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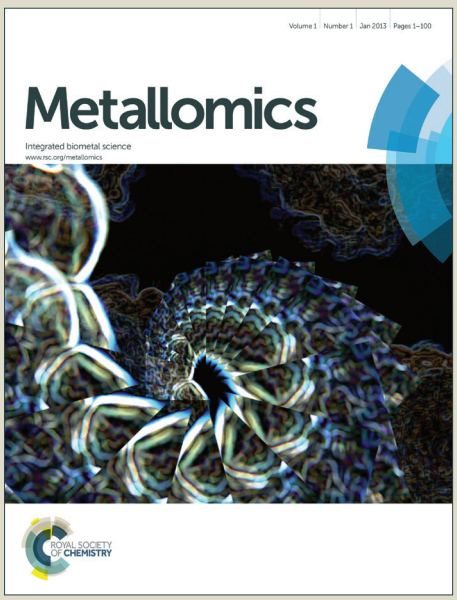





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**Minireview article**

# Is interaction of amyloid β-peptides with metals involved in cognitive activity?

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**Abstract**

Metal ions, i.e., $Zn^{2+}$ and $Cu^{2+}$, are released from neuron terminals in the hippocampus, which plays important roles for spatial and declarative memory, and may serve as a signal factor. Synaptic homeostasis of metal ions is critical for cognitive activity in the hippocampus. Amyloid-β (Aβ) is a causative candidate for the pathogenesis of Alzheimer's disease (AD) and Aβ-induced synapse dysfunction is easy to emerge along with normal aging and leads to the cognitive decline and memory loss in the pre-dementia stage of AD. Because Aβ interacts with $Zn^{2+}$ and $Cu^{2+}$, it is likely that these metal ions are involved in Aβ-induced modification of synaptic function. There is evidence to indicate that the inhibition of the interaction of Aβ with $Zn^{2+}$ and $Cu^{2+}$ may ameliorate the pathophysiology of AD. Interaction of extracellular $Zn^{2+}$ with Aβ in the hippocampus is involved in transiently Aβ-induced cognition deficits, while interaction of extracellular $Cu^{2+}$ reduces bioavailability of intracellular $Cu^{2+}$, followed by increase in oxidative stress, which may lead to cognitive deficits. It is likely that $Zn^{2+}$ and $Cu^{2+}$ play as a key-mediating factor in pathophysiology of synaptic dysfunction in which Aβ is involved. On the basis of the idea that understating Aβ-induced changes in synaptic plasticity are important to prevent AD, the present paper summarize the interaction of Aβ with metal ions in cognition.

## 1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly and is a progressive neurological disease. In AD, the pathogenesis is thought to need the progress for 20–30 years before clinical onset.[1,2] The clinical disease stages of AD have been divided into three phases.[3,4] First is a pre-symptomatic phase in which individuals are normal in cognition but some have pathological changes in AD. Second is a prodromal phase of AD, commonly referred to as mild cognitive impairment (MCI),[5] which is characterized by the onset of the earliest cognitive symptoms. The symptoms are typically episodic memory deficits and do not meet the criteria for dementia. The final phase in the evolution of AD is dementia, defined as impairments in multiple cognitive domains that are severe enough to produce loss of function. Individuals with MCI have 32% fewer neurons in the entorhinal cortex and show synaptic loss in the dentate gyrus, which correlates with cognitive deficits.[6-8] This cellular disconnection suggests that the dentate dyrus may be one of the earliest sites to display synaptic dysfunction.[9] Postmortem studies also suggest that the hippocampus and entorhinal cortex are the first brain regions to be affected.[10]

Neurofibrillary tangles and neuritic senile plaques are the two pathological hallmarks that define AD. Tau protein is the major component of neurofibrillary tangles. β-Amyloid (Aβ) is the major component of neuritic senile plaques, which plays a key role in AD pathogenesis. The amyloid cascade hypothesis has proposed that the key event in AD development is the extracellular accumulation of insoluble, fibrillar Aβ.[11–13] This hypothesis has later modified to acknowledge soluble Aβ oligomers as pathogenic agents.[14-16] A heterogeneous pool of monomeric Aβ varying in length from 37 to 49 amino acids is produced by proteolytic cleavage from the transmembrane amyloid precursor protein (APP) by β- and γ-secretases.[17,18] Aβ is

prone to self-assemble into multiple aggregates that are termed oligomers, protofibrils or mature amyloid fibrils based on their appearance by electron or atomic force microscopy (Fig. 1). Aβ1-40, which comprises 40 amino acids, and Aβ1-42, C-terminally extended by two residues, are the two most abundant isoforms. Aβ1-40 is approximately 10 times as abundant as Aβ1-42 in human plasma and cerebrospinal fluid.[19] Importantly, Aβ1-42 has high tendency to form the aggregates and is neurotoxic more than Aβ1-40. A lot of research has aimed at understanding the Aβ aggregation mechanism and identifying the intermediate species that occur along the aggregation pathway. The current amyloid cascade hypothesis suggests that AD pathogenesis is mediated by soluble Aβ oligomers.[20,21]

On the other hand, homeostasis of metals in the brain is perturbed in AD patients. Metal ions such as copper, zinc, and iron have been implicated in the pathogenesis of AD through a variety of mechanisms including increased Aβ affinity and redox effects. Zinc, copper and iron are detected at high levels (Zn: 1.1 mM, Cu: 0.4 mM, Fe: 0.9 mM) in the senile plaques in the AD-affected brain compared to those in neuropils in non-demented brains (Zn: 0.4 mM, Cu: 0.07 mM, Fe: 0.3 mM).[22] In the pathogenesis of AD, furthermore, each of the major protein participants has physiologically important interactions with these metals: APP is the neuronal iron export ferroxidase with a major interaction with ferroportin. Presenilins function as a part of the γ-secretase intramembrane protease complex and are involved in the import of copper and zinc into neurons. Tau is involved in the export of neuronal iron by facilitating the trafficking of APP to the membrane surface.[23,24] Therefore, amyloid and tau pathophysiology are closely linked to changes in metal dynamics in the brain, which may contribute to the pathogenesis in AD.

Memory function normally declines along with aging, and is believed to decline initially due to changes in synaptic function rather than loss of neurons.[25] Some

individuals are led to develop AD with progressive neurodegeneration. One of the pathophysiological mechanisms mediated with Aβ is to alter synaptic function such as long-term potentiation (LTP) and long-term depression (LTD), which are believed to be cellular mechanisms of learning and memory, prior to visible appearance of neuronal loss. It has been reported that soluble Aβ oligomers inhibit LTP induction and facilitate LTD induction.[26-29] The cognitive deficit and memory loss occurring before any prominent neuronal loss is observed in patients with MCI or early-phase AD. On the other hand, Aβ is normally produced in the brain, where the in vivo concentration in the rodent has been estimated to be in the picomolar range.[30] It is reported that Aβ levels in the brain extracellular fluid is linked to cognitive activity.[31,32] The evidence suggests that the analysis on the interaction between Aβ and metals focused on the changes in synaptic plasticity is important to understand cognitive function under both pathological and physiological conditions and also to pursue the strategy to prevent cognitive deficits in the pre-dementia stage of AD. The present paper deals with insight into interaction of Aβ-peptides with metals in cognition.

## 2. Aβ-mediated modification in synaptic plasticity and cognition

Synaptic activity dynamically regulates extracellular Aβ levels in the brain. The relationship between synaptic activity and extracellular Aβ levels may be related to presynaptic activity[31]; extracellular Aβ levels are directly linked to synaptic vesicle exocytosis (Fig. 1). Synaptic vesicle release appears to be the primary mediator of dynamic changes in extracellular Aβ levels that are linked with synaptic activity and are independent of changes in APP processing.

Some studies using normal young animals show that endogenous Aβ is involved in learning and memory[33] ant that endogenous Aβ1-42 is a critical player in LTP and memory within the normal brain.[32] In a preparation containing both monomers and oligomers of Aβ1-42, the low picomolar concentrations cause significant enhancement of LTP in the hippocampus, while the high nanomolar concentrations cause well-established attenuation of LTP.[34] The action of synthesized Aβ in hippocampal LTP and reference memory shows bidirectional dose-response curves.[35]

In postmortem AD cortex, which include monomers and various oligomers, significant correlations are observed between the levels of Aβ oligomers and cognitive decline (MMSE scores) as well as the neuropathological hallmarks of AD.[36-38] Shankar et al.[39] report that soluble Aβ oligomers, which are extracted from the cortex of typical AD subjects, inhibit LTP and enhance LTD in hippocampal slices prepared form normal rodents. They indicate that soluble Aβ oligomers extracted from the AD brains impair synapse function as well as synapse structure and that dimers are the smallest synaptotoxic species. Intracerebroventricular injection of the extracts of soluble oligomers also disrupts memory after learning in normal rats. In contrast, insoluble amyloid plaque cores from the same AD brains did not impair LTP unless they are first solubilized to release Aβ dimers and other oligomers, suggesting that plaque cores have low bioactivity but sequester Aβ dimers that can be synaptotoxic if released.[39]

Aβ oligomers increase extracellular levels of glutamate[40,41] and the increase occurs via inhibition of glutamate reuptake by astrocytes and neurons after the release,[42] although it is unclear whether the inhibitory effect of Aβ oligomers on glutamate reuptake is due to direct binding to glutamate transporters. Soluble Aβ oligomers perturb synaptic plasticity by altering glutamate recycling at the synapse and promoting synapse depression.[42] Furthermore, Li et al. show that excess activation of extrasynaptic NR2B-containing NMDA (N-methyl-D-aspartate) receptors is involved

in Aβ oligomer-mediated inhibition of LTP without desensitization of synaptic NMDA receptors.[43]

On the other hand, intraneuronal Aβ is associated with the formation of reactive oxygen species and reactive nitrogen species, and induces calcium-dependent excitotoxicity, impairment of cellular respiration via mitochondrial dysfunction, and synaptic dysfunctions associated with cognitive deficits.[44] Oxidative stress associated with mitochondrial dysfunction has been implicated in the pathogenesis of MCI and AD.[45] The activation of anti-oxidation system ameliorates Aβ-induced cognitive deficits,[46,47] suggesting that activation of anti-oxidation system is a potential therapeutic target for cognitive decline in AD patients.

Aβ-induced inflammatory damage also leads to energy failure and synaptic dysfunction.[44] Activated microglia and reactive astrocytes localize to fibrillar plaques, and their biochemical markers are elevated in the brains of AD patients.[48] Initially, the phagocytic microglia engulf and degrade Aβ. However, chronically activated microglia release chemokines and a cascade of damaging cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor $\alpha$ (TNF-$\alpha$).[49] Inflammatory cytokines and chemokines, as well as reactive oxygen species and reactive nitrogen species, can alter synaptic activity, followed by dysfunction and loss of synapses, which correlates with cognitive decline.[50]

## 3. Metal-mediated modification in synaptic plasticity and cognition

Homeostasis of metals in the brain is strictly regulated by the brain barrier system, i.e., the blood-brain and blood-cerebrospinal fluid barriers. However, the blood-brain barrier is modified and/or disrupted in the process of neurological diseases including AD,[51,52] probably resulting in dyshomeostasis of metals in the brain

extracellular fluid followed by that in the brain parenchema. Although the exact concentrations of zinc, copper and iron in the brain extracellular fluid are unknown, it is reported that the concentrations of zinc, copper and iron in the human cerebrospinal fluid are 0.38 μM, 0.34 μM, and 0.54 μM, respectively.[53,54] A portion of these metals serves as metal ions in the brain extracellular fluid. $Zn^{2+}$ level in in the brain extracellular fluid is estimated to be 10 nM[55] and that in the intracellular (cytosol) compartment is estimated to be less than 1 nM,[56,57] while the levels of $Cu^{2+}$($Cu^{+}$) and $Fe^{3+}$($Fe^{2+}$) in both the extracellular and intracellular compartments are unknown.

The synaptic vesicles of presynaptic terminals are labeled by Timm's sulfide-silver staining method, suggesting that synaptic vesicles contain metals such as zinc and copper. The trisynaptic circuits in the hippocampus are glutamatergic and a subclass of glutamatergic neurons is zincergic. It has been reported that synaptic $Zn^{2+}$ release, which serves as a signal factor in both extracellular and intracellular compartments, is involved in LTP, i.e., mossy fiber LTP and CA1 LTP.[58-61] Even in non-zincergic synapses, intracellular $Zn^{2+}$ signaling in dentate granule cells, which mainly originates in the internal stores containing $Zn^{2+}$, is required for both LTP and object recognition.[62] On the other hand, excess intracellular $Zn^{2+}$ signaling in CA1 pyramidal cells, which is linked with $Zn^{2+}$ release from neuron terminals, affects CA1 LTP and transiently affects object recognition.[63] For synaptic neurotransmission, extracellular $Zn^{2+}$ levels in the hippocampus may be strictly regulated even during LTP induction.

$Cu^{2+}$ dynamics at the synapses and its involvement in cognition is poorly understood. $Cu^{2+}$ is released post-synaptically after NMDA receptor activation, which causes the rapid and reversible trafficking of Menkes ATPase to neuronal processes.[64] Menkes ATPase is directly required for the copper efflux. Synaptosomal copper release[65] and copper-mediated modulation of neuronal excitability[66] suggest $Cu^{2+}$

concentration is increased in the synaptic cleft during synaptic excitation. It is possible that extracellular $Cu^{2+}$ and/or intracellular $Cu^{2+}$ is involved in synaptic function such as changes in plasticity. Thus, extracellular $Cu^{2+}$ levels might also be strictly regulated in the hippocampus for synaptic neurotransmission.

## 4. Metal-mediated Aβ aggregation

Synthetic Aβ1-42 aggregates more readily than Aβ1-40 and Aβ1-42 readily seeds the in vitro aggregation of Aβ1-40. Aβ1-42 is likely to be more amyloidogenic and readily assembles into soluble oligomers and consequent fibril deposits.[67,68] $Cu^{2+}$ and $Zn^{2+}$ accelerate both Aβ1-40 and Aβ1-42 deposition but resulted only in the formation of amorphous (nonfibrillar) aggregates that is transformed to amyloid fibrils upon prolonged incubation. $Cu^{2+}$- or $Zn^{2+}$-induced aggregates of Aβ, i.e., soluble oligomers, have been implicated as the neurotoxic form of the peptides against synapse function and structure. In contrast, $Fe^{3+}$ induces the deposition of fibrillar amyloid plaques at neutral pH.[68] Aβ directly coordinates $Cu^{2+}$ and $Zn^{2+}$, but not $Fe^{3+}$ or other metal ions, within the cores of plaques. Iron is found in the plaque periphery and primarily bound to ferritin in the neuritic plaques.[69] Interestingly, rat and mouse Aβ possesses amino acid substitutions that reduce the interactions of metal ions, perhaps explaining why these animals are exceptional among mammals for not accumulating cerebral Aβ amyloid with aging.[70]

Aβ possesses selective affinity $Zn^{2+}$ binding sites, which are mediated with histidine. The original reported Kd of high affinity $Zn^{2+}$ binding is around 100 nM and that for low affinity binding is around 5 μM.[70] Although the values are controversial, $Zn^{2+}$ is bound to Aβ in the extracellular compartment because the micromolar concentration of $Zn^{2+}$ may be released from zincergic synapses.[71] Aβ also possesses

selective affinity $Cu^{2+}$ binding sites and the binding affinity of $Cu^{2+}$ is higher than that of $Zn^{2+}$.[70] $Cu^{2+}$ is also bound to Aβ in the extracellular compartment, although the concentration of $Cu^{2+}$ is controversial and potentially nanomolar. Equimolar amounts of $Zn^{2+}$ and $Cu^{2+}$ are bound to Aβ at pH 7.4 and Aβ binds up to 2.5 equivalents of either $Cu^{2+}$ or $Zn^{2+}$. Under acidic conditions (pH 6.6), however, $Cu^{2+}$ completely displaces $Zn^{2+}$ from Aβ. Sharma et al.[72] show that $Zn^{2+}$ forms insoluble amorphous aggregates and $Cu^{2+}$ induces formation of soluble Aβ1-42 oligomers and that $Zn^{2+}$ decreases Aβ1-42 toxicity by formation of non-toxic insoluble amorphous aggregates, while $Cu^{2+}$ enhances toxicity due to forming neurotoxic soluble Aβ1-42 oligomers.

## 5. Metal-Aβ-mediated modification in synaptic plasticity and cognition

Interestingly, the formation and accumulation of Aβ oligomers is enhanced at synaptic terminals after electrical and chemical stimulation when Aβ1-42 oligomers (500 nM) were added to hippocampal slices from rats and mice. Aβ oligomers are colocalized at the cell surface with NR2B receptor subunits. Clioquinol, an 8-hydroxyquinoline, is a lipophilic chelator for $Zn^{2+}$ and $Cu^{2+}$, and reduces the enhanced colocalization of Aβ oligomers.[73] Clioquinol has been shown to decrease Aβ deposits and to improve cognitive deficits in AD animal models. Clioquinol does not interact directly with Aβ, while is selectively bound to the Aβ-metal ion complexes and dissociates the metal ion from Aβ.[74] The colocalization is also reduced in brain slices from the synapse-specific vesicular zinc transporter ZnT-3 knockout mice.[73] These evidence suggests that synaptic $Zn^{2+}$ and $Cu^{2+}$ play critical roles in the formation of Aβ oligomers (Fig. 1), followed by glutamate excitotoxicity via activation of glutamate receptors containing NR2B subunits.

It is likely that crosstalk between metals and Aβ occurs under dynamic synapse environment. However, the involvement of the crosstalk in synaptic plasticity and cognitive activity is poorly understood, while it has been reported that preclinical and clinical data show the potential for metal chelation-based drug therapy for AD: Clioquinol reduces zinc accumulation in neuritic plaques and inhibits the amyloidogenic pathway in the AβPP/PS1 transgenic mouse brain.[75] Clioquinol also promotes the degradation of metal-dependent Aβ oligomers to restore endocytosis and ameliorates Aβ toxicity.[76] Multitarget-directed selenium-containing clioquinol derivatives show higher hydrogen peroxide scavenging and intracellular antioxidant activity than clioquinol, suggesting that the clioquinol derivatives are promising for the treatment of AD.[77] Furthermore, PBT2, a copper/zinc ionophore and second-generation 8-hydroxyquinoline analog, significantly lowers Aβ levels in the cerebrospinal fluid and improves cognitive performance over baseline in several key executive function tests.[78,79] On the other hand, it is important to assess crosstalk between metals and Aβ in short-term memory loss for the prevention of AD pathogenesis. We examined an idea that short-term cognition is transiently affected by a state of confusion in $Zn^{2+}$ transport system due to local increase in Aβ1-42 concentration in the normal brain. Aβ-mediated $Zn^{2+}$ influx into dentate granule cells transiently induces a short-term cognitive deficit via the attenuation of dentate dyrus LTP.[80] The attenuation of LTP is enhanced by addition of exogenous $Zn^{2+}$, while the cognitive deficit is rescued by the treatment with either extracellular or intracellular $Zn^{2+}$ chelators, suggesting that extracellular $Zn^{2+}$ in the dentate gyrus may play a key role in Aβ-induced cognition deficits (Fig. 1) and is a potential target to prevent short-term cognitive deficits. In contrast, it is possible that crosstalk between $Zn^{2+}$ and Aβ is involved in learning and memory under the appropriate circumstance because of physiological phenomena of Aβ action.[32,33]

It is unknown whether crosstalk between $Cu^{2+}$ and Aβ is involved in in synaptic plasticity (Fig. 1). $Cu^{2+}$ accentuates distinct misfolding of Aβ1-40 and Aβ1-42 peptides, and potentiates membrane disruption via Cu-Aβ1-42 oligomers.[81] Inhibition of human high-affinity copper importer Ctr1 orthologous in the nervous system of drosophila ameliorates Aβ1-42-induced AD-like symptoms, suggesting that $Cu^{2+}$ may play a causative role in AD pathogenesis, as either Aβ oligomers or aggregates are less toxic in the reduced copper environment or one with less copper binding.[82] A new copper-specific chelating agent, a bis-8-aminoquinoline PA1637, efficiently induces episodic memory recovery in a non-transgenic AD model after a single intracerebroventricular injection of Aβ1-42,[83] suggesting that $Cu^{2+}$ play a key role in Aβ-induced cognition deficits. In both the normal brain and the brain in the pre-dementia stage of AD, crosstalk between $Cu^{2+}$ and Aβ in cognition is an important issue to be clarified.

Intranasal administration of deferoxamine, a high-affinity iron chelator, reverses iron-induced memory deficits and inhibits amyloidogenic APP processing in a transgenic AD model mouse. The data suggest that intranasal treatment of deferoxamine may be useful for AD.[84] Amelioration of iron homeostasis is a potential strategy for prevention and treatment of AD. On the other hand, copper and iron are redox active and generate reactive oxygen species via Fenton reaction and the Haber–Weiss reaction. Aβ become pro-oxidant when bound to copper or iron, which can generate hydrogen peroxide.[85] APP or Aβ interactions with $Cu^{2+}$ induce reduction to $Cu^{+}$ in vitro, promoting neurotoxic hydrogen peroxide production.[86] Interaction of $Cu^{2+}$ and Aβ in the extracellular compartment reduces bioavailability of intracellular $Cu^{2+}$, followed by increase in oxidative stress via the reduced expression of anti-oxidative proteins such as superoxide dismutase,[87] which may influence cognitive activity.[88] Although hydrogen peroxide has a bidirectional role in the regulation of

hippocampal LTP,[89] it is possible that the interaction of intraneuronal Aβ with copper and iron affects synaptic function such as LTP via generation of reactive oxygen species (Fig. 1). Furthermore, extracellular Aβ oligomers with $Zn^{2+}$ and $Cu^{2+}$ may directly interact with astrocytes and microglia.[87] Thus it is also possible that inflammatory cytokines and chemokines, which are produced by their interaction, affects synaptic function such as LTP.

## 6. Perspective

It is likely that $Zn^{2+}$ and $Cu^{2+}$ are dynamically linked to neurotransmission at the synapses and that Aβ is also involved in synaptic function. However, the dynamics of metal ions and Aβ at the synapses and their interaction is poorly understood (Fig. 1), especially in the in vivo circumstance. Dynamics of Aβ is diversely changed by the circumstance. We need to pay attention to the difference in the interaction between the in vitro and in vivo circumstance. The strategy focusing on the Aβ-mediated changes in extracellular dynamics of $Zn^{2+}$ and $Cu^{2+}$ may be important to understand cognitive decline and memory loss in the pre-dementia stage of AD and also in normal aging. The strategy can lead to preventing the pathogenesis of AD.

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**Figure legend**

Figure 1. Cognitive decline via interaction of metals and Aβ at the synapses

Aβ is bound to $Zn^{2+}$ and $Cu^{2+}$ in the extracellular compartment, which often originates in the release form the presynaptic and postsynaptic terminals, and forms oligomers. Zn-Aβ1-42 oligomers may be taken up into postsynaptic neurons and transiently induce cognitive decline. Increase in intracellular $Zn^{2+}$, which is induced by the release from Zn-Aβ1-42, may lead to cognitive decline. Cu-Aβ1-42 oligomers may potentiate membrane disruption. Intraneuronal Aβ interacts with $Cu^{2+}$ and $Fe^{2+}$, and generates reactive oxygen species, potentially followed by the involvement in cognitive decline.