Amyloid-β peptide active site: theoretical Cu K-edge XANES study

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Abstract. This article is dedicated to the local atomic structure analysis of the copper binding site in amyloid-β peptide. Here we considered two possible structural models that were previously obtained by means of EXAFS analysis and density functional theory simulations. We present the calculations of Cu K-edge XANES spectra for both models and make comparison of these spectra with experiment.

1. Introduction

Alzheimer's disease (AD) is a progressive, irreversible brain disease that destroys memory and thinking skills. AD is the most common form of dementia. Most often, AD is diagnosed in people older than 65 years [1]. However, the disease is not associated with the age only. A small percentage of people have early-onset AD in their 40 or 50 years. An estimated 5.4 million Americans of all ages have AD in 2012 and of these 5.2 million people aged 65 and older [2] and 200 thousands individuals are under the age of 65 years [3].

Currently, AD is incurable, and treatments for symptoms are available only. Today, many scientists are searching for new and more effective treatments that can help delay the onset of the disease, alleviate the symptoms and prevent its appearance.

At the cellular level, the AD is related to the death of nerve cells and loss of synapses in the cerebral cortex. The disease is also characterized by the presence of misfolded protein depositions or amyloid plaques in the brain. The main component of these plaques is the amyloid-β peptide (Aβ). Its predominant length is 42 amino acids: DAEFRH6DSGYEVH13H14QKLVFFAEDVGSNKGAIIGLMVGVVIA. The Aβ peptide is cleaved from the membrane-bound amyloid precursor protein (APP) by the β/γ-secretase pathway. In vitro Aβ can bind to Cu, Zn and Fe with relatively high affinity and increased content of these metals are found in AD amyloid plaques [4,5]. There is evidence that the neurodegeneration observed in AD is related to the toxicity from reactive oxygen species (ROS) produced in the brain by the Aβ peptide bound to copper ions [5,6]. The ROS oxidize essential cellular components such as proteins, lipids and DNA. It leads to cell damage and oxidative stress. According to studies [5,7] the Aβ-Cu²⁺ complexes can participate in extensive redox chemical reactions that produce H₂O₂ and other ROS from molecular oxygen. As a result of these redox reactions the copper ion Cu²⁺ is reduced to Cu¹⁺. Knowledge of the electronic and atomic structures of the nearest environment of Cu²⁺ is required for deeper understanding this process. Recent researches show...
particular interest to the role amyloid-β redox for oxidative stress [8-11]. It may be critical to the etiology of AD.

2. Models and method
This article focuses on the structure of the Aβ-Cu²⁺ active site. We consider two model (Model 1 and Model 2) which structures were described in [8]. These models were proposed by means EXAFS analysis of the Aβ peptides complexed with Cu²⁺ in solution under a range of buffer conditions and density functional theory (DFT) simulations.

In both models Cu²⁺ is six-coordinated. In the Model 1 the copper ion is surrounded by three nitrogen atoms belonging to the imidazole rings of histidines (His-6, His-13, His-14), two oxygen atoms belonging to the carboxylate side chain of glutamic acid (Glu-11) and water molecule (W1). The Cu²⁺ coordination environment is similar octahedral, the three nitrogens and one oxygen of glutamic acid (O\text{Glu-11}^1) are located in the equatorial plane and other oxygen of glutamic acid (O\text{Glu-11}^2) and water molecule are located on the axis. This model includes two additional solvent oxygen (O_{sol}) atoms, which may belong to the carboxylate group from the N-terminal amino acids such as asparagine. They may be involved in a hydrogen bonding with water molecule W1 to stabilize the copper binding site.

The Model 2 also includes the three imidazole equatorial rings (His-6, His-13, His-14), axial water molecule W1, and solvent oxygen atoms. But carboxylate side chain of Glu-11 is replaced by phenol ring of tyrosine (Tyr-10) and second axial water molecule is added. The distances between the copper ion and the neighboring atoms are listed in ‘table 1’.Both models with the notation adopted above are shown in ‘figure 1’.

![Figure 1](image.png)
Table 1. The distances between the copper ion and the neighboring atoms (in Å) for two model structures of the copper binding site in Aβ [9].

|                  | Model 1 | Model 2 |
|------------------|---------|---------|
| d(Cu²⁺-N_His-6)  | 1.91    | 1.91    |
| d(Cu²⁺-N_His-13)| 1.99    | 1.97    |
| d(Cu²⁺-N_His-14)| 2.09    | 2.11    |
| d(Cu²⁺-O_W1)    | 2.03    | 2.32    |
| d(Cu²⁺-O₉₆_Glu-11)| 1.94 |         |
| d(Cu²⁺-O₈₅_Glu-11)| 2.27 |         |
| d(Cu²⁺-O_Tyr-10)|        | 1.97    |
| d(Cu²⁺-O_W2)    |        | 1.96    |

In order to study these models the XANES spectroscopy was used. The theoretical XANES spectra were calculated using two one-electron approaches. The first one is based on the real space Green’s function formalism in considering the full multiple scattering a photoelectron wave on a self-consistent potential of muffin tin (MT) type and is implemented in the FEFF9.03 code [12]. The second one uses the finite difference method to solve the Schrodinger equation and is carried out in the FDMNES program [13].

3. Results and discussions

The ‘figure 2’ shows the comparison of the experimental Cu K-edge XANES spectrum of the truncated Aβ₁₋₁₆-Cu²⁺ complexes dissolved in phosphate buffer [8] with the theoretical spectra for both models calculated with FEFF9.03 program. The radius of the cluster in which the multiple scattering procedure is performed, was equal to 8 Å and the cluster included all atoms of each model (30 for Model 1 and 32 for Model 2). The exchange part of the scattering potential was Hedin-Lundqvist type [14]. The position of the absorption edge is determined by the self-consistent calculations, but in the ‘figure 2’ both theoretical spectra are shifted by 5.1 eV to lower energies. This is done in order to make the comparison of theory with experiment easier. Most likely the cause of this shift is the incompleteness of models, which don’t have hydrogen atoms. Hydrogen atoms can influence the self-consistent procedure and lead to a shift of the Fermi level.

The experimental spectrum has a number of features which are identified in ‘figure 2’ as A, B, C, D, E and F. The low-intensity peak A (at ≈8979 eV) is caused by the 1s→3d quadrupole-allowed transitions in Cu²⁺ [15,16]. The small shoulder B (at ≈8987-8988 eV), which occurs from either a vibronic allowed 1s→4s transition or 1s→4p transitions with a metal-ligand charge-transfer shakedown, has been explained mainly to scattering by the axial ligands [16]. The main peaks C and D (at ≈9897 eV and ≈9001 eV, respectively) is assigned to the 1s→4p (or 1s→continuum) transition. There are also features E and F, which are located at ≈9010 eV and 9045 eV. They are related to multiple scattering in first shell ligands [15,17], and its form and position can depend, for example, on the orientation of the imidazole rings [16].

The shoulder B is observed in both the theoretical spectra, but for Model 1 is more pronounced. The shape of peaks C and D is very different between the models. The intensity ratio of the peaks C and D for Model 2 more agrees with the experimental curve. But the feature E, which is observed in spectrum of Model 1 is absent or greatly reduced for Model 2. The peak F in both cases is shifted to lower energies (by 11 eV for Model 1 and by 5 eV for Model 2).
Figure 2. The comparison of simulated Cu K-edge XANES spectra for both models of the copper binding site in Aβ (FEFF9.03) with experimental spectrum of the truncated Aβ1-16-Cu2+ complexes in phosphate buffer [9]. From top to bottom: Model 2 (red line), Model 1 (blue line), experiment (black line). The spectral features are marked as A, B, C, D, E and F.

These deviations of the theoretical spectra from the experiment may be associated with features of the considered models and/or the calculation method. In order to answer the question, we calculated the spectra using a method, which is based on the finite difference method to solve the Schrödinger equation (code FEMNES). This approach is more time-consuming, but at the same it has the opportunity to perform calculations beyond the MT approximation, which in this case is not entirely justified. The calculation results are presented in the ‘figure 3’. The Schrödinger equation was solved on a grid of points in real space, which is a sphere centered on the Cu2+ and the radius of 5.5 Å. Thus the calculation included 27 atoms for Model 1 and 29 atoms for Model 2. It should be noted that the Model 1 curve has a near-edge feature A and this peak is shifted to higher energies on $\approx 1$ eV. The spectrum of Model 2 doesn’t have the peak A, but a new peak A’ is observed there (at $\approx 8985-8986$ eV). In the experimental spectrum it is not explicitly pronounced. Perhaps the peak A’ is smoothed by experimental resolution. The shoulder B on the theoretical spectra is located closer to the main maximum C than on the experimental spectrum. Its position and shape differ between the two calculated spectra and this is in agreement with the nature of this feature [16]. The feature D is absent on the theoretical spectra and there is a small dip in its place for Model 2. The shoulder E is also absent on the spectrum for Model 1. The maximum F for both models is shifted to lower energies by 6 eV relative to corresponding experimental peak.
Figure 3. The comparison of non-MT theoretical Cu K-edge XANES spectra for both models of the copper binding site in Aβ (FDMNES) with experimental spectrum of the truncated Aβ(1-16)-Cu²⁺ complexes in phosphate buffer [9]. From top to bottom: Model 2 (red line), Model 1 (blue line), experiment (black line).

4. Conclusions
We calculated the Cu K-edge XANES spectra for the two models of the copper binding site in Aβ-Cu²⁺ complex using the two approaches: full multiple scattering with MT approximation (FEFF9.03) and finite difference method to solve the Schrodinger equation (FDMNES). It was found that spectra simulated by FEFF9.03 program show better overall agreement with experimental XANES spectrum with some preference to Model 2. The intensity ratio of the peaks C and D for Model 2 more agrees with the experimental curve (‘figure 2’). However, the statistical analysis of EXAFS fittings of three two models gave preference to Model 1 [8] which is in agreement with other biophysical studies. For example, recent spectroscopic measurements have confirmed that Tyr-10 and Met-35 are not key residues in metal binding [18-20]. This inability to precisely define the exact metal coordination sites in Aβ suggests that metal coordination may be pleomorphic [19] and we should model a superposition of different binding modes. Also, the use of MT approximation in this case is not entirely justified, since the structure of the Aβ-Cu²⁺ active site has cavities in which the potential is not constant would be more appropriate. However, these calculations are very useful for preliminary analysis and identification of the most significant effects. Next step will be analysis of the spectra calculated beyond the MT approximation in FEFF9.03.

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