EUROPEAN SYNCHROTRON RADIATION FACILITY
ESRF User Office
Experimental Report for 20151029

Interference of Zinc in Copper removal from the amyloid-β peptide, a key feature in Cu chelation-based therapies against Alzheimer's disease?

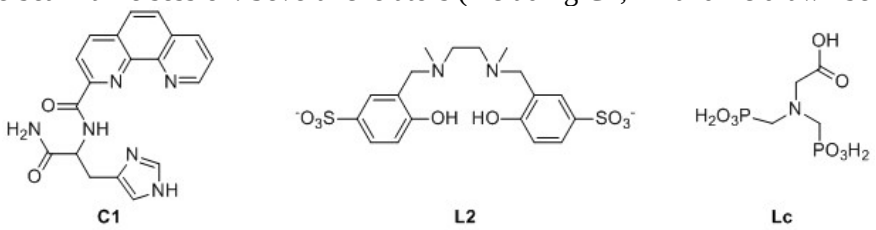
Scope of the project:

Alzheimer's disease (AD) is the most common neurodegenerative disorder. While fibrils of the amyloid-β (Aβ) peptide are found in the senile plaques in AD's brains, the monomereic soluble form of the peptide is found in healthy brains. This places the aggregation of Aβ at the core of the pathologic process. Metallic ions such as Cu(I/II) and Zn(II) can alter the Aβ aggregation: Cu(II) seems to stabilize toxic low molecular weight oligomers while Zn(II) stabilizes fibrils considered to be less toxic. In addition, Cu(I/II) (in contrast to Zn(II)) ions are redox active and can produce ROS (Reactive Oxygen Species). Therefore Cu(II) can be considered as "the" therapeutic target in the context of AD. Thus a possible therapeutic approach is to remove Cu(II) from Aβ with a chelator (noted L). L has to full-fill several conditions, including: i) the Cu(L) complex should not produce ROS on its own, ii) Zn(II) which is present in the synaptic cleft in high concentration compared to Cu(II) should not prevent Cu removal by the chelator. In other words the following equation has to be full-filled:

$$\mathbf{Cu(A\beta) + Zn(L) \rightarrow \quad Cu(L) + Zn(A\beta)} \ (1)$$

This corresponds to a Cu(II) chelator with a Cu(II) over Zn(II) selectivity (corresponding to the ratio between the affinity for Cu(II) and the affinity for Zn(II) for a given chelator) higher than the Cu(II) over Zn(II) selectivity of Aβ that is quite high (4 orders of magnitude at pH 7.1).

During the experiment 20151029, we took advantage of XANES to consider reaction 1. Indeed, the Zn(II) ion is silent in most of the spectroscopic techniques and Cu and Zn K-edges are close enough to be recorded during the same beam-time session. Several chelators (including C1, L2 and Lc drawn below) were tested.



Results:

A) C1 is chelator from O. Iranzo's group and L2 is a chelator synthetized in our team. We have monitored the swap of metallic ions between Cu(Aβ) and Zn(L) with these two ligands (Figures 1 and 2, respectively). The Cu and Zn K-edges data evidence that both C1 and L2 can remove Cu(II) from Aβ, even in presence of Zn(II).

Regarding the L2 ligand, the spectra indicate that Cu(II) removal from Aβ is total even in presence of Zn(II) in line with the known value of the Cu over Zn selectivity for this ligand (8 orders of magnitude).

Regarding the C1 ligand, for which the Cu over Zn selectivity is not known, analysis of the spectra indicates that all Cu(II) is removed from Aβ in absence of Zn(II) while 10% remains bound to Aβ in presence of Zn(II).

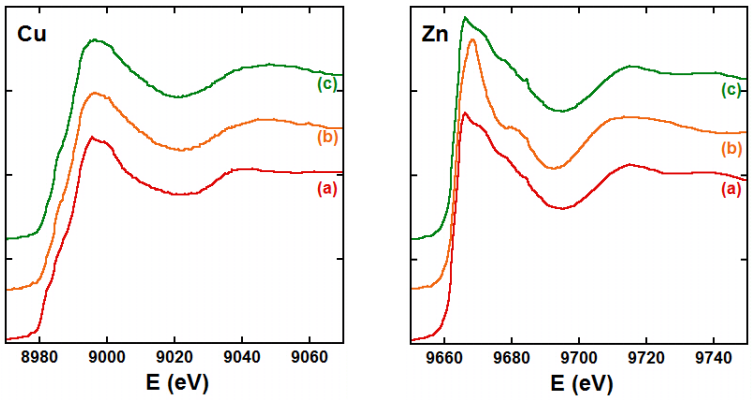


Figure 1. Cu and Zn K-edges spectra of (a) Cu(Aβ) or Zn(Aβ), (b) Cu(L2) or Zn(L2) and (c) Cu(Aβ) + Zn(L2). HEPES buffer 100 mM pH 7.1, [Cu] = [Aβ16] = [L2] = [L2-Zn] = 1 mM. 10 % glycerol are used as a cryoprotectant. *T* = 20 K.

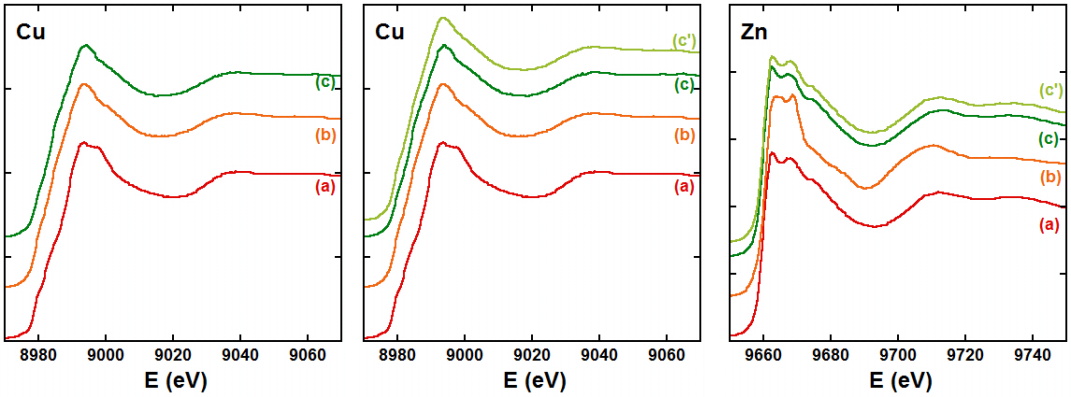


Figure 2. Cu and Zn K-edges spectra of (a) Cu(Aβ) or Zn(Aβ), (b) Cu(C1) or Zn(C1), (c) Cu(Aβ) + C1 (left panel) or Cu(Aβ) + Zn(C1) (middle and right panels) and (c') convolution of 10% of spectra (a) and 90% of spectra (b) to reproduce spectra (c). HEPES buffer 100 mM pH 7.1, [Cu] = [Aβ28] = [L2] = [L2-Zn] = 1 mM. 10 % glycerol are used as a cryoprotectant. *T* = 20 K.

B) For the third commercial chelator (Lc) the results are more complicated. Indeed, there is formation of a ternary complex, between Aβ, Lc and Cu(II) or Zn(II) (Figure 3, left and middle panels), where imidazole groups from the peptide complete the coordination sphere of the metallic centres. The Cu K-edges signatures of the different samples are not helpful because of their high similarity (Figure 3, left panel). In contrast, analysis of the Zn K-edge features reveals that 60% of Cu(II) remains bound to Aβ in presence of Zn(II) (Figure 3, right panel) while EPR shows that Lc is able to remove all Cu(II) from Aβ in absence of Zn(II) (data not shown). This is in line with the weak Cu over Zn selectivity of the Lc ligand (2 orders of magnitude).

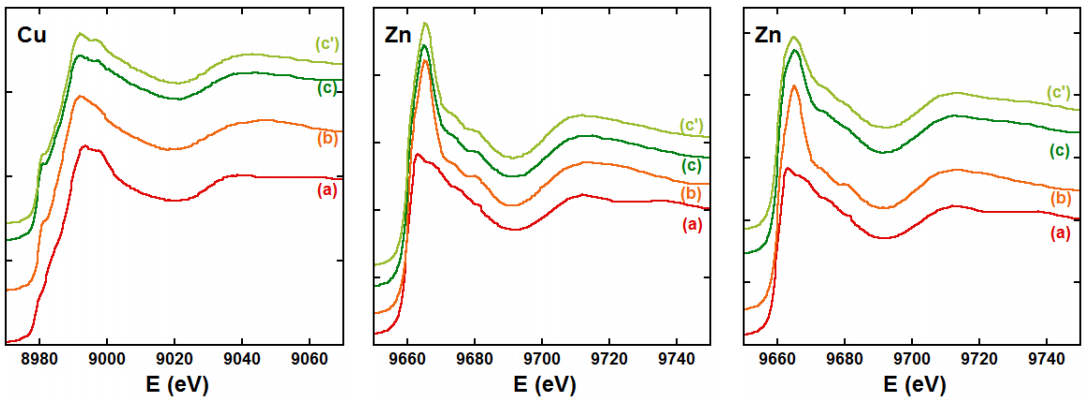


Figure 3. Cu and Zn K-edges spectra of (a) Cu(Aβ) or Zn(Aβ), (b) Cu(Lc) or Zn(Lc), (c) Cu(Lc) or Zn(Lc) + Aβ and (c') Cu(Lc) or Zn(Lc) + imidazole (left and middle panels) or (b) Zn(Lc) + Aβ, (c) Cu(Aβ) + Zn(Lc) and (c') convolution of 40% of spectra (a) and 60% of spectra (b) to reproduce spectra (c) (right panel). HEPES buffer 100 mM pH 7.1, [Cu] = [Aβ16] = [L2] = [L2-Zn] = 1 mM. 10 % glycerol are used as a cryoprotectant. *T* = 20 K.

Conclusion: We have tested several ligands during this beam-time including C1, L2 and Lc. These experiments show that C1 and L2 are efficient in the removal of Cu(II) from Aβ, even in the presence of Zn(II) while Lc is able to appreciably remove Cu(II) from Aβ only in absence of Zn(II). We thus have illustrated with various examples how Zn(II) can interfere in Cu(II) removal from Aβ. This may be of interest and significance in the context of AD where the Cu ions can be anticipated as the most pertinent biological target. Alongside, we show that XANES is the method of choice to study such metal swap.

Experimental details: Zn and Cu K-edges XANES spectra were recorded on the FAME beamline during a 18-shifts session in October 2015. The measurements were performed on ~mM solution 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 Zn and Cu foil spectra in transmission. For each sample, at least 3 XANES spectra were recorded and averaged.

Publications: We expect to publish two papers on the Cu(II) removal from Aβ study, a first one with the L2 and Lc ligands, in order to show the importance of Zn in the removal of Cu(II) from Aβ which is a proof of concept (manuscript submitted) and a second one with other ligands, including ligands for which the Cu over Zn is not already known (or difficult to determine).