ESRF	**Experiment title:** Coordination of Copper(I) by cysteine-based biomimetic compounds which are potential drugs to treat metal overload	**Experiment number**: 30-02-1024
Beamline:	**Date of experiment**: from: 16/11/2011 to: 22/11/2011	**Date of report**: 07/02/2012
Shifts: 15	**Local contact(s)**: Denis Testemale	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Objective & expected results:

This proposal was aimed at proving the coordination of Cu in complexes of cysteine-containing chelators synthesized in our laboratory, which afford several sulfur donors. Indeed, we are designing efficient metal complexing agents to treat people suffering from copper overload, like in Wilson's disease.[1] These potential drugs mimic the high affinity of cysteine sulfur donors for Cu(I) evidenced in proteins trafficking or sequestering this metal in cells. The copper complexes have been fully characterized by analytical and spectroscopic methods to determine the thermodynamics and speciation of Cu species in water solution. *EXAFS can now provide a direct proof of the coordination mode of Cu in these cysteine-containing biomimetic compounds, which is critical for the understanding of the chelation properties but also for the design of optimized chelating agents.*

Results and the conclusions of the study:

The XAS spectra were collected on frozen solutions at copper K-edge and 5-15 K to avoid any structural transformations, especially those caused by beam damage. **We validated the preparation of the samples** analyzed by XAS, which showed no oxidation. We have now a well-established protocol: (1) preparation the complexes in a glovebox in our lab, (2) transfer to ESRF, (3) deposition of drops on the sample holder and immediate freeze in liquid nitrogen and (4) spectra acquisition at low temperature.

The first series of samples containing Cu(I) complexes with the sulfur-based ligands presented in the proposal were prepared with a Cu concentration of **3-6 mM**. This first analysis demonstrated the impact of the total concentration on the speciation of Cu(I) complexes. Indeed, it appeared clearly that the formation of polymetallic complexes was favored in comparison to the diluted samples which we previously studied with various spectroscopic tools.[2-4]

Therefore a second series of samples was prepared with a lower Cu(I) concentration, down to **0.6 mM**. Of course these samples required a longer acquisition time to get spectra with a good signal to noise ratio, and gave results in accordance with the speciation previously determined by other technics.

We focused mainly on the interpretation of the EXAFS data collected on the copper complexes with **pseudopeptides affording 3 sulfur donors**, to obtain the direct and final proof of copper coordination in these sulfur-only chemical environments which show the **larger affinities for Cu(I)**.

The preliminary analysis of these data demonstrates that:

- **Cu(I) is coordinated by three sulfur in a trigonal sulfur-only coordination mode CuS_3** in all the samples with tripodal ligands. The Cu-S distance is 2.25-2.27 Å in accordance with values found in similar complexes in the literature (Figure 1.).
- A change in the Cu environment is observed when the Cu/L ratio increases: the Cu coordination evolves from a mononuclear mode (pure CuS_3) to **polynuclear environments** characteristic of cluster-type complexes $(Cu_2S_3)_x$ evidenced by a strong Cu---Cu interaction at 2.70-2.73 Å (Figure 2.).

Conclusions. These data corroborate our previously published spectrocopic results about Cu(I) complexes with tripodal ligands. The EXAFS signatures are currently being fitted in collaboration with Denis Testemale (Fame) to try to have a better insight into these cluster-type complexes.

Eventually, these experiments allowed us to prove the trigonal sulfur-only coordination of Cu(I) by cysteine-based biomimetic compouds of potential interest for treating metal overload. We demonstrated that data can be aquired at low Cu concentration – less than 1 mM – and that **concentration was a critical parameter to study the speciation**. An important result is the proof that Cu(I) coordination is CuS_3 in all the samples with tripodal ligands. We will soon have a correct interpretation of the EXAFS spectra of the two main complexes – the monometallic complex CuS_3 and the cluster $(Cu_2S_3)_x$ cluster – formed in solution. We are now planning to analyze a series of samples with various Cu to ligand ratios to study and quantify the equilibrium between these two species. Besides, preliminary experiments on other tripodal ligands based on other sulfur donors gave very encouraging results.

Perspectives. Performing another set of experiments at ESRF (BM30) will allow us to go further into this study as regards to the following points:

- To improve the signal to noise ratio for diluted samples studied in November 2011 at ESRF.
- To shed light on the relationships between the Cu(I) concentration and the nuclearity of the complexes formed, which calls for multiplying the concentration-dependent experiments.
- To study other sulfur derivatives-based scaffolds which have shown interesting Cu(I) complexation properties, detected by NMR and UV-spectroscopy.

Figure 1. $Cu_{0.25}L1$- planar trigonal model- CuS_3 coordination ([L] = 2.7 mM- [Cu] = 0.68 mM).

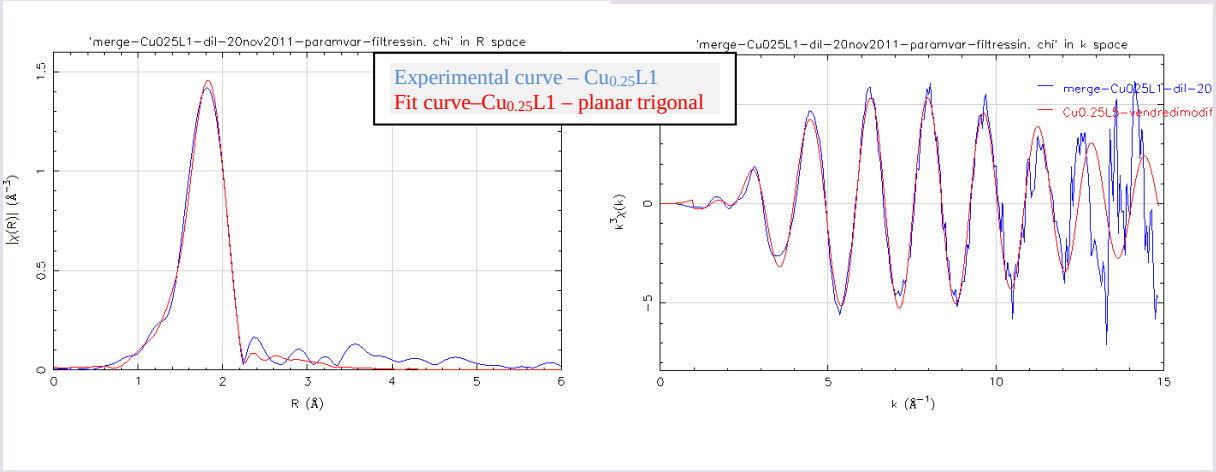
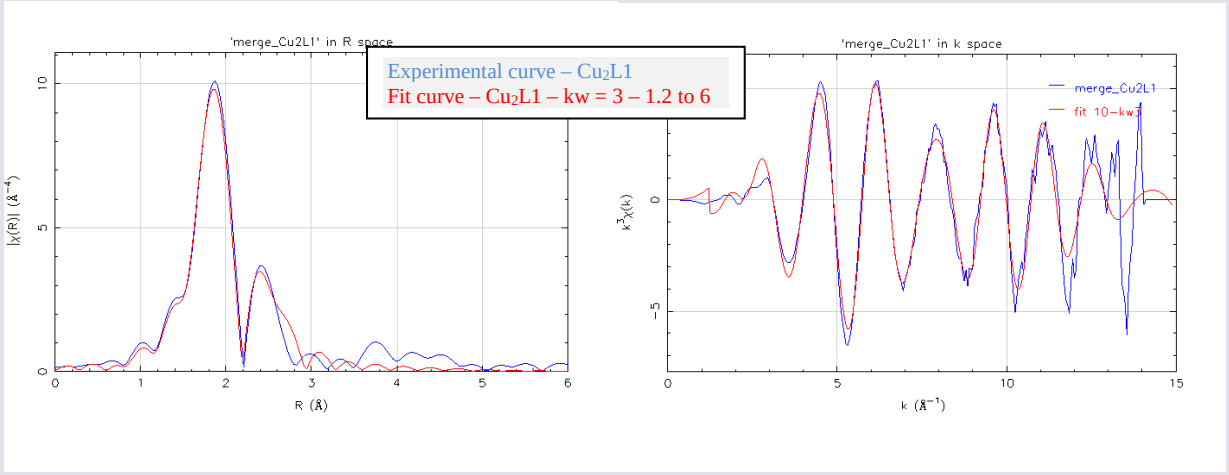


Figure 2. Cu_2L1- planar trigonal model- 1Cu Z- CuS_3 coordination with Cu/Cu interactions ([L] = 3 mM- [Cu] = 6 mM).



Justification and comments about the use of beam time (5 lines max.):
This beam time allocation afforded many results. First, it allowed us to adjust the experimental conditions to perform the XAS acquisitions, i.e. the preparation of the samples and the concentration used. Second, we were able to prove Cu coordination in the complexes with the very high affinity tripodal chelator, which is always trigonal planar CuS_3 either in mononuclear or cluster-type species. These results are of great interest for the design of even more efficient chelating agents.

Publication(s):

(1) Delangle, P.; Mintz, E. *Dalton Trans.* **2012**, DOI:10.1039/C2DT12188C.
(2) Pujol, A. M.; Gateau, C.; Lebrun, C.; Delangle, P. *Chem. Eur. J.* **2011**, *17*, 4418-4428.
(3) Pujol, A. M.; Gateau, C.; Lebrun, C.; Delangle, P. *J. Am. Chem. Soc.* **2009**, *131*, 6928-6929.
(4) Pujol, A. M.; Cuillel, M.; Renaudet, O.; Lebrun, C.; Charbonnier, P.; Cassio, D.; Gateau, C.; Dumy, P.; Mintz, E.; Delangle, P. *J. Am. Chem. Soc.* **2011**, *133*, 286-296.