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Application for beam time at ESRF – Experimental Report for CH-3015

X-ray Absorption Spectroscopy studies of the interaction of copper and zinc ions with peptides relevant for Alzheimer's disease.

Scope of the project: The global goal of the project is to get structural insight into the binding site of the essentiel metal ions (Cu, Zn and Fe) to the peptide amyloid-β. This metal-peptide interaction is supposed to be directly linked to Alzheimer's disease and hence of fundamental and applicative interests. The initial specific aim of the project was to make use of XAS (XANES and EXAFS) to get an insight into the coordination of Fe^{2+} to the amyloid-β (Aβ) peptide involved in Alzheimer disease (AD). However, obtaining such very reactive reduced complexes (Fe^{2+}-Aβ) was made impossible without the use of a gloves box (not available for this project). As a consequence, we performed **extensive studies of Cu^{2+} and Zn^{2+} coordination to Aβ and to a series of relevant mutants**. We also obtained **very promising first results concerning the simultaneous binding of Cu^{+} and Zn^{2+} to Aβ**. During the 18-shifts session, we recorded about thirty useful spectra: XANES and EXAFS spectra for Zn^{2+}-Aβ and only XANES spectra for the Cu^{2+}-Aβ complexes due to partial photo-reduction of the Cu^{2+} samples during the experiment, in spite of the use of the He-cryostat. These results will help to better describe the metal centre environment when bound to the Aβ peptide, which is still an open debate (especially for Zn^{2+}) and which is an important issue regarding the importance of metal ions in the development of AD.

Results: The results obtained on Cu^{2+} and Zn^{2+} coordination to Aβ and to a series of relevant mutants are exemplified in Figure 1 where the XANES spectra of Cu^{2+} and the XANES and EXAFS spectra of Zn^{2+} bound to the human Aβ (h-Ab, black lines) and to one among the ten Aβ mutants studied (Ab-mutant 1, red lines) are shown. In Figure 1, are also compared the XANES and EXAFS spectra of Zn^{2+} bound to the human Aβ in absence (black lines) and in presence of one equivalent of Cu^{+} (h-Ab + Cu^{+}, blue lines).

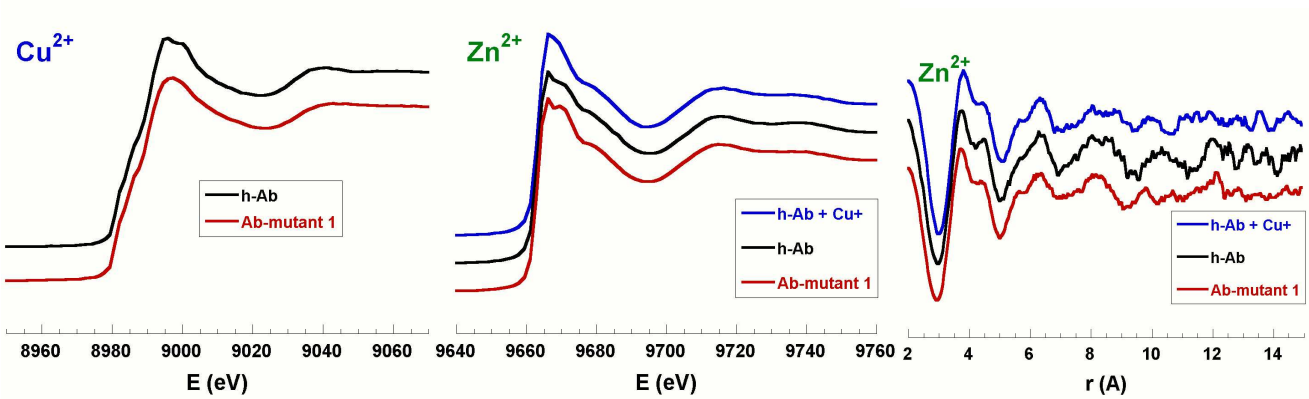

Figure 1. XANES spectra of Cu^{2+} (left panel) and the XANES (middle panel) and EXAFS (right panel) spectra of Zn^{2+} bound to the human Aβ (h-Ab, black lines) and to mutant 1 (Ab-mutant 1, red lines) and in presence of one equivalent of Cu^{+} (h-Ab + Cu^{+}, blue lines).

In the examples chosen in Figure 1, for both metals there are significant differences between the spectroscopic signatures of the metal bound to h-Aβ and mutant 1. By studying a series of mutants, it is then possible to discriminate which are the amino-acids residues involved in the metal center environment. Thus, we have identified several key residues for the coordination of Cu^{2+} and Zn^{2+} to h-Aβ. More structural data will be obtained for the Zn^{2+} species, for which the EXAFS data are fully analyzable. These studies should bring some insights into the structural modification encountered in the mutants compared to

the h-Aβ. It is also clear that the presence of Cu^{+} significantly modifies the XAS signature of Zn^{2+} bond to h-Aβ, thus indicating i) the possibility of simultaneous binding of the two metal centers to Aβ and ii) their inter-dependence.

Experimental details: Cu and Zn K-edge XAS spectra were recorded on the BM30B beamline during a 18-shifts session in Feb. 2010. The measurements were performed on ~mM solution or aggregated samples at low temperature (He-cryostat) in the fluorescence mode using a 30-element high-purity Ge detector. The energy was calibrated by the measurement of Cu and Zn foils spectra in transmission mode. For each sample, two to five spectra were recorded with varying the postion of the beam. Possible X-ray photo-reduction of the Cu^{2+}(Aβ) samples was evidenced by checking, on two consecutive scans, the appearance of the XANES feature at 8984 eV which is typical of Cu^{+} species. After 1 scan of 40 minutes, the feature is not negligible (we estimated that Cu^{+} was about 30% after one scan), indicating that the EXAFS data can not be correctly analyzed. However, the XANES spectra are usable since in this region less than 10% of Cu^{2+} are photoreduced.

Publications: On the basis of data obtained during this session, we expect to publish at least three papers: two in which XANES results on Cu^{2+} coordination to Aβ will be integrated in more general studies including other spectroscopic techniques (EPR, NMR, CD…) and at least one, in which XAS results (XANES and EXAFS data on Zn^{2+} coordination to Aβ) will be the principal topic of the paper.