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X-ray Absorption Studies of Metal Binding to the Amyloid β-peptide

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Introduction

Alzheimer's disease (AD) is progressive neurodegenerative disorder that is characterised by the presence of misfolded protein deposition, described as amyloid plaque. This structural transition from the native state to a β-sheet aggregated form is accompanied by a gain of toxic function. The major constituent of AD plaques is the 4.3kDa amyloid beta peptide (Aβ) that is cleaved from the membrane-bound amyloid precursor protein (APP). *In vitro*, Aβ binds metal ions including Cu^{2+}, Zn^{2+}, and Fe^{3+} giving rise to peptide aggregation and extensive redox chemical reactions. Since elevated levels of these metals are found in amyloid deposits in AD-affected brains, the oxidative stress causing cellular damage may be related to the production of reactive oxygen species by metallated forms of Aβ [1,2].

NMR and EPR results [2] indicate the coordination sphere about the Cu^{2+} and Zn^{2+} ions may be similar to that observed in superoxide dismutase (SOD) with His6, His13 and His14 involved. The presence of bridging histidine between two metal ions, as found in the active site of SOD, was inferred.

The studies carried out so far provide an insight into the nature of the metal binding to Aβ but this is an incomplete view. Our recent XAFS studies on Aβ–metal complexes have been focused in characterization of Cu(II) and Zn(II) binding to the Aβ peptide under a range of conditions varying metal/peptide concentrations, pH, and duffers.

Experimental

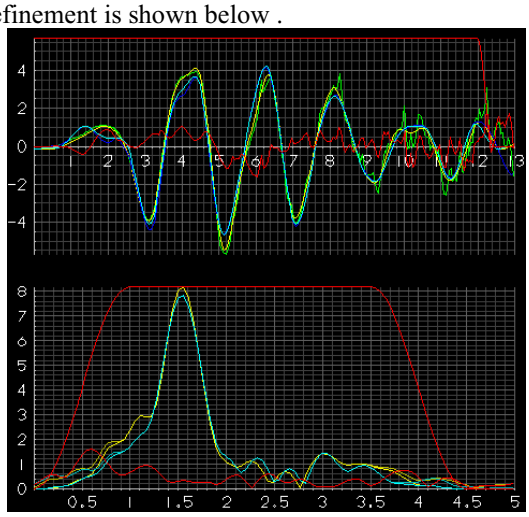
Due to limited solubility of the Aβ itself in the presence of metals in an aqueous environment the shortened Aβ(1-16) and Aβ(1-28) forms have also been used in experiments. Shorter forms contain all the residues for metal binding and are soluble at millimolar concentrations with Cu or Zn bound. EPR suggests that metal binding is the same for the shorter peptide. Samples with equimolar Aβ(1-42) and Zn^{2+}, Aβ(1-28) and (Zn^{2+} + Cu^{2+}) in PBS, pH 6.4; Ab(1-28) and Cu^{2+} in PBS, pH 7.4, and in Ethyl Morpholine, pH 7.0, were synthesized as described previously [2]. There is enough evidence in the literature to suggest that there are pH driven changes in the metal coordination sphere. There are also buffer driven differences in the coordination sphere; although these are likely to be more obvious with the longer peptides with greater aggregation tendencies

The samples were transported and kept in liquid nitrogen prior the experiments. The solution volumes of 0.1-0.2mL were injected into specially designed solution cells [4] made of Teflon with the Kapton tape sealed windows matching the size of the beam. The solution cells were inserted into a Crydone REF-1577-D22 closed-cycle cryostat and were maintained at ~10K with a Neocera LTC-11 temperature controller unit.

The Cu K-edge XAS was measured at the Australian National Beamline Facility (ANBF) on bending magnet beamline 20B using a Si(111) monochromator. XAFS data analysis was performed using the XFIT package [5]. The scans were averaged using weights based on mismatch between the XAFS and the average XAFS. A background correction was applied by fitting spline curves. The background-subtracted, normalized data were converted to k^3 space, where k is the photoelectron wave vector, to enhance the amplitude of the high k oscillations.

Results and Discussion

Physically sensible structural models have been obtained using restrained refinements (R-factor range of 20-25%). Example of the Aβ(1-42)+Zn^{2+} sample refinement is shown below .



Preliminary structural models obtained from EXAFS data are consistent with recent independent EPR measurements and models proposed in [3]. Further accurate measurements will be required to check reproducibility of results obtained.

References

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