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

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Introduction

The amyloid β-peptide (Aβ) is a 4.3kDa peptide, normally soluble and found in all biological fluids. However, it accumulates as the major constituent of the extracellular deposits that are the pathologic hallmarks of Alzheimer's disease (AD). *In vitro*, Aβ binds metal ions including Zn^{2+}, Cu^{2+}, and Fe^{3+} giving rise to 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. In contrast, EPR spectroscopy study [3] indicates that ligands coordinating the Cu^{2+} ion in a square-planar arrangement likely include the N-terminal amino group and N groups of His13 and His14. The fourth ligand is from His6 rather than Tyr10. No evidence of a dimeric species was found.

The studies carried out so far provide an insight into the nature of the metal binding to Aβ but this is an incomplete view. The focus of this work has been to characterize more reliably the structural consequences of Cu binding to the Aβ peptide using the X-ray absorption (EXAFS/XANES) technique.

Experimental

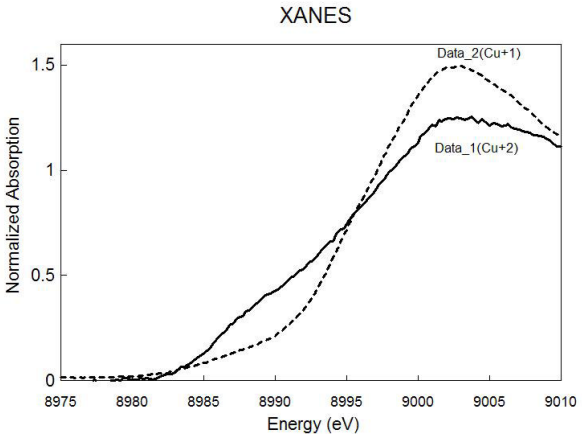
Due to limited solubility of the Aβ itself in the presence of metals in an aqueous environment the shortened Aβ1-16 form has been used in experiments. Aβ1-16 contains all the residues for metal binding and is soluble at millimolar concentrations with Cu bound. EPR suggests that metal binding is the same for the shorter peptide. Both oxidized Aβ1-16-Cu^{2+} and reduced Aβ1-16-Cu^{1+} forms were synthesized as described previously [2]. The samples were transported and kept in liquid nitrogen prior the experiments. Metal concentrations in solutions were greater than or equal to 1mM. 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. Eight scans were recorded for each sample in fluorescence mode using standard N_2-filled ionization chambers.

XAFS data analysis was performed using the XFIT program [5]. The scans were averaged using weights based on mismatch between the XAFS and the average XAFS. This scheme is particularly appropriate for weak and noisy data. XAFS spectra were checked individually before averaging, and any monochromator glitches were removed. 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

Preliminary results show that, for example, the normalized Cu K-edge XANES spectrum below for oxidized Aβ1-16-Cu^{2+} exhibits features similar to those observed in SOD, however spectrum for reduced Aβ1-16-Cu^{1+} significantly differs from that expected for Cu^{1+} species with trigonal coordination as in reduced SOD. Further more accurate measurements will be required to check reproducibility of results obtained.



References

[1] A. I. Bush, TINS, 26, 207 (2003).
[2] C. C. Curtain et al., JBC, 276, 20466 (2001).
[3] C. D. Syme et al., JBC, 279, 18169 (2004).
[4] S. Best, Private Communication.
[5] P. J. Ellis et al., J. Synch. Rad. 2, 190 (1995).
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