Characterization of the Cu (II) and Zn (II) binding to the Amyloid-β short peptides by both the Extended X-ray Absorption Fine Structure and the Synchrotron Radiation Circular Dichroism spectroscopy

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Abstract. Alzheimer’s disease (AD) is a progressive and devastating neurodegenerative pathology, clinically characterized by dementia, cognitive impairment, personality disorders and memory loss. It is generally accepted that, misfolding of Aβ peptides is the key element in pathogenesis and the secondary structure of Aβ can be changed to major β-strand with reasons unknown yet. Many studies have shown that the misfolding may be linked with some biometals, mainly copper and zinc ions. To characterize interactions of Aβ and metal ions, we utilized both the extended X-ray fine structure spectroscopy (EXAFS) and the synchrotron radiation circular dichroism spectroscopy (SRCD). Aβ (13-22), Aβ (13-21), Aβ (E22G) and Aβ(HH-AA) were selected to study the mechanism of copper and zinc binding to Aβ. We found that Cu interaction with H13 and H14 residues led to the disappearance of the PPП, while the Cu binding E22 residue caused a remarkable conformation change to β-sheet enrichment. The Zn ion, in contrast, made little effect on the conformation and it coordinated to only one histidine (H residue) or not.

1. Introduction
It is generally believed that polypeptide amyloid-β, the main component of plaque deposits in Alzheimer’s disease (AD), is probably a causative of AD [1, 2]. In our bodies, Aβ produced by sequential proteolytic cleavage of the amyloid precursor protein (APP) is normal and soluble with little content of β-strand [2]. While, it is yet unknown that how soluble Aβ is transformed into insoluble, fibrous and eventually aggregated one which can possibly lead to AD [2]. X-ray diffractions on the amyloid fibrils of Aβ indicate a cross-β conformation with its β-sheet and backbone hydrogen bonds directing perpendicular and parallel to the fibril axis respectively [1]. The conformation changes of native Aβ may be linked with some biometals, such as copper and zinc. There is evidence that copper and zinc are elevated in amyloid plaque deposits with concentrations of 0.4 mM and 1 mM, respectively [1]. In vitro, Aβ deposits induced by copper can be dissolved backwards after the additions of metal chelators [3]. Mean while, the amyloid precipitate in vivo extracted from the postmortem tissue of AD patient behaves the same above after being doped by metal chelators [4]. Recently, researches attract much interest in the short-range structure of metal ions coordinating to Aβ. Histidines (H6, H13 and H14) are considered to be the most attracting binding sites for metal ions.
The conformation structure around metal ions sensitively depends on the solution condition, such as buffer and pH [5, 6, 7]. Furthermore, Electron Paramagnetic Resonance (EPR) spectroscopy on copper after binding to Aβ demonstrates that in low pH, copper can coordinate to two histidines [8].

In our studies, four short sequences of human Aβ, Aβ (13-22), Aβ (13-21), Aβ (E22G) and Aβ (HH-AA) were selected as short peptide models for detection of conformation changes triggered by copper and zinc in vitro. Synchrotron radiation circular dichroism spectroscopy (SRCD), as one of the most sensitive tool for measurement of protein’s secondary structure, was used to detect long-range structure of Aβ after being coordinated to metal ions. While short-range structure around metal ions was probed by the extended X-ray fine structure spectroscopy (EXAFS) fitting analysis. By both SRCD and EXAFS analysis, we found that metal ions binding to at least two histidines led to the disappearance of PPΠ [9], a secondary structure of protein.

2. Materials and Methods
Aβ (13-22), Aβ (13-21), Aβ (E22G) and Aβ (HH-AA), as four short peptides, were chosen with purity exceeding 98%. The sequence of Aβ (13-22) is HHQKLVFFAE. Ac- and -NH₂ represent acetylation of the N-terminal amino group and amidation of the C-terminal amino group, respectively. The stock solution of Aβ short peptides (~2mM) has been prepared by dissolving them in MES (2-(N-Morpholino) ethanesulfonic acid). The pH of the solution was adjusted to pH 5.6 by adding NaOH stock solution. The stock solution were stirred, sonicated for 10 minutes and centrifuged for 3 minutes at 12000 g. The supernatant was mixed by copper or zinc at mole ratio of 1:0.5. The mixture was centrifuged (3minutes at 12000 g) to get supernatant for measurements of SRCD and EXAFS.

SRCD spectra about measurements of Aβ were performed at the beamline 4B8 of the Beijing Synchrotron Radiation Facility (BSRF) [10]. CaF₂ sample cell with optic length of 0.05 mm was used for holding solution Aβ. CD spectrum of each sample was recorded at least three times, with baseline to be extracted later. Online analysis [11, 12] for secondary structures of Aβ was followed.

The Cu and Zn K-edge EXAFS spectra were collected at the 1W1B beamline of BSRF. The monochromator is double-crystal Si (111). Data was collected in fluorescence mode using Lytle ionization chamber filled by Ar. Ni and Cu filter were inserted before the detector to inhibit scattering signal for Cu and Zn EXAFS data collecting, respectively. Athena was used for subtraction and normalization. While, Artemis was used for fitting analysis of k or R space spectra with a predicted model

3. Results and Discussion
Figure 1 displays the SRCD spectra of the four short peptides of Aβ (13-22), Aβ (13-21), Aβ (E22G) and Aβ (HH-AA) after being reacted with Cu²⁺. First of all, the SRCD shapes (solid curves) of Aβ short peptides in their native state are almost the same and no obvious conformation distinction about their secondary structure can be found, except for the Aβ (HH-AA). The spectra of the four Aβ sequences similarly show the CD positive peak at about 220nm, which manifests possibly a considerable PPΠ helix contribution. However, the CD patterns (dotted lines) in Fig. 1 display obvious differences for the four Aβ short peptides after reaction with Cu²⁺. In contrast, their spectra show almost the same CD negative peak at about 220nm, except for the Aβ (HH-AA). The changes of CD peak at ~220nm after addition of copper demonstrate that reactions of copper and Aβ can lead to the disappearance of PPΠ and two histidines play significantly important role in that process.

Figure 2 exhibits the Cu K-edge EXAFS spectra in k space for the four Aβ short peptides. All the EXAFS experimental spectra display the similar split peak at almost 4Å⁻¹, except for the Aβ (HH-AA), in which histidine has been substituted by A. The split peak at ~4Å⁻¹ is generally considered as a fingerprint that metals coordinate to the histidines in BioXAS. Therefore copper binds to H residue, except for the Aβ (HH-AA). Data has been best fitted by the 2-histidine geometry model (data not shown). So, combined with the results of SRCD spectra in Fig. 1, EXAFS patterns in Fig. 2 elucidates that copper coordinating to two histidines can lead to the disappearance of PPΠ.
Figure 1. The SRCD spectra of Aβ (13-22), Aβ (13-21), Aβ (E22G) and Aβ (HH-AA) after additions of Cu$^{2+}$ at molar ratio of 1:0.5.

Figure 2. Cu K-edge EXAFS spectra of Aβ (13-22), Aβ (13-21), Aβ (E22G) and Aβ (HH-AA) after additions of Cu$^{2+}$ at molar ratio of 1:0.5.

Figure 3. The SRCD spectra of Aβ (13-22), Aβ (13-21) and Aβ (E22G) after additions of Zn$^{2+}$ at molar ratio of 1:0.5.

Figure 4. Zn K-edge EXAFS spectra of Aβ (13-22), Aβ (13-21) and Aβ (E22G) after additions of Zn$^{2+}$ at molar ratio of 1:0.5.
Figure 3 displays the SRCD spectra of Aβ short peptide models of Aβ (13-22), Aβ (13-21) and Aβ (E22G) after reaction with Zn²⁺. For CD positive peak at about 220 nm, there are no obvious changes for all the Aβ short peptides after interaction with zinc. That is to say, the content of PP remains nearly the same after the additions of Zn²⁺. So, reactions of zinc and Aβ fail to trigger the disappearance of PPII secondary structure.

Figure 4 shows the Zn K-edge EXAFS spectra in k space for Aβ short peptides of Aβ (13-22), Aβ (13-21) and Aβ (E22G). Compared to Cu K-edge EXAFS spectra in Fig. 2, there are no split peak at ~4 Å⁻¹ for Zn EXAFS spectra in Fig. 4, which manifests that zinc may not coordinate to histidine. Data has been best fitted by the 1-histidine geometry model (data not shown). So, combined with our results of SRCD spectra in Fig. 3, the Zn EXAFS spectra elucidate that zinc binding to at most one histidine can’t lead to the disappearance of PPII.

4. Conclusion
In summary, using SRCD and EXAFS spectroscopy, we present long-range and short-range structure change of Aβ short peptide models after reaction with metal ions, respectively. SRCD demonstrates that PPII disappears after reaction with copper, while EXAFS elucidates that copper has coordinated to two histidines. So, it shows that Cu²⁺ binding to two histidines leads to the disappearance of PPII. However, PPII remains the same after reaction with zinc by SRCD and Zn²⁺ binds to at most one histidine by EXAFS. Compared to copper, Zn²⁺ binding to at most one histidine can’t trigger the disappearance of PPII.

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