EUROPEAN SYNCHROTRON RADIATION FACILITY
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Experimental Report for 20130207

X-ray Absorption Spectroscopy pH dependent study of zinc interactions with the Alzheimer's peptide (Aβ): insights into zinc induced aggregation of Aβ.

Scope of the project:

(A) The initial aim of the project was to make use of XAS (XANES and EXAFS) to gain insights into the coordination of Zn(II) to the amyloid-β (Aβ) peptide, both involved in Alzheimer disease (AD). Indeed, due to the intrinsically disordered nature of the Aβ peptide, it is impossible to obtain X-ray characterizations of the binding site. Knowing the binding site of Zn(II) to the Aβ peptide is necessary to understand its impact in the Zn-induced Aβ aggregation, an event considered to be crucial in the development of AD. In the proposal, it was proposed to study coordination of Zn(II) at two different pH values, i.e. 7.0 and 9.0. Indeed, we have recently acquired some data showing that the Zn(II) binding to the Aβ peptide is pH dependent and that two forms coexist near physiological pH.[1, 2] One of the form (latter noted species I) is predominant near pH 7 while the second one (noted species II) is predominant near pH 9. In contrast to Cu(II) for which pulsed EPR methods combined to the use of ^{13}C and ^{15}N isotopically labelled Aβ[3] and NMR[4] analysis can directly probe the metal centre environment to the Aβ peptide, Zn(II) coordination sphere can only be indirectly deduced from the study of Zn(II) binding to various pertinent modified Aβ peptides. In previous experiments, we have recorded the XANES spectra of a wide series of Zn(II)-modified Aβ peptides at pH 7.4, a pH value at which the two species I and II coexist, thus precluding a straightforward interpretation of the data. In the present experiment, we recorded about 20 samples at pH 7.0. Recording of samples at pH 9.0 was prevented due to the formation of $Zn(OH)_2$ that is favoured compared to the formation of the Zn(II)-peptide complexes at this pH value. We tried to decrease the pH (attempts at pH 8.6, 8.3 and 8.0) but unfortunately, $Zn(OH)_2$ was still present at pH 8.0. Hence we cannot gain insights into the Zn(II) coordination to the Aβ peptide in species II, but have all the XANES data required to obtain a picture of Zn(II) binding at pH 7.0 (species I). These results are crucial to describe the Zn(II) environment when bound to the Aβ peptide (at pH 7.0), which is still an open debate and which is an important issue regarding the importance of metal ions in the etiology of AD.

(B) With the time saved due to the aborted recording of the high pH data, we have investigated another project relying on the Cu chelation from Aβ peptide. Several systems were studied focusing on the chelation of Cu(I), for which the XANES spectroscopy is the most suitable method to investigate such Cu(I) transfer from the Aβ peptide to the chelator. Very interesting results were obtained with the PTA ligand (1,3,5-Triaza-7-phosphaadamantane), which is able to reduce Cu(II) bound to Aβ and to extract the Cu(I) from the Aβ. This is a very promising result, since copper chelation is one of the possible therapeutic approach currently developed but the studies reported so far exclusively deal with Cu(II) chelation.

Results:

(A) The results obtained on Zn(II) coordination to Aβ and to a wide series of relevant mutants are shown in Figure 1 where the XANES spectra of Zn(II) bound to the human Aβ and to 9 modified Aβ are depicted. From the analysis of these data and of *in house* NMR and affinity data, the binding site of Zn(II) at pH 7.0 can be proposed.[1, 2] The Zn(II) ion is bound to the His6 and His13 or His14 in equilibrium, and to the carboxylato groups from Asp1 and Glu11.

(B) The Cu(II) reduction and removal from Aβ can be easily probed by XANES spectroscopy in which the signatures of Cu(II)-Aβ, Cu(I)-Aβ and Cu(I)-$(PTA)_4$ complexes are significantly different (Figure 2). From these data, it appears that the PTA ligand is able to reduce Cu(II) and to retrieve it to form the Cu(I)-$(PTA)_4$ complex. Because we know from other studies that PTA cannot bind to Cu(II), we can propose the following mechanism for this reaction :

$$\text{Cu(II)-A}\beta + 4\ \text{PTA} \rightarrow \text{Cu(I)-A}\beta + 4\ \text{PTA}^{ox} \rightarrow \text{Cu(I)-PTA}^{ox},$$

where oxidation of the PTA ligand (leading to PTA^{ox}) does not involve the coordinating phosphorus atom.

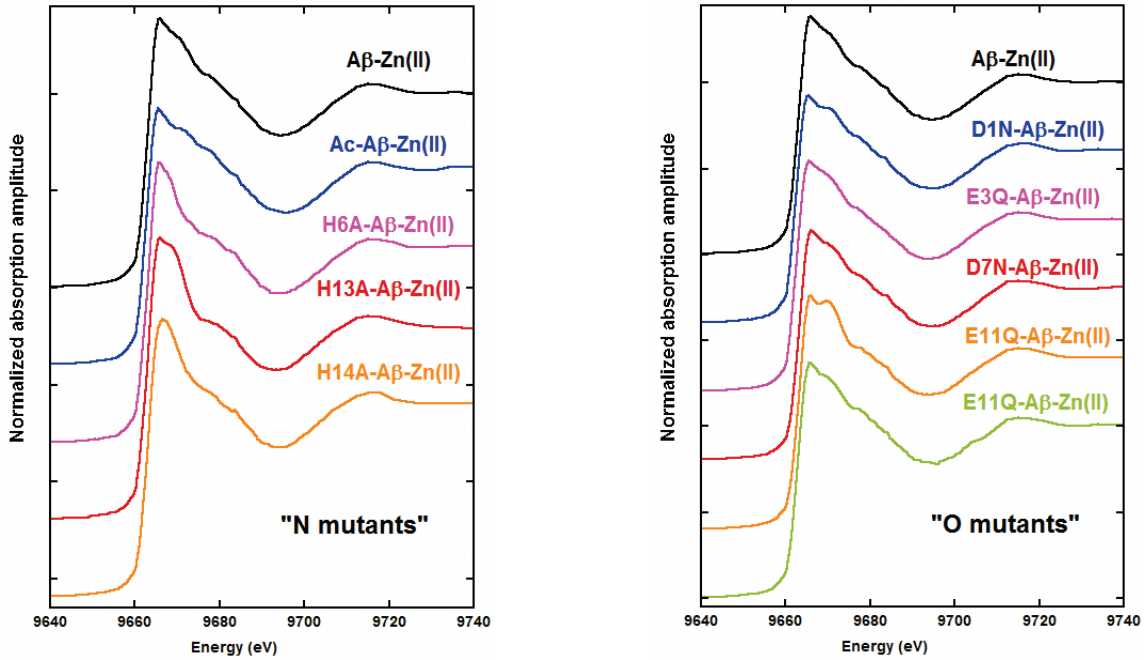

Figure 1. XANES spectra of Zn(II) bound to the human Aβ (black line) and to N mutants (left panel) and O mutants (right panel), hepes buffer 50 mM pH 6.9, [Zn] = 1 mM, [peptide] = 1.1 mM. T = 10 K.

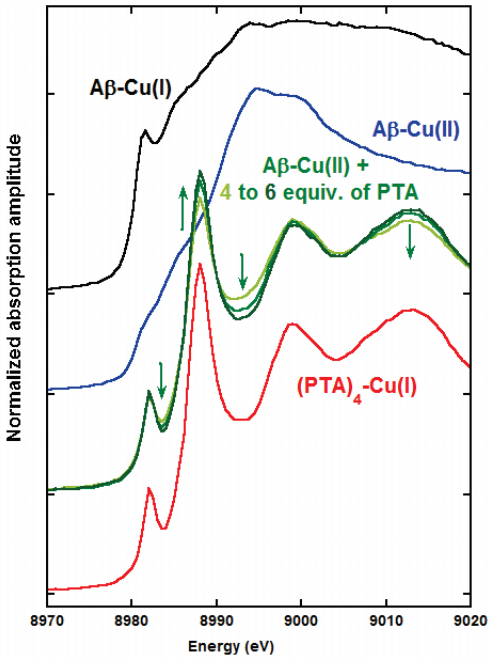

Figure 2. XANES spectra of Cu(I) (black line) and Cu(II) (blue line) bound to the human Aβ, to the Cu(I)-$(PTA)_4$ complex (red line) and to the Cu(II)-Aβ sample to which 4 to 6 equiv. of PTA ligand were added (green lines), phosphate buffer 50 mM pH 7.4, [Cu] = 1 mM, [peptide] = 1.1 mM. T = 10 K. It is worth noting that addition of more than 4 equivalents of PTA ligand does not change the XANES signature significantly in line with the oxidation of the ligand elsewhere than on the coordinating phosphorus ligand.

Experimental details: Zn and Cu K-edge XANES spectra were recorded on the FAME beamline during a 15-shifts session in October 2013. 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, about 4 to 6 XANES spectra were recorded and averaged.

Publication: On the basis of the data we obtained during this session, we expect to complete the manuscript regarding Zn(II) binding to Aβ in species I at pH 7.0 that we already have in preparation (ref 1) and to write a paper on the Cu reduction/extraction mechanism from Aβ by the PTA ligands, for which other already available data can be added.

References.

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