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Application for beam time at ESRF – Experimental Report for CH-3245

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

Scope of the project: The aim of the project was to make use of XAS (XANES and EXAFS) to gain insights into the simultaneous coordination of Zn(II) / Cu(II) or Zn(II) / Cu(I) to the amyloid-β (Aβ) peptide involved in Alzheimer disease (AD). So far, no studies are reported dealing with the simultaneous Zn(II) and Cu(I) or Cu(II) binding to Aβ. Due to the possibility to detect simultaneously Zn and Cu K-edges and because Cu(I) and Zn(II) are silent in most of other spectroscopies, XAS is the method of choice for such studies.

Results:

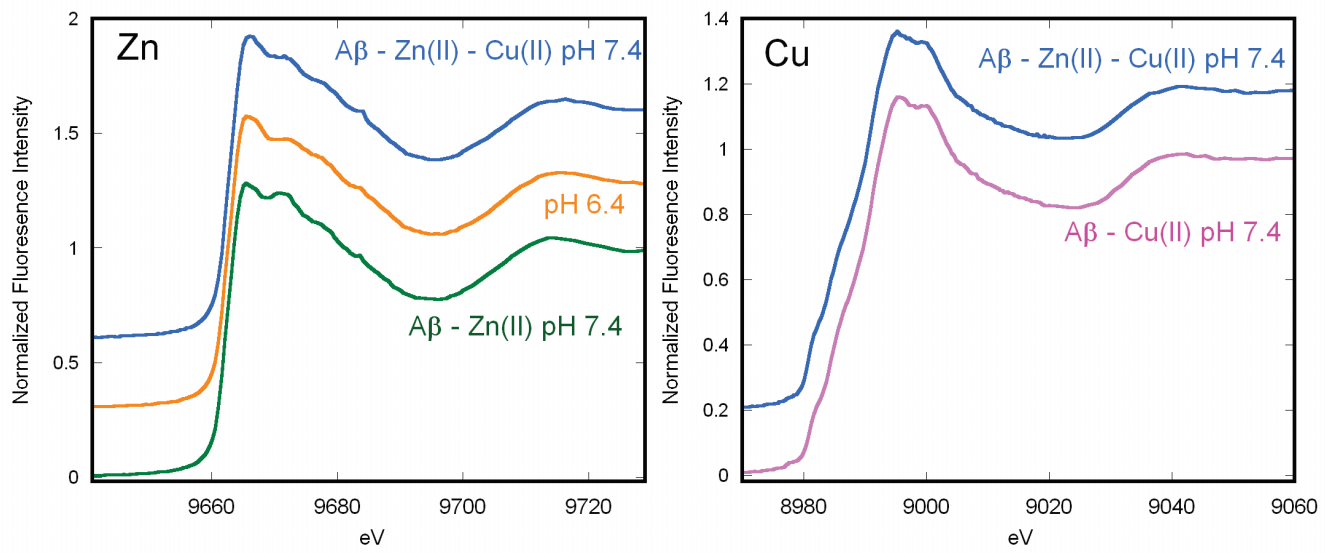

Figure 1. XANES spectra at Zn K-edge (left panel) and at Cu K-edge (right panel) of Aβ in presence of Zn(II) at several pH (green and orange left panel), in presence of Cu(II) (pink, right panel) and with simultaneous presence of Zn(II) and Cu(II) (blue, left and right panels).

In the Figure 1 are shown the Zn K-edge (left) and Cu K-edge (right) spectra of the Aβ-Zn(II)-Cu(II) sample (blue) at pH 7.4. They are compared to the XANES spectra of Aβ-Zn(II) at pHs 6.4 (orange) and 7.4 (green) and of Aβ-Cu(II) (pink) at pH 7.4. No difference is observed between the XANES spectra of Aβ-Cu(II) (pink) and Aβ-Zn(II)-Cu(II) (blue), meaning that Cu(II) coordination does not change when Zn(II) is bound to Aβ. On the contrary, XANES spectra of Aβ-Zn(II) (green) and Aβ-Zn(II)-Cu(II) (blue) are clearly different, suggesting that the Zn(II) coordination sphere evolves in presence of Cu(II) ion. It is also worth noting that Zn(II) remains bound to the Aβ peptide, since the XANES signature of Zn(II) in the buffer is clearly different to the XANES trace observed here. To precise this modification, the XANES spectra of Aβ-Zn(II) at pH 6.4 was recorded and the signature obtained was close to that of Aβ-Zn(II)-Cu(II) species at pH 7.4 (blue). This strongly suggest that at pH 7.4 in presence of Cu(II), the Zn(II) environment is shifted towards that observed at lower pH in absence of Cu(II).

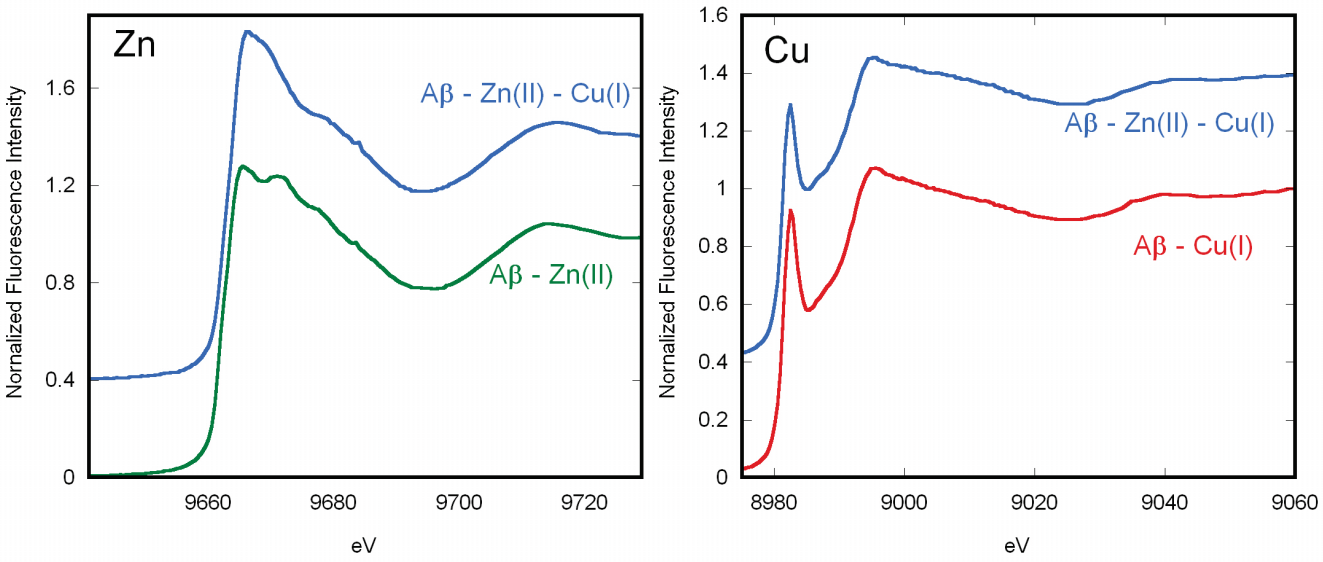

Figure 2. XANES spectra at Zn K-edge (left panel) and Cu K-edge (right panel) of Aβ in presence of Zn(II) (green) or Cu(I) (red) or with simultaneous presence of Zn(II) and Cu(I) (blue)

In the Figure 1 are shown the Zn K-edge (left) and Cu K-edge (right) spectra of the Aβ-Zn(II)-Cu(I) sample (blue) at pH 7.4. Whereas Zn(II) coordination to Aβ is affected by the presence of Cu(I) (left panel), Cu(I) coordination to Aβ in presence of Zn(II) remains the same (right panel). This very interesting result could be explained by the displacement of Zn(II) from its initial binding site by concomitant coordination of the Cu(I) ion.

It is worth noting that the XANES spectrum at Zn K-edge of Aβ–Zn(II)–Cu(I) (Fig.2 left panel) is different from that of Aβ-Zn(II)–Cu(II) (Fig.1 left panel), meaning that binding site of Zn(II) is not the same when Zn(II) is displaced by Cu(I) or by Cu(II) ions. This may result from the amino-acid residues involved in Cu(II) and Cu(I) coordination. By using other techniques (RPE and NMR), we previously demonstrated the square planar Cu(II) coordination to Aβ with the implication of N-terminal, O from amide of Asp1, His6 and His13/His14 in equilibrium. In case of Cu(I), coordinated in linear fashion, main ligands are His13 and His14. As a consequence, Zn(II) has to adjust its binding site depending of which ligands remain available for metal coordination.

The results obtained in this XAS session are very promising, and show the ability of both Cu(II) and Cu(I) to displace Zn(II) from its initial binding site. They also prove the simultaneous binding of couple Zn(II)/Cu(I) and Zn(II)/Cu(II) to Aβ.

Note that corresponding EXAFS data have been recorded and are currently under analysis.

Experimental details: XAS spectra were recorded on the FAME beamline during a 18-shifts session in september 2011. Air sensitive samples were prepared and introduced in degazed sample-holder before freezing them in liquid nitrogen. Zn K-edge and Cu K-edge XANES spectra have been recorded on the same sample. The measurements were performed on ~mM ions metal contaning solution, at low temperature ~25°K (He-cryostat) in the fluorescence detection mode using a 30-element high-purity Ge detector. The energy was calibrated by the simultaneous measurement of a Zn foil spectrum in transmission detection mode.

Publication: On the basis of the data we obtained during this session, we expect to publish one paper where the XAS results (XANES and EXAFS on Zn(II) coordination to Aβ in presence of either Cu(I) or Cu(II) ion) will be the principal topic.