| ESRF | **Experiment title: Structure of the metal binding domain of the copper metalloprotein Atx1 and peptidic analogs in presence of Cu(I), Co(II), Cd(II) and Hg(II).** | **Experiment number**: 30-02/650 |
|---|---|---|
| **Beamline**: BM30B | **Date of experiment**: from: 04.21.04 to: 04.27.04 | **Date of report**: 09.01.04 |
| **Shifts: 9** | **Local contact(s)**: J-L Hazemann and O. Proux | *Received at ESRF:* |

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## Report:

The initial aim of this experiment was the study of the coordination of some heavy metal ions (namely Cu(I), Hg(II) and Cd(II)) by the yeast metallochaperone protein Atx1, and by Atx1-derived biomimetic peptides as described in the corresponding proposal. But, due to some technical problems at the beginning of the allocated beamtime, we could only study the interactions with the Cu(I) ion. The XANES and EXAFS signals were recorded at room temperature.

So far, we have focused our study on the complexation of Cu(I) by the Atx1 protein. Some preliminary results have shed light on the glutathione-assisted Cu(I)-Atx1 interaction. Atx1 is supposed to form a homodimer in presence of glutathione, each monomer complexed to a Cu(I) ion and interacting with a glutathione. The nature of the interaction (coordination number of the Cu(I) ions and geometry of the metal sites) remains uncertain and has to be characterized. Two models (figure 1) have been hypothesised to account for the formation of the $(CuAtx1\text{-}glutathione)_2$ dimer. In the first model each Cu(I) ion is 3-coordinated by 3 cysteine terminal thiolate, whereas in the second one, each Cu(I) is 4-coordinated by 2 cysteine thiolate groups (from the Atx1 proteins) and by 2 bridging thiolates (from the glutathione molecules).

Then we have used three model compounds corresponding to the three possible coordination of Cu(I) in this case: a linear 2-coordinate structure ($[Et_4N][Cu(SAd)_2]$, SAd: adamantane-1-thiolate), a planar trigonal structure ($[Et_4N]_2[Cu(SC_6H_4Cl)_3]$) and a distorted 4-coordinate one in a dicopper(I) cluster ($[Cu(SC_5H_5N)_3]_2[Cl]_2$).
As observed in figure 2, the model Cu(I) complexes exhibit a wide range in peak energies and intensities in the pre-edge region that can be correlated with the coordination number of the metal.

The dicopper(I) Atx1-glutathione homodimer exhibits a low-energy absorption peak at 8980 eV whose amplitude and feature suggest the presence of a single geometry for both copper(I) centres, and the Cu(I) ions are 3-coordinated.

The $k^3$-weighted experimental EXAFS spectrum of the $(CuAtx1\text{-glutathione})_2$ dimer and its Fourier transform (reported on figure 3) are compared with their fitted value computed from the $CuS_3$ model complex. The spectrum is dominated by the first shell contribution which is best simulated by three equivalent sulfur atoms at 2.24 Å (goodness-of-fit parameter $F = 3.9\ 10^{-2}$). This Cu-S distance is typical of a 3-coordinate Cu(I) ion, as evidenced by *ab initio* quantum mechanics calculations.

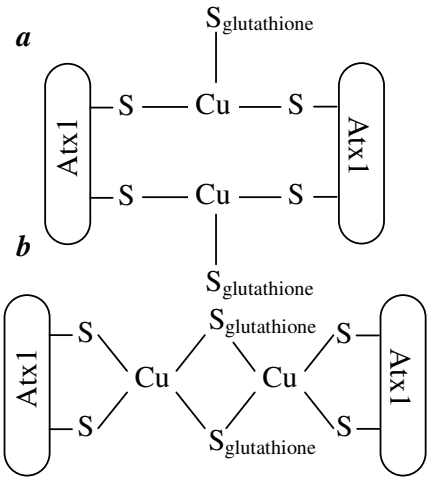


**Figure 1.** Models of the complexation of Cu(I) by Atx1 in solution in presence of glutathione. In model *a*, each Cu(I) ion is 3-coordinated whereas in model *b*, the ions are coordinated in a tetrahedral fashion.

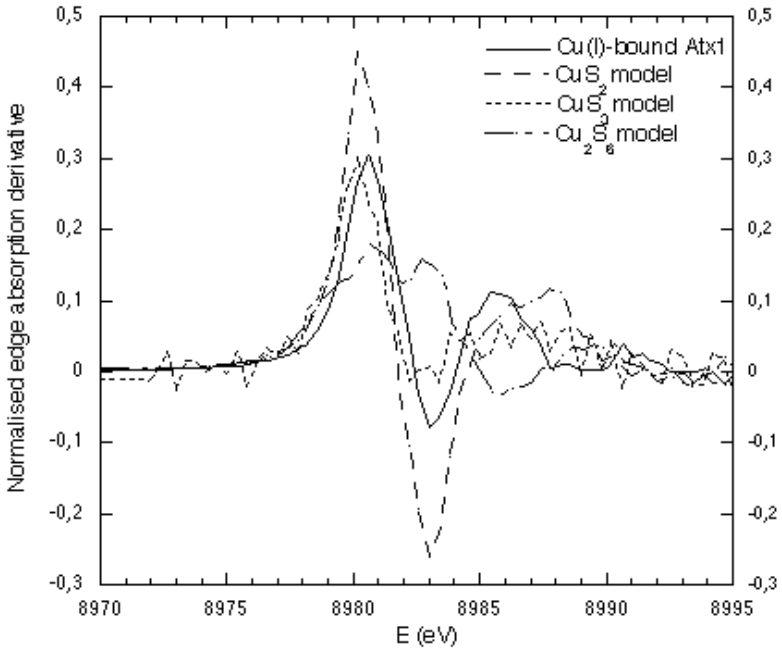


**Figure 2.** Normalised edge absorption derivative spectra of the Cu(I)-bound Atx1 in presence of glutathione and of the three Cu(I) model complexes of the metal site.

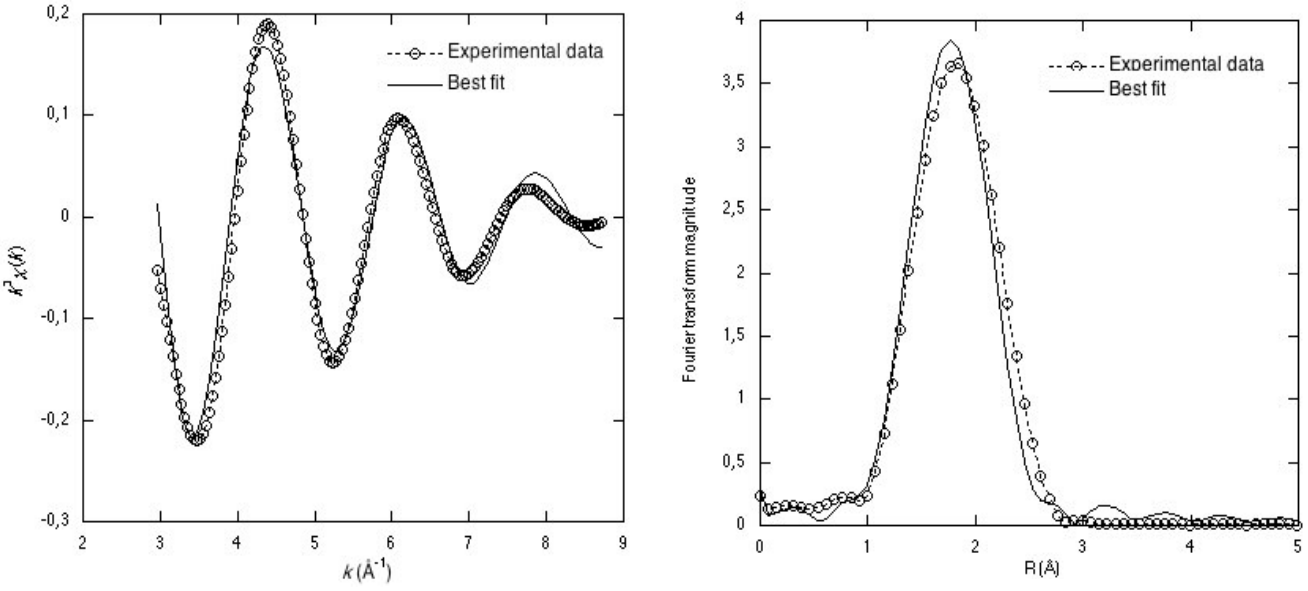


**Figure 3.** EXAFS spectrum (left) and Fourier transform (right) of the $(Cu(I)Atx1\text{-glutathione})_2$ dimer in solution. The best fit (with a 3-coordinated Cu(I) complex) and the experimental data are reported.