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Zinc-mediated modulation of the configuration and activity of complexes between copper and amyloid-β peptides

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ABSTRACT

A growing body of Alzheimer's disease (AD) research is concerned with understanding the interaction between amyloid-β (Aβ) peptides and metal ions (e.g., Cu, Zn, and Fe) and determining the biological relevance of the metal–Aβ complexes to essential metal homeostasis and neuronal cell loss. Previously, many studies have dealt with the interaction between Aβ and "single" but not "multiple" metal ions in terms of binding affinity and coordination chemistry. In the present work, we found that Zn(II) ions modified the configuration of Aβ–Cu(II) by forming Zn(II)–Aβ–Cu(II) ternary complexes. As a result, the catalytic activity of Aβ–Cu(II) against a biological ascorbic acid species was repressed by Zn(II) binding. The formation of the ternary complex can therefore explain the protective role of Zn(II) in AD.

1. Introduction

The main pathological hallmarks of Alzheimer's disease (AD) include the formation of senile plaques that are composed primarily of 39–43 amino acid amyloid-β (Aβ) peptides, the dramatic loss of neuronal cells, and extensive oxidative stress [1,2]. More intriguingly, high levels of metals (such as Zn, Fe, and Cu) accumulate inside the senile plaques [3,4]. Consequently, there has recently been substantial interest in the interaction between metals and Aβ and in the possible roles of the metal–Aβ complexes in AD pathogenesis [5–12].

It is becoming evident that the metal ions are involved in the aggregation and deposition of the Aβ peptides [7,13]. Moreover, the metal–Aβ complexes (Aβ–Cu and Aβ–Fe) can induce the production of reactive oxygen species (ROS) and the aggravation of oxidative stress [2,11,14]. Although many studies have been performed to determine the major metal binding sites on the Aβ peptides (for reviews, see ref [6] and [8]), the literature has not reached a consensus about the stoichiometry and binding affinity of Aβ with metals, such as Cu(II) and Zn(II). For example, at least two Aβ–Cu(II) binding modes with varying binding affinities have been proposed, and investigations into the detailed binding modes have continued [9,10,15–17]. The binding (affinity) constants that have been reported thus far have ranged from micromolar to attomolar [12,18–22], with most values being within the submicromolar to nanomolar range [12,17,19,22]. Moreover, it has also been reported that Aβ can host more than one Cu(II) [23,24] or Zn(II) [23,25]. Despite these conflicting results, there is a general agreement that the hydrophilic domain (residues 1–16) of Aβ binds to copper through the three histidine residues at positions 6, 13, and 14, whereas the C-terminal region, which contains hydrophobic amino-acid residues, is not believed to be involved in direct interactions with metal ions [8]. It has commonly been suggested that ROS produced by redox-active Aβ–Cu(II) could be directly involved in the toxicity of Aβ and in the development of AD. In contrast, the effect of the redox-inactive Zn(II) is neuroprotective. Nevertheless, how Zn(II) and Cu(II) bind to Aβ and how metal binding influences the aggregation and chemical reactivity of Aβ remain to be determined. Most previous studies have been focused on the interactions of "single" metal ions with Aβ, but little research has been reported regarding interactions with "multiple" metal ions. For this reason, in the present work, we have investigated the effects of Zn(II) on the configuration and activity of Aβ–Cu(II) using fluorescence, ultraviolet visible spectroscopy (UV–vis) and electrochemical techniques. We also present the biological implications of our findings and discuss the mechanism of the neuroprotective role of zinc compared to copper in AD.

2. Materials and methods

2.1. Materials

Lyophilized Aβ(1–16) (DAEFRHDSGYEVHHQK) was synthesized by Genemed Synthesis (San Antonio, TX) and purified in-house using HPLC. Aβ(1–42) (DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAII

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GLMVGGVVI) was purchased from American Peptide Co. Inc. (Sunnyvale, CA). Ascorbic acid (AA), dimethyl sulfoxide (DMSO), $ZnCl_2$, $CuSO_4$, $K_2HPO_4{\cdot}3H_2O$, KH_2PO_4, NaOH, and Na_2SO_4 were all of AR grade and were purchased from either Aldrich Chemicals or Beijing Chemical Reagent Co. (Beijing, China). The Microcon YM-1 centrifugal filter units were purchased from Millipore Corp. (Billerica, MA). The stock solutions of 5 mM Zn(II) and Cu(II) were prepared by dissolving an appropriate amount of $ZnCl_2$ or $CuSO_4$ in 5 mM H_2SO_4. The 1 mM solution of Aβ(1–16) was freshly prepared by dissolving the lyophilized samples in 1 mM NaOH, while the Aβ(1–42) samples were dissolved in DMSO and subsequently diluted with phosphate buffer to the desired concentrations. All aqueous solutions were prepared using deionized water treated by a water-purification system (Simplicity Plus, Millipore Corp., Billerica, MA).

2.2. Fluorescence spectroscopy

The fluorescence spectra were carried out at room temperature using a Hitachi F-4600 spectrofluorimeter (Hitachi High-Tech. Co., Japan). The excitation wavelength was set at 280 nm, and the emission spectra were recorded from 290 to 400 nm for the tyrosine residue in Aβ(1–16). Before the assay, Aβ(1–16) was diluted to 100 μM in 10 mM Tris–HCl buffer (pH 7.4).

2.3. Kinetic measurements

The AA oxidation rate was determined by monitoring the change in AA absorbance at 265 nm using a UV–vis spectrophotometer (Shimadzu Co., Japan). The absorbance values contributed by the individual peptides at 265 nm (measured in separate peptide solutions at the same concentrations) have been excluded from the plotted data. The kinetic experiments were conducted at 25 °C in phosphate-buffered saline (PBS buffer, 50 mM pH 7.4), as described previously [14]. At this pH, AA exists in the ascorbate ion form and shows a maximum absorption (ε) of $1.5 \times 10^4\,M^{-1}\,cm^{-1}$.

2.4. Detection of hydrogen peroxide

The H_2O_2 detection kit was obtained from Bioanalytical System Inc. (West Lafayette, IN). The final solutions were injected through a six-port rotary valve (Valco, Houston, TX) into a flowing stream of PBS buffer (50 mM, pH 7.4), as delivered by a syringe pump (KD Scientific, Holliston, MA) at a flow rate of 6 mL/h. The concentrations of H_2O_2 generated were determined by comparing the measured current to that of a calibration curve constructed using H_2O_2 standard solutions.

2.5. Electrochemical measurements

The electrochemical experiments were performed using a CHI 660 electrochemical workstation (CH Instruments, Austin, TX) with a homemade plastic electrochemical cell. A glassy carbon (GC) disk electrode and a platinum wire were used as the working and counter electrodes, respectively. All of the potentials were measured against Ag/AgCl. The potential of the Ag/AgCl reference electrode was 0.196 V with respect to the normal hydrogen potential (NHE). The electrolyte solution for the metal–Aβ complex was a 10 mM phosphate buffer (pH 7.4) containing 0.1 M Na_2SO_4. To assess Zn(II) and Cu(II), the reaction mixtures containing Aβ(1–16) and Zn(II)/Cu(II) were filtered through a 25-mm diameter YM-1 membrane at 13000 rpm for 30 min. The Zn(II)/Cu(II) ions eluted through the membrane were analyzed by differential pulse voltammetry (DPV). Prior to each experiment, the glassy carbon electrode was polished with 15- and 3-μm diamond pastes and alumina pastes with diameters of 1- and 0.3-μm. The Cu(II) ions were detected directly using a GC electrode with a potential scan between −0.2 and 0.6 V. Before the detection of the Zn(II) ions, mercury films were deposited onto the GC electrode surface by holding the electrode potential at −0.4 V for 300 s in a N_2-degassed 1% HNO_3 solution containing 5 mM Hg^{2+}. In the case of the DPV detection, a deposition time of 180 s at −1.3 V was used to deposit zinc onto the mercury film prior to the potential scan between −1.3 and −0.8 V.

3. Results

3.1. Formation of ternary complexes between Cu(II), Zn(II) and the Aβ(1–16) peptide

Previously, Garzon-Rodriguez et al. have shown that Aβ fluorescence is quenched upon the formation of Cu(II) complexes [26]. Using a similar approach, Maiti et al. determined that the binding constant between Aβ(1–16) and Cu(II) is $1.0 \times 10^6\,M^{-1}$ [20]. Consistent with literature, we found that the fluorescence peak at 310 nm decreased upon binding to Cu(II) (see the difference between curve I and V in Fig. 1). Interestingly, with the addition of different concentration of Zn(II), the fluorescence intensity of Aβ(1–16)–Cu(II) increased (e.g., compare curve V with curve II, III and IV). Therefore, the fluorescence experiment clearly confirms that Zn(II) interacts with Aβ and alters the configuration of Aβ(1–16)–Cu(II). There are two mechanisms that could mediate this interaction: (1) the configuration of Aβ(1–16)–Cu(II) is modified by the additional Zn(II) binding, or (2) Zn(II) is forced to interact with Aβ by displacing Cu(II) from its favored metal-binding site. To clarify this point, we determined the amounts of free metal ions in the Zn(II)/Aβ/Cu(II) mixtures using DPV. The mixtures were filtered through MY-1 centrifugal filter membranes with a molecular cutoff 1 KDa. The molecular weight of Aβ is 1955 Da, while the weights of Zn(II) and Cu(II) are each below 1 KDa. Therefore, the Aβ or metal–Aβ complexes remained at the top of the filter membrane, whereas the free metal ions were eluted through the pores of the membrane and became separated from the metal complexes.

Fig. 2A presents the DPVs of the anodic stripping of Zn(II) eluted from the membranes in the absence (curve I) or presence (curve II) of the Aβ(1–16)–Cu(II) species. The anodic peaks at approximately −1.127 V can be attributed to the stripping of the Zn deposited onto the Hg film. As can be seen, the anodic peak in curve I is much smaller than in curve II, thereby suggesting that Zn(II) ions are largely sequestered by Aβ. The small amount of zinc released in curve II can be ascribed to the breakage of the Zn(II)–Aβ bonds.

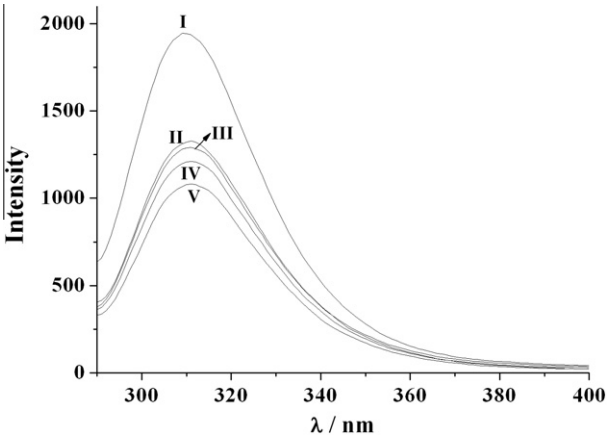

Fig. 1. Fluorescence spectra of 100 μM Aβ(1–16) in 10 mM Tris–HCl buffer (pH 7.4) (curve I) and of 100 μM Aβ(1–16) solutions containing 100 μM Cu(II) (curve V), 100 μM Cu(II) and 50 μM Zn(II) (curve IV), 100 μM Cu(II) and 100 μM Zn(II) (curve III), and 100 μM Cu(II) and 200 μM Zn(II) (curve II).

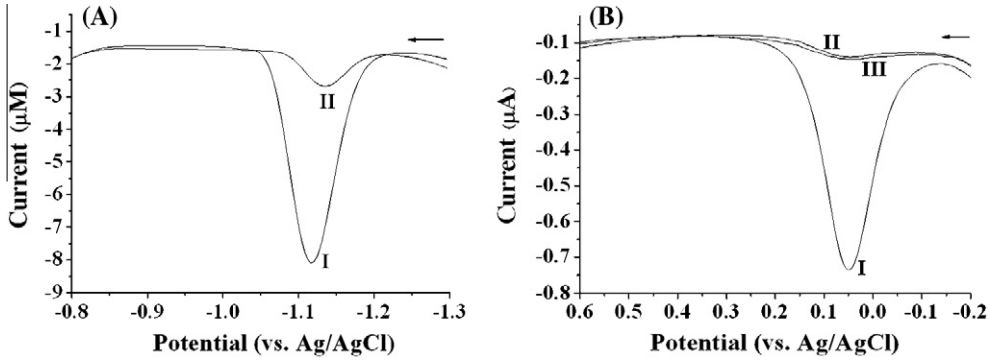

Fig. 2. DPVs of Zn(II) ions at the thin mercury film electrode (A) and Cu(II) ions at the GC electrode (B). In panel A, curves I and II represent the DPVs of the material eluted through the YM-1 membrane from the Zn(II) and Zn(II)/Aβ(1–16)/Cu(II) solutions, respectively. In panel B, curves I, II and III represent those from the Cu(II), Aβ(1–16)/Cu(II) and Zn(II)/Aβ(1–16)/Cu(II) solutions, respectively. The initial concentrations of Aβ(1–16), Zn(II) and Cu(II) were 100 μM each. Before each assay, the eluents were diluted 20-fold in Tris–HCl buffer (pH 7.4). Pulse height: 50 mV; pulse width: 50 ms. The arrow indicates the scan direction.

To examine the influence of Zn(II) on Cu(II) binding, we performed DPV to determine the amount of free Cu(II) released from the Aβ–Cu(II) complex. As shown in Fig. 2B, the signal at 0.052 V is related to the oxidation of Cu at the GC electrodes. Comparing the anodic peak in curve III to the peaks in curves I and II, we found that the addition of Zn(II) did not result in the release of Cu(II) from Aβ(1–16)–Cu(II). The small anodic peaks that were exhibited in curves II and III may be due to the breakage of the Aβ–Cu(II) bonds. Overall, these results indicate that Zn(II) ions form a ternary complex with Aβ–Cu(II) but do not displace the Aβ-bound Cu(II) ions. This results in a change in the configuration of Aβ(1–16)–Cu(II) and the fluorescence increase shown in Fig. 1. Our results are also strongly supported by the CD and EPR spectroscopic studies carried recently by Damante et al. [27].

3.2. Influence of Zn(II) on the activity of Aβ–Cu(II)

Through voltammetric and spectroscopic studies of complexes of Cu(II) with Aβ or prion peptide as a ligand (denoted as L), we have demonstrated that the biological species ascorbic acid (AA) can be catalytically oxidized to dehydroascorbate (DA) by the Cu(II) complexes to produce H_2O_2 [14,28]:

$$2L - Cu(II) + AA + H_2O = 2L - Cu(I) + DA + 2H^+ \quad (1)$$

$$2L - Cu(I) + O_2 + 2H^+ = 2L - Cu(II) + H_2O_2 \quad (2)$$

To investigate the influence of Zn(II) on the activity of Aβ–Cu(II), we determined the kinetics of AA oxidation by O_2 in the presence of different Cu(II) species by monitoring the change in AA absorbance at 265 nm. As can be seen in Fig. 3A, in the absence of Cu(II)-containing species, the rate of AA auto-oxidation is exceptionally slow (0.050 ± 0.005 nM s^{-1}). However, AA is rapidly consumed when free Cu(II) (122.8 ± 14.5 nM s^{-1}) is present. As reported previously, Aβ(1–16)–Cu(II) and Aβ(1–42)–Cu(II) catalyze the oxidation of AA at a rate of 40.4 ± 5.8 and 38.7 ± 4.1 nM s^{-1}, respectively. Interestingly, we found that the rates decreased to 19.5 ± 2.6 and 14.5 ± 2.1 nM s^{-1} upon the addition of Zn(II) into the solutions of Aβ(1–16)–Cu(II) and Aβ(1–42)–Cu(II), respectively. To determine the amount of H_2O_2 generated from the O_2 reduction, we used the same commercial electrochemical detection kit employed in our previous studies on H_2O_2 generation by Cu(II)–prion peptide complexes. As depicted in Fig. 3B, after a 1-h reaction, the amount of H_2O_2 generated in the presence of the Cu(II) species increased in the order of Zn(II)–Aβ(1–42)–Cu(II) < Zn(II)–Aβ(1–16)–Cu(II) < Aβ(1–42)–Cu(II) ≈ Aβ(1–16)–Cu(II). From these results, we conclude that the incorporation of Zn(II) decreases the catalytic activity of Aβ–Cu(II). It has been suggested that the reactivity of Aβ–Cu(II) depends on the length of the peptide because of steric hindrance to the accessibility of the Cu(II) center to oxygen [14]. In this study, we propose that the change in the Cu(II) coordination environment, induced by Zn(II) binding, may underlie the difference in the activity.

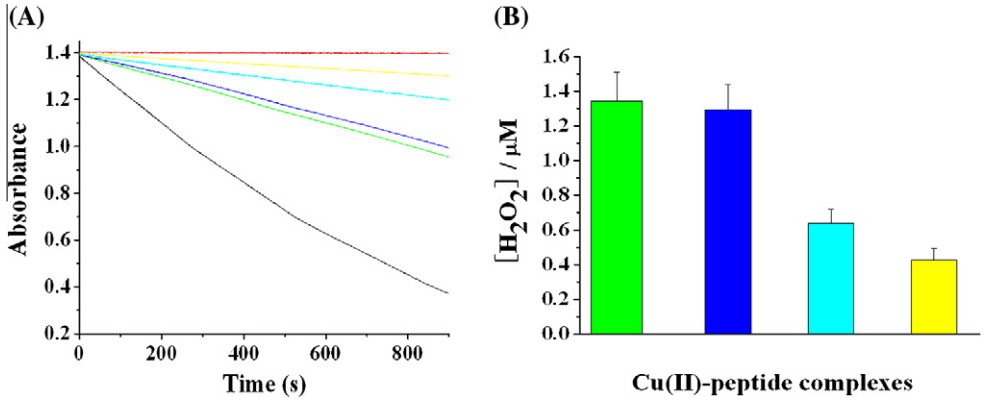

Fig. 3. (A) The change in AA (100 μM) absorbance as a function of reaction time in the absence (red curve) and the presence of different Cu(II)-containing species: Aβ(1–16)–Cu(II) (green curve), Aβ(1–42)–Cu(II) (blue curve), Zn(II)–Aβ(1–16)–Cu(II) (cyan curve), Zn(II)–Aβ(1–42)–Cu(II) (yellow curve) and free Cu(II) (black curve). (B) The amounts of H_2O_2 generated by Aβ(1–16)–Cu(II) (green bar), Aβ(1–42)–Cu(II) (blue bar) (cyan bar), Zn(II)–Aβ(1–16)–Cu(II) and Zn(II)–Aβ(1–42)–Cu(II) (yellow bar). The concentrations of Aβ(1–16), Aβ(1–42), Cu(II) and Zn(II) were all 5 μM. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

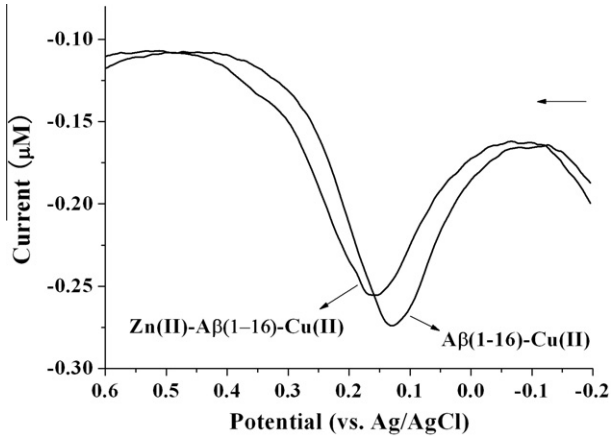

Fig. 4. The DPVs of Aβ(1–16)–Cu(II) and Zn(II)–Aβ(1–16)–Cu(II). The concentrations of Aβ(1–16), Cu(II) and Zn(II) were 100 μM each. The arrow indicates the scan direction.

Electrochemical studies can provide direct evidence to define the aforementioned reactivity of the Cu(II) complexes. Therefore, it would be informative to determine if the redox potential of Aβ–Cu(II) is affected by the coordination of Zn(II) ions. Accordingly, we performed DPV to determine the oxidation potential of the Aβ(1–16)–Cu(II) complex in the presence or absence of Zn(II) ions. As shown in Fig. 4, the signal at 0.132 V can be attributed to the anodic peak of Aβ(1–16)–Cu(II). There is an apparent potential shift to 0.168 V with the addition of Zn(II) ions. The positive shift indicates that Zn(II)–Aβ(1–16)–Cu(I) is more stable than Aβ(1–16)–Cu(I), and the oxidation of Cu(I) to Cu(II) by O_2 is kinetically slow. Thus, the rate of reaction 2 becomes slower. This result explains the above-mentioned kinetic data extremely well.

4. Discussion

Although it is commonly accepted that the interaction between Zn(II) and Cu(II) and Aβ is linked to AD, how the metal ions function and why they bind to Aβ only in AD but not under healthy conditions remain poorly understood. In the present work, we have identified the formation of a Zn(II)–Aβ(1–16)–Cu(II) ternary complex and investigated the influence of Zn(II) on the activity of Aβ(1–16)–Cu(II). The obtained results indicate that Zn(II) ions depress the catalytic activity of the Cu(II) center, due to the formation of mixed metal complex.

Our finding has significant biological relevance and may offer some insight into the role of Aβ and metal ions in the etiology of AD. It has been estimated that concentrations of up to 300 μM Zn(II) are present at the synaptic cleft of glutaminergic neurons [29]. This so-called synaptic Zn(II) pool is thought to be the source of the Zn(II) bound to amyloid plaques in AD. The concentration of Zn(II) is higher than that of Cu(II) in the same region. We hypothesize that in healthy brains, Zn(II) ions play a neuroprotective role by binding to Aβ monomers or aggregates in the presence or absence of bound Cu(II). Although Zn(II)–Aβ–Cu(II) can promote the production of low levels of H_2O_2 in vivo, low levels of H_2O_2 are readily scavenged by enzymes, such as catalase and glutathione peroxidase, as well as by antioxidants, such as AA and uric acid. When most of the Zn(II) ions are sequestered by Aβ species in the brain, excess Aβ monomers or aggregates deprive Cu(II) from other physiologically relevant Cu(II)–peptide and –protein complexes to form Aβ–Cu(II) aggregates. The high propensity of Aβ to aggregate and precipitate facilitates the accumulation of Cu(II) near the cell membrane. Without the protection of Zn(II), Aβ–Cu(II) aggregates promote the incessant production of large quantities of H_2O_2 in the proximity of the cell membrane, causing more severe membrane damage and resulting in faster neuronal cell death.

References

[1] J. Hardy, D.J. Selkoe, Medicine – The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics, Science 297 (2002) 353–356.
[2] S. Varadarajan, S. Yatin, M. Aksenova, et al., Review: Alzheimer's amyloid beta-peptide-associated free radical oxidative stress and neurotoxicity, J. Struct. Biol. 130 (2000) 184–208.
[3] A.I. Bush, The metallobiology of Alzheimer's disease, Trends Neurosci. 26 (2003) 207–214.
[4] G. Liu, W. Huang, R.D. Moir, et al., Metal exposure and Alzheimer's pathogenesis, J. Struct. Biol. 155 (2006) 45–51.
[5] J. Dong, J.E. Shokes, R.A. Scott, et al., Modulating amyloid self-assembly and fibril morphology with Zn(II). J. Am. Chem. Soc. (2006).
[6] P. Faller, Copper and zinc binding to amyloid-β: coordination, dynamics, aggregation, reactivity and metal-ion transfer, Chem. BioChem. 10 (2009) 2837–2845.
[7] C. Ha, J. Ryu, C.B. Park, Metal ions differentially influence the aggregation and deposition of Alzheimer's β-amyloid on a solid template, Biochemistry 46 (2007) 6118–6125.
[8] R. Jakob-Roetne, H. Jacobsen, Alzheimer's disease: from pathology to therapeutic approaches, Angew. Chem. Int. Ed. 48 (2009) 3030–3059.
[9] J.W. Karr, L.J. Kaupp, V.A. Szalai, Amyloid-β binds Cu^{2+} in a mononuclear metal ion binding site, J. Am. Chem. Soc. 126 (2004) 13534–13538.
[10] J. Shearer, V.A. Szalai, The amyloid-β peptide of Alzheimer's disease binds cu[I] in a linear Bis–His coordination environment: insight into a possible neuroprotective mechanism for the amyloid-β peptide, J. Am. Chem. Soc. 130 (2008) 17826–17835.
[11] A. Sigel, H. Sigel, R.K.O. Sigel, Metal Ions in Life Sciences, John Wiley & Sons, West Sussex, 2006.
[12] C.D. Syme, R.C. Nadal, S.E.J. Rigby, et al., Copper binding to the amyloid-beta peptide associated with Alzheimer's disease, J. Biol. Chem. 279 (2004) 18169–18177.
[13] M.A. Lovell, J.D. Robertson, W.J. Teesdale, et al., Copper, iron and zinc in Alzheimer's disease senile plaques, J. Neurol. Sci. 158 (1998) 47–52.
[14] D. Jiang, X. Li, L. Liu, et al., Reaction rates and mechanism of the ascorbic acid oxidation by molecular oxygen facilitated by Cu(II)-Containing amyloid-β complexes and aggregates, J. Phys. Chem. B 114 (2010) 4896–4903.
[15] S.C. Drew, C.J. Noble, C.L. Masters, et al., Pleomorphic copper coordination by Alzheimer's disease amyloid-beta peptide, J. Am. Chem. Soc. 131 (2009) 1195–1207.
[16] P. Faller, C. Hureau, Bioinorganic chemistry of copper and zinc ions coordinated to amyloid-β peptide. Dalton Trans. (2009) 1080–1094.
[17] N.C. Maitti, D. Jiang, A.J. Wain, et al., Mechanistic studies of Cu(II) binding to amyloid-β peptides and fluorescence and redox behaviors of the resulting complexes, J. Phys. Chem. B 112 (2008) 8406–8411.
[18] L.Q. Hatcher, L. Hong, W.D. Bush, et al., Quantification of the binding constat of copper(II) to the Amyloid-Beta peptide, J. Phys. Chem. B 112 (2008) 8160–8164.
[19] Q.-F. Ma, J. Hu, W.-H. Wu, et al., Characterization of copper binding to the peptide amyloid-beta(1–16) associated with Alzheimer's disease, Biopolymers 83 (2006) 20–31.
[20] N.C. Maiti, D. Jiang, A.J. Wain, et al., Mechanistic studies of Cu(II) binding to amyloid-b peptides and fluorescence and redox behaviors of the resulting complexes, J. Phys. Chem. B 112 (2008) 8406–8411.
[21] C.J. Sarell, C.D. Syme, S.E.J. Rigby, et al., Copper(II) binding to amyloid-β fibrils of Alzheimer's disease reveals a picomolar affinity: stoichiometry and coordination geometry are independent of Aβ oligomeric form, Biochemistry 48 (2009) 4388–4402.
[22] V. Tõugu, A. Karafin, P. Palumaa, Binding of zinc(II) and copper(II) to the full-length Alzheimer's amyloid-beta peptide, J. Neurochem. 104 (2008) 1249–1259.
[23] C.S. Atwood, R.C. Scarpa, X. Huang, et al., Characterization of copper interactions with Alzheimer amyloid-β peptides: identification of an attomolar-affinity copper binding site on amyloid β_{1-42}, J. Neurochem. 75 (2000) 1219–1233.
[24] L. Guilloreau, L. Damian, Y. Coppel, et al., Structural and thermodynamical properties of CuII amyloid-beta16/28 complexes associated with Alzheimer's disease J, Biol. Inorg. Chem. 11 (2006) 1024–1038.
[25] A. Clements, D. Allsop, D.M. Walsh, et al., Aggregation and metal-binding properties of mutant forms of the amyloid Aβ peptide of Alzheimer's disease, J. Neurochem. 66 (1996) 740–747.
[26] W. Garzon-Rodriguez, A.K. Yatsimirsky, C.G. Glabe, Binding of Zn(II), Cu(II), and Fe(II) ions to Alzheimer's all peptide studied by fluorescence, Bioorg. Med. Chem. Lett. 9 (1999) 2243–2248.
[27] C.A. Damante, K. Ösz, Z. Nagy, et al., Zn^{2+}'s ability to alter the distribution of Cu^{2+} among the available binding sites of Aβ(1–16)-polyethylenglycol-ylated peptide: implications in Alzheimer's disease, Inorg. Chem. 50 (2011) 5342–5350.
[28] L. Liu, D. Jiang, A. McDonald, et al., Copper redox cycling in the prion protein depends critically on binding mode, J. Am. Chem. Soc. 133 (2011) 12229–12237.
[29] E.L. Que, D.W. Domaille, C.J. Chang, Metals in neurobiology: probing their chemistry and biology with molecular imaging, Chem. Rev. 108 (2008) 1517–1549.