ESRF	**Experiment title:** Zinc binding effect on Copper coordination mode of synthetic peptides mimicking Prion Protein sequence.	**Experiment number**: **SC- 2747**
Beamline: BM30	**Date of experiment**: from: 1.3.2009 to: 10.3.2009	**Date of report**: 24.2.2010
Shifts: 18	**Local contact(s)**: Jean-louis Hazemann Olivier Proux	*Received at ESRF:*

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Report:

XAS data have been acquired on various samples of PrP-tetra-octarepeat peptides in the presence of both Cu^{2+} and Zn^{2+} ions.

In a previous work (Morante et al., 2004) XAS spectroscopy has been used to explore the Cu^{2+} site geometry in PrP peptides containing one, two and four-octarepeat and in the whole recombinant form of bovine PrP (BoPrP). In the paper a model of Cu^{2+} coordination mode has been proposed.which depends on the [Cu]: [octarepeat] concentration. The model has been found to be in nice agreement with the EPR results from (Chattopadhyay et al., 2005) showing that Cu^{2+} can be found in three different coordination modes thus indicating the presence of three types of "components" whose relative content depends on the [Cu]: [octarepeat] concentration ratio. More recently, new EPR measurements carried out by the same group (Walter et al., JACS 2007) have shown that the presence of Zn^{2+} modulates the Cu^{2+} binding mode to the synthetic PrP tetra-octarepeat peptide. In particular it has been suggested that, even if Zn^{2+} is not able to displace Cu^{2+}, increased Zn^{2+} concentration can progressively change the way Cu^{2+} is bound to the tetra-octarepeat. At the same time, it is observed that the Cu^{2+} titration curve is different according to whether Zn^{2+} is present or not.

In the present study we exploit the possibility offered by XAS spectroscopy of directly looking at the structure around the two metal ions when they are simultaneously present in the same sample (it should be recalled that Zn^{2+} is EPR silent). We acquired XAS data on Cu and Zn-prion protein complexes at both metals edges at the beamlime BM30B of the ESRF Grenoble.

Starting from a 0.2 mM tetra-octarepeat solution, three samples containing Cu^{2+} at 0.16 mM, 0.4 mM and 0.6 mM concentrations have been prepared (samples S_1, S_2 and S_3 respectively). To an aliquot of each one of these samples 0.6 mM Zn^{2+} is added (samples S_1_Zn, S_2_Zn and S_3_Zn respectively).

Qualitative data analysis:

To gain a first understanding of the structural properties of the metal (Zn^{2+} and Cu^{2+}) coordination modes, similarities and differences among the many measured spectra are examined.

In Fig.1 the comparison of the XANES spectrum (left panel) of Cu^{2+} in buffer with the XANES spectra of samples at different Cu^{2+} concentrations in the absence of Zn^{2+} is shown. It is quite evident, mainly looking at the shape of the white line that, at all Cu^{2+} concentrations we have considered, the spectrum of Cu^{2+} in buffer is rather different from that of Cu^{2+} bound to the peptide.

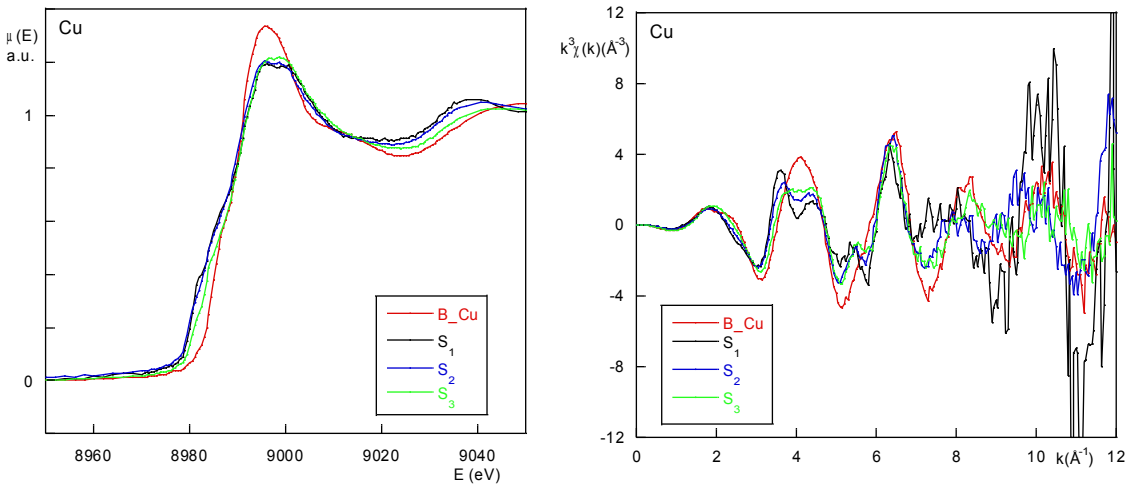

Figure 1

In the right panel of Fig.1 the same comparison is presented in the EXAFS region. The Cu^{2+} buffer spectrum is again definitely different from that of all the other samples, but now we can also notice that S_2 and S_3 spectra are very similar between themselves, but significantly different from S_1 spectrum. This qualitative observation is in agreement with EPR results. In fact, at low Cu^{2+} concentration (sample S_1) only component C3 is present, the fraction of which decreases with increasing Cu^{2+} concentration. Indeed, the fraction of component C3 is already very low in sample S_2 and completely disappears in sample S_3.

Analogously, the spectra registered at the Zn K-edge at different Cu concentrations are compared with the buffer (B_Zn) spectrum (Fig.2).

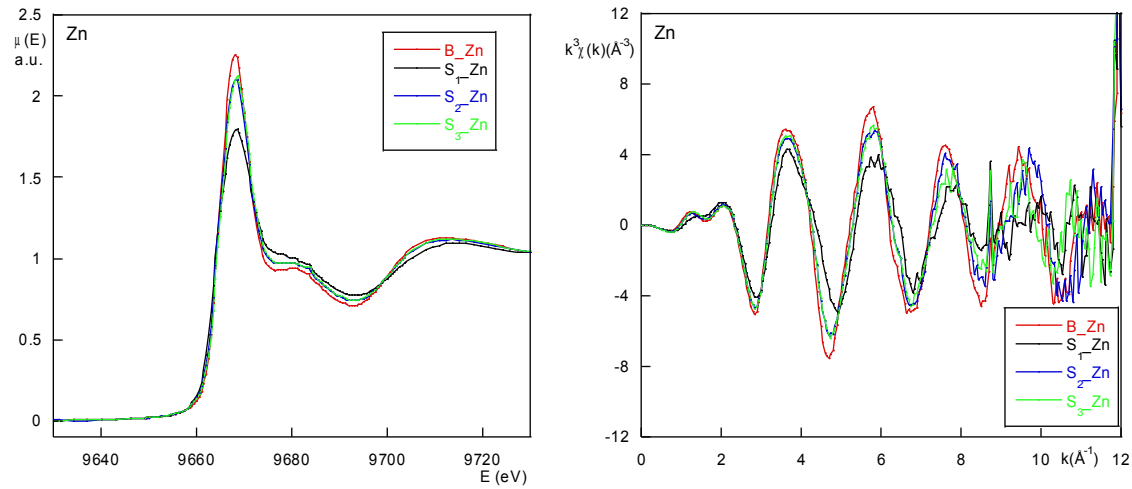

Figure 2

The left panel of Fig.2 shows that the XANES spectrum of S_1_Zn (which is the sample with the lowest Cu^{2+} concentration) is significantly different from that of Zn^{2+} in buffer, while spectra of the samples where the Cu^{2+} concentration is higher (S_2_Zn and S_3_Zn) are almost identical between themselves and very similar to the spectrum of Zn^{2+} in buffer. In other words, the local Zn^{2+} environment in the S_1_Zn sample is significantly different from that of Zn^{2+} in solution, thus proving that at least a fraction of Zn^{2+} is bound to the peptide.

Quantitative data analysis:

Taking into account the qualitative observations reported above, we proceed to a more quantitative analysis by making the assumption that the Zn^{2+} is present in our samples in two different structural configurations: one corresponding to Zn^{2+} in solution and a second one corresponding to Zn^{2+} in complex. In this second case we made the further hypothesis that Zn^{2+} can be bound to a different number of His residues. For the analyss we chose the S_1_Zn spectrum where, as shown by EPR data (Walter et al., JACS 2007) and confirmed by our qualitative XAS analysis, it is the one with the lowest amount of Zn^{2+} in solution.

We used B_Zn and we found that the best fit of the EXAFS data is obtained by assuming that Zn is coordinated to 6 oxygen atoms located at a distance of 2.07 Å in an octahedral geometry.

For the S_1_Zn EXAFS spectrum four different fits (Fig. 3) have been performed, each with a different assumed number of His residues bound to Zn^{2+}.

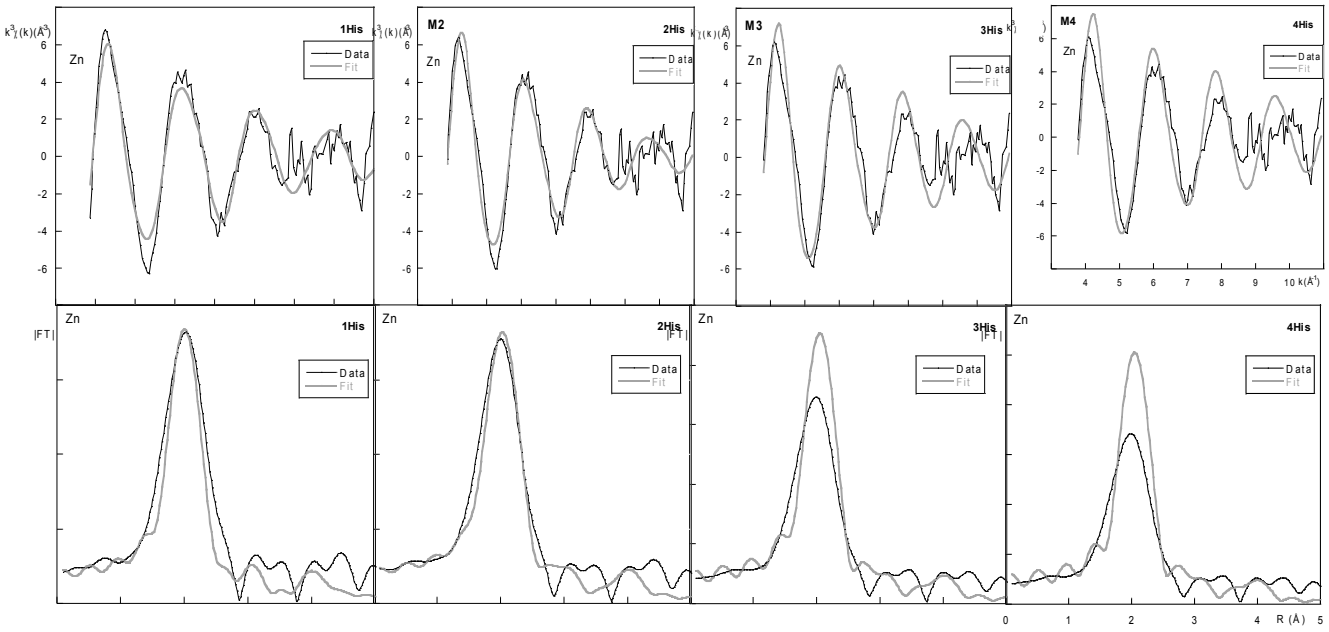

Figure 3

It is rather clear from Fig.3 that, by increasing the number of assumed bound His residues, the quality of the fit significantly decreases. Indeed the fit is found to be reasonable only in the case of 1 or 2 bound His residues.

In conclusion, even within the crude approximation we made in building our structural models, we find sufficient qualitative and quantitative reasons to discard for the Zn^{2+} ions, in the presence of 0.16 mM Cu, the binding modes involving 3 or 4 His residues, although we have not enough "resolution" to distinguish between the model with 1 or 2 bound His.

The important point is that in the experiments we performed within this proposal all the samples in the presence of Zn^{2+} have been obtained by adding Zn^{2+} to samples where Cu^{2+} was already present. Given the higher affinity of Cu^{2+} with respect to Zn^{2+}, we are almost sure that the Zn^{2+} could not replace Cu^{2+} in peptide binding. However, from the biological point of view, given the high natural concentration of Zn^{2+}, it would be interesting to look at the Zn^{2+} binding mode in the absence of Cu^{2+} and how it can be affected by Cu^{2+} presence. To this purpose we are submitting a continuation of the proposal.

References

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