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### Report:

We collected experimental XANES and EXAFS spectra of three peptides (cyclo(Lys-His-βAla-His), cyclo(Lys-DHis-βAla-His), cyclo(Gly-βAla-Gly-Lys)) at low temperature in order to study the oxidation state of copper, the average Cu-N distance, and to get information on the local geometry around copper in the studied peptides as a function of peptide composition.

To this aim, also a suite of well characterised copper model compounds with known oxidation state ( $\text{Cu}^+$ ,  $\text{Cu}^{2+}$  and  $\text{Cu}^{3+}$ ) and coordination geometries (linear, tetrahedral, square pyramidal, octahedral) so as to be able to parametrise pre-edge peak intensity and energy, edge energy, and other spectral features as a function of oxidation state. Moreover, theoretical XANES calculations are being performed on model compounds in order to test how confidently the experimental features can be reproduced. Then calculations will be performed also on the studied peptides in order to get information on the local geometry.

The model compounds studied show a consistent shift in the edge energy as a function of Cu oxidation state. Moreover, there also a shift of a peak in the edge region (labelled A in fig. 1). However, in order to quantify relative abundances of  $\text{Cu}^{2+}$  and  $\text{Cu}^{3+}$  we found that pre-edge peak (see inset of Fig. 1) is much more useful. In particular, monovalent Cu compounds do not display pre-edge peak (as expected for a  $d^{10}$  compound), whereas divalent Cu compounds display a well defined pre-edge peak consistently at 8978 eV (integrated area =0.01) and trivalent model compounds display a pre-edge peak shifted at higher energy (8979.3 eV) and with a higher integrated intensity (0.04). Despite the very low pre-edge peak intensity, the

very good signal to noise ratio of the collected spectra allow to discriminate perfectly the pre-edge peaks of the samples investigated.

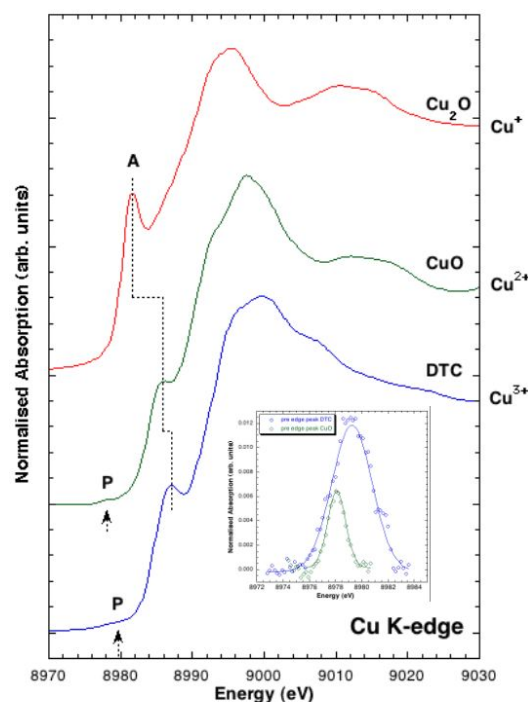


Fig. 1 Cu K-edge XANES spectra of selected Cu model compounds with different oxidation state. It is apparent the energy shift of the peak A, as well as the presence of a pre-edge peak for divalent and trivalent Cu model compounds (see inset). In particular, the pre-edge peak of trivalent Cu model compounds (DiTellurate Cuprate shown here) is shifted at higher energy with respect to divalent Cu and displays relatively higher integrated intensities.

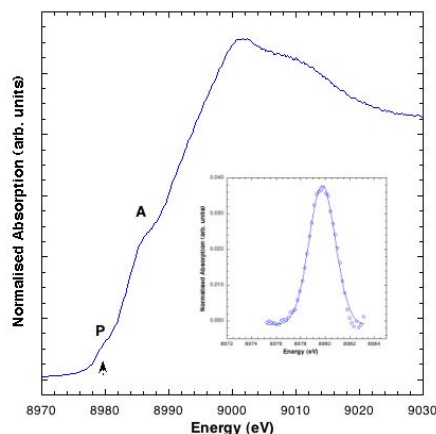


Fig. 2 Cu K-edge XANES spectrum of cyclo(Lys-DHis-βAla-His) peptide and detail of the background subtracted pre-edge peak P ( shown in the inset along with a fitted pseudo Voigt curve). Both the pre-edge peak position and integrated intensity, as well as the position of peak A, are consistent with the presence of  $\text{Cu}_{3+}$ .

The spectra of the cyclo-peptides have been collected in fluorescence mode by means of a Ge multidetector. The use of a He cryostat helped in preventing radiation-induced damage of the studied peptides. The spectra were acquired in quick EXAFS mode. For each samples, a preliminary check has been performed with a time scan to check how much time could the sample could be irradiated before observing a change in the edge position (e.g. possibly due to photoreduction). Spectra have been collected moving the beam to a new position 0.5 mm away from the previous position every about 1000 sec. total measuring time. As the sample did not show any form of alteration after even 1500 seconds, this procedure allowed to collect many tens of scans for each sample and to obtain an excellent signal to noise ratio both at the XANES and EXAFS regions of the spectra.

Two of the investigated cyclo-peptides (cyclo(Lys-His-βAla-His) and cyclo(Lys-DHis-βAla-His)) show pre-edge peak energy position and integrated intensity consistent with those of  $\text{Cu}^{3+}$  model compounds. Also edge energy is consistent with the presence of trivalent Copper. EXAFS spectra also show well definite oscillations well above noise level up to  $10 \text{ \AA}^{-1}$ . EXAFS data analysis is now in progress using the GNXAS program package in order to get average Cu-N distances.

Moreover, theoretical XANES spectra will be calculated for the studied peptides with the MXAN code in order to get information on the local geometry around Cu ions.