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Cu(II) binding to various forms of amyloid-β peptides. Are they friends or foes?

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\textbf{Abbreviations:}


\textbf{Abstract:}

In the present micro-review, we describe the Cu(II) binding to several forms of amyloid-β peptides, the peptides involved in Alzheimer’s disease. It has indeed been shown that in addition to the “full-length” peptide originating from the precursor protein after cleavage at position 1, several other shorter peptides do exist in large proportion and may be involved in the disease as well. Cu(II) binding to amyloid-β peptides is one of the key interactions that impact both the aggregating properties of the amyloid peptides and the Reactive Oxygen Species (ROS) production, two events linked to the etiology of the disease. Binding sites and affinity are described in correlation with Cu(II) induced ROS formation and Cu(II) altered aggregation, for amyloid peptides starting at position 1, 3, 4, 11 and for the corresponding pyroglutamate forms when they could be obtained (i.e. for peptides cleaved at positions 3 and 11). It appears that the current paradigm which points out a toxic role of the Cu(II) – amyloid-β interaction might well be shifted towards a possible protective role when the peptides considered are the N-terminally truncated ones.
1. Introduction

1.1 Alzheimer’s disease (AD)

Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by progressive cognitive and memory impairments. AD is the most common type of dementia in the elderly population representing 60-80% of all cases. There are two neurohistological hallmarks of AD: the amyloid (or senile) plaques, which are extracellular deposits of insoluble amyloid-β (Aβ) peptides under aggregated forms and the intracellular tau tangles. The mechanisms underlying the triggering and progression of the disease are still unclear. Nevertheless, according to the amyloid cascade hypothesis first proposed in the 90’s, the imbalance between production and clearance of the Aβ peptides is at the origin of the accumulation of peptides that further leads to the aggregation of the soluble monomeric peptide into fibrils and amorphous aggregates detected in the senile plaque. The amyloid cascade is an early event and plays a causal role in AD (Scheme 1). The complete aggregation pathway from the monomeric peptide to the fibrils contains several steps including oligomers, protofibrils and fibrils. Oligomeric species have been proposed to be the most toxic species, instigating further pathological events, including the formation of intracellular neurofibrillary tangles, destruction of synaptic connections, which would lead ultimately to neuronal cell death and dementia. Metal ions have been involved in the amyloid cascade process based on the detection of high levels of copper and zinc in the senile plaques and on in vitro studies probing their abilities to modify the aggregation process. In addition, the redox activity of copper is responsible for the production of Reactive Oxygen Species (ROS) that contribute to the oxidative stress observed in AD.
Scheme 1. Toxic events in the framework of the amyloid cascade hypothesis: (over)-production of the amyloidogenic Aβ peptide, formation of oligomeric species and ROS production associated to the formation of metal-Aβ complexes.

1.2 The amyloid-β peptides

Several Aβ peptides of various lengths are found in vivo and while the Aβ1-40/42 have been the focus of most of the reported studies, N-terminally truncated forms are also of interest since they represent a significant amount of the Aβ content in the brain (> 60%). In addition, the N-terminal modifications do alter dramatically the Cu(II) binding abilities of the peptides. This will be the focus of the next paragraphs.

a. APP cleavage

The Aβ peptides originate from the cleavage of the transmembrane Amyloid Precursor Protein (APP) by the β and γ-secretases, acting at the N- and C-terminal sites, respectively (Scheme 1). Because the C-terminal part of the Aβ sequence is buried in the membrane, proteolysis of the APP by the γ-secretase occurs at several sites and Aβ1-38 to Aβ1-43 peptides can be processed.\(^1\)

b. The full-length peptides

Among the “full-length” peptides (corresponding in the present review to Aβ1-38 - Aβ1-43) the 40 and 42 amino-acid residues forms are the most abundant in the Cerebro-Spinal Fluid (CSF), with the Aβ1.

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\(^1\)The peptides are noted Aβ\(_{n-m/p/q}\). This corresponds to a peptide starting at position \(n\) and ending at position \(m\), or \(p\) or \(q\) and so on.
representing 5-10% of the Aβ_{1-40} amount.\textsuperscript{[2]} The longer form is the most toxic in line with a higher propensity to aggregate. The Aβ peptides encompasses a N-terminal binding site (spanning from residues 1 to 16) and a C-terminal hydrophobic sequence involved in the aggregation process. Hence, it is considered that the Aβ_{1-16} peptide is an appropriate model with regard to metal ion binding. This sequence is classically used in spectroscopic studies due to its higher solubility that eases its handling. It has also been extensively used to study the coordination of Cu(II), Cu(I), Zn(II) and Fe(II) to Aβ.\textsuperscript{[16]}

c. N-terminally truncated peptides

As for the C-terminal site, the N-terminal site undergoes some cleavage heterogeneity with peptides starting at positions between 1 and 5.\textsuperscript{[9, 24-26]} The reason for such heterogeneity is unclear. In seminal reports by Masters and coworkers,\textsuperscript{[27, 28]} the quantity of Aβ_{4-n} was found to be predominant in the amyloid deposits of AD patients. This was further confirmed by mass-spectrometry analyses revealing that the dominating Aβ species in AD brains are Aβ_{4-42}, Aβ_{1-40} and Aβ_{1-42},\textsuperscript{[25]} and suggesting that N-terminally truncated peptides are abundant in aged human brains.\textsuperscript{[24]} Aβ_{11-n} peptides are also detected in plaques from sporadic AD brains representing up to 20% of the total content of Aβ peptides.\textsuperscript{[29]}

When the N-terminal deletion occur at position 3 or 11 corresponding to Glu residues, the formation of pyroglutamate groups can occur, leading to Aβ_{p3-n} and Aβ_{p11-n}, respectively. The pyroglutamate is generated by the action of the enzyme glutaminyl cyclase\textsuperscript{[30]} and/or spontaneously but slowly over time.\textsuperscript{[29]}

The various forms of the Aβ peptides discussed in the text are reminded in Scheme 2.

\textbf{Scheme 2.} Sequence of N-terminally truncated Aβ peptides discussed in the present review.

d. Cu(II) binding to the Aβ peptides

In the present micro-review, we focus on Cu(II) binding to the various Aβ peptides. It is indeed anticipated that the N-terminal modifications impact significantly the Cu(II) site and affinity that will further induce changes in ROS production and aggregation of the Cu-peptide complexes, two key events linked to the amyloid cascade.
2. Techniques to study Cu(II) coordination, affinity, ROS production and peptide aggregation

2.1 Coordination sites

There are several techniques to probe the Cu(II) site in a peptide, Electron Paramagnetic Resonance (EPR) being the most appropriate due to its easy access and the large quantity of information that can be extracted from a spectrum. Indeed the values of $g_\parallel$, $g_\perp$, and of the hyperfine and super-hyperfine couplings give access to the geometry and the nature of the binding atoms.\textsuperscript{31-34} Other techniques include UV-Vis and CD, X-ray absorption spectroscopy (both EXAFS and XANES),\textsuperscript{35} and NMR.\textsuperscript{32} The redox properties of the Cu-peptide complexes can be evaluated by cyclic or square-wave voltammetries.\textsuperscript{32, 36, 37}

2.2 Affinity

There are mainly two ways of evaluating the affinity of Cu(II) for a peptide: direct titration or indirect competition experiments. In general, for direct titration experiment, quenching of the fluorescence of Tyr10 (in case of Aβ, but could also be Trp residues) or calorimetric (ITC) measurements are used.\textsuperscript{38, 39} For competition experiments, any competitor having an affinity close to the one of the peptide and leading to a Cu(II) complex with a spectroscopic signature (in EPR, UV-Vis or CD) different than that the Cu(II) peptidic species can be used (see for instance, ref. \textsuperscript{40}). Potentiometry is another accurate and direct method of establishing affinity constants. Protonation and stability constants for the Cu(II) complexes of short Aβ peptides, such Aβ\textsubscript{x-16}, can be calculated from pH-metric acid-base titrations.\textsuperscript{39}

2.3 ROS production

ROS production that corresponds to the incomplete (Cu-catalyzed) reduction of O\textsubscript{2} can be monitored by several indirect methods and as a function of time (Scheme 3). Consumption of ascorbate, the most classically used reductant, can be followed by UV-Vis spectroscopy at 265 nm.\textsuperscript{22} It is almost impossible to probe the formation of superoxide due to its high rate of reaction with ascorbate (and reduced ascorbate). Superoxide was detected via the reduction of CytC only when the Cu(Aβ) was reduced by electrolysis.\textsuperscript{41} There are several tools to detect the formation of hydrogen peroxide,\textsuperscript{22} such as the amplex red assay (formation of the resorufin luminophore) and H\textsubscript{2}DCF assay (formation of 2,7-dichlorofluorescein) and the formation of the hydroxyl radical, such as the CCA assay (formation of the fluorescent 7-OH-CCA adduct) and TBARS assay.\textsuperscript{22}
Scheme 3. Catalytic production of ROS by Cu(Aβ), from dioxygen and ascorbate as reductant and some methods to detect the production of the various ROS by spectrophotometry.

2.4 Aggregation

The aggregation of amyloid peptides is driven by the auto-catalytic self-assembly of monomeric peptides leading to the formation of β-sheet rich fibrils. While the kinetic of fibrils formation is mathematically described\(^{[42]}\) by a sigmoid-like curve (see equation 1 and Scheme 4), the mechanism of amorphous aggregates formation is not well-understood.

The fibril formation displays three distinct phases. The initial phase is the lag phase, as related to the nucleation process, followed by a steep growing phase corresponding to the elongation process up to a stationary phase when the system have reached the thermodynamic equilibrium (Scheme 4). The first stage (lag phase linked to the nucleation process) is the kinetically limiting step where monomeric peptides dimerize and/or oligomerize. When a critical amount of β-sheets is reached, fast auto-assembly occurs (elongation step) finally leading to fibrils. As the system is now at its equilibrium, no more change is observed, this is the stationary phase. In order to describe the fibrils formation, two parameters are generally considered: the quantity of fibrils formed for a given starting concentration of monomeric peptides and the half-time \((t_{1/2})\), which is the time needed to reach half of the maximum of fibrils content.\(^{[43-46]}\)

Equation 1:
Where \( k \) is the elongation rate, \( A \) the amplitude, \( t_{1/2} \) the time point when half of the maximal intensity is reached, and \( F_0 \) the baseline before aggregation.

In vitro, the Aβ aggregation process can monitored as a function of time by several spectroscopic techniques: \(^{[47]}\) (i) CD by probing β-sheets content, \(^{[48]}\) (ii) raman and infra-red spectroscopy, which provide information on secondary and tertiary structures changes at all stages of fibrillation, \(^{[49]}\) (iii) nuclear magnetic resonance (NMR); liquid and solid state, \(^{[50-52]}\) (iv) turbidity and light scattering, \(^{[53, 54]}\) (v) native gel electrophoresis \(^{[55]}\) and (vi) by addition of chromophores either changing their UV-visible absorption upon interactions with β-sheets (like Congo Red for instance) \(^{[56]}\) or their fluorescent properties. \(^{[57, 58]}\) A very classical assay relies on the use of Thioflavin-T (ThT), a dye that shows a characteristic red shift in the excitation spectrum and strong enhancement of fluorescence quantum yield upon binding to fibrillar structures. \(^{[59-62]}\)

Amyloid fibrils are characterized by laminated β-sheets whose strands run perpendicular to the long-axis of the fibril. \(^{[59, 62, 63]}\) This amyloid composition produces the characteristic ~5 Å and ~10 Å reflections observed in X-ray diffraction experiments, which are attributed to the strand spacing within and between β-sheet layers, respectively. \(^{[64]}\)

The detection of intermediates species, especially oligomers, is more challenging. Nevertheless, various techniques can be used. \(^{[65]}\) These methods include recognition by specific antibodies, \(^{[66]}\) mass spectrometry, \(^{[45]}\) FRET, \(^{[58]}\) and electrophoresis on native gel. \(^{[67]}\)

Furthermore, morphology of the aggregates formed can be evaluated by microscopy (Transmission Electron or Atomic Force Microscopy). \(^{[49, 68]}\)

Each of these techniques offers advantages but also drawbacks in terms of technique-related artifacts, \(^{[45]}\) which can anyway be overcome by using multiple independent analytical techniques in parallel.
Scheme 4. Scheme of the Aβ peptide self-assembly process. Applicability range for the techniques used to monitor the different phases of the aggregation process.

3. Cu(II) and “full-length” Aβ peptides

The human Aβ binds preferentially one Cu(II) ion in the N-terminal domain. The rest of the sequence can modify the affinity maybe via second sphere interactions. The Aβ116 has been recognized as a trustworthy model for the study of Cu(II) binding to the full-length peptide. Binding of additional equivalents of Cu(II) ion has been reported[69] but is likely not biologically relevant and thus will not be commented on in the present micro-review.

Coordination site. The Cu(II) binding sites to human Aβ peptides have been extensively studied with various complementary techniques (e.g. EPR and advanced EPR, NMR, UV-Vis and CD, Raman and potentiometry) and has been recently reviewed[16, 33, 34]. Briefly, around neutral pH, the Cu(II)-(Aβ) complex exists in two main forms, noted component I (predominant at lower pH) and II (predominant at higher pH) (Scheme 5.A). Component II is obtained from component I by the deprotonation of an amide bond of the peptide backbone and subsequent binding to the Cu(II). The pKa corresponding to the transition between I and II is near pH 7.8. Both forms bind the Cu(II) in a distorted square planar geometry. In component I, Cu(II) is equatorially bound to Asp1 via the terminal amine, the adjacent CO from the Asp1-Ala2 peptide bond, a N atom from imidazole ring of His6 and another one from His13 (component Ia) or from His14 (component Ib) or two N atoms from the His13 and 14 (component Ic). The apical position may be occupied by an oxygen atom, coming either from a water molecule,[70] or from carboxylate groups from side chains of Asp or Glu.[71] In component II, the main equatorial Cu(II) ligands are the N-terminal amine, the amidyl function from the Asp1-Ala2 peptide...
bond, the adjacent CO from the Ala2-Glu3 peptide bond, and one N atom from the imidazole ring of one of the three His. The carboxylate group from Asp1 has been proposed as apical ligand,\[^{[70]}\] likely via a water molecule as bridge.

**Scheme 5.** Proposed coordination sites of Cu(II)-hAβ (A), Cu(II)-Aβ\(_{3-16}\) (B), Cu(II)-Aβ\(_{p3-16}\) (C), Cu(II)-Aβ\(_{4-16}\) (D) and Cu(II)-Aβ\(_{11-16}\) (E).

**Affinity.** As stated above, the high affinity Cu(II) binding site is located in the first 16 N-terminal residues. There are many reports on the evaluation of the Cu(II) affinity for the Aβ peptide. The divergence in the affinity value between studies originated from the use of different evaluation methods.\[^{[39]}\] This has recently been sorted out,\[^{[38, 40]}\] and a conditional value of 1.6 \(10^9\) M\(^{-1}\) at pH 7.1 and of 1.1 \(10^{10}\) M\(^{-1}\) at pH 7.4 for the Aβ\(_{1-16}\) make consensus (the conditional value is the value at a
given pH with no competing buffer).\textsuperscript{[72]} The Cu(II) affinity seems to weakly increase with the length of peptide.\textsuperscript{[38, 40, 72, 73]}

**ROS production.** The ability of Cu(Aβ) complexes to generate ROS is in line with the hypothesis that they participate in the oxidative stress observed in AD brains post mortem.\textsuperscript{[74]} Such an ability relies on the possibility to have a reaction between the reductant (the physiological one is ascorbate) and the Cu\textsuperscript{II}(Aβ) and between the Cu\textsuperscript{I}(Aβ) and dioxygen, superoxide and/or hydrogen peroxide. Those reactions were recently probed by several methods (see § 2.3) and it was shown that (i) the Cu(Aβ) do produce ROS, (ii) the rate of ROS production is weaker than for “loosely bound” Cu, corresponding to Cu in buffer and (iii) the rate is significant if compared to other biologically relevant peptides, such as GHK and DAHK.\textsuperscript{[74-79]} A direct comparison between ROS production by “loosely bound” Cu and Cu(Aβ) species is difficult since it will depend on the experimental conditions (concentrations in Cu, ascorbate, dioxygen and peptides). Hence, the ascorbate consumption rate of Cu(Aβ) is about half that of “loosely bound” Cu. The length of the peptide has almost no impact on the ROS production rate, but because the peptide can itself be oxidized by the ROS produced, the quantity of ROS escaping the system is weaker when the peptide is longer.\textsuperscript{[74]}

**Aggregation.** The effect of the Cu(II) on Aβ\textsubscript{1-40/42} aggregation has been discussed widely,\textsuperscript{[17, 43, 80]} and consensus takes long time to be reached. Indeed, a major controversial point is due to the intrinsic complex nature of Aβ aggregates. Amorphous or/and fibrillar aggregates could be formed from monomeric Aβ. Depending of analytical techniques used, researchers were able (or not) to discriminate the nature of the aggregates. Also, experimental parameters such Cu(II) / Aβ stoichiometry or pH seem to have a dramatic impact on the outcome of aggregation assays. Nowadays, studies seem to converge on different aspects. On one hand, the presence of sub-stoichiometric Cu(II) seems to accelerate the formation of fibrillar aggregates\textsuperscript{[81]} likely due to a metal-mediated dimerization,\textsuperscript{[82, 83]} and a stabilization of oligomers.\textsuperscript{[84, 85]} On the other hand, in excess, Cu(II) generates more amorphous and less fibrillar aggregates.\textsuperscript{[86-88]} It has also been shown that removing Cu(II) from Aβ amorphous aggregates using an external chelator restore fibrillary-type aggregates.\textsuperscript{[89]} Another key parameter in the Aβ aggregation is the pH. It has been shown that Aβ aggregation occurs faster at acidic pH,\textsuperscript{[90, 91]} and Cu(II) is able to interact with Aβ aggregation even at mildly acid pH,\textsuperscript{[92]} in a rapid time frame.\textsuperscript{[93]} Nevertheless, below pH=5, Cu(II) do not impact Aβ aggregation,\textsuperscript{[82]} likely due to its inability to strongly interact with Aβ.\textsuperscript{[73]}
4. N-truncated peptides

4.1 ATCUN type motifs

For a long time the “full-length” Aβ\(_{1-40/42}\) peptides (or the corresponding C-terminally truncated forms Aβ\(_{1-16/28}\)) have been the primary focus of Cu(II) binding studies.\(^{[16]}\) However, it has been reported that the N-truncated Aβ\(_{4-42}\) peptide is one of the major form of Aβ peptides detected in healthy and diseased brains.\(^{[24-28]}\)\(^{[4-9]}\) The Aβ\(_{4-40/42}\) and Aβ\(_{11-40/42}\) peptide share a common feature with respect to Cu(II) binding site, since they present a N-terminal ATCUN (amino-terminal copper and nickel) motif characterized by the sequence H\(_2\)N-Xxx-Yyy-His.\(^{[97, 98]}\) It is worth noting that a main difference between the Aβ\(_{4-x}\) and Aβ\(_{11-x}\) is the possibility for the latter to form pyroglutamate counterparts. Hence, while the Aβ\(_{11-40/42}\) have been detected in large quantity in the CSF and amyloid plaques,\(^{[29, 94, 99-101]}\) they can degrade rapidly into pyroglutamate forms.\(^{[25, 94]}\)

Such N-terminal truncations, which can also happen at other sites between Asp\(_1\) and Arg\(_5\), are linked to the enzymatic cleavage activity of secretases that depends on many factors as reviewed in ref.\(^{[9]}\).

The formation of the Aβ\(_{11-40/42}\) peptide is due to the activity of a secondary β-secretase (β\(^+\)).\(^{[95, 96, 102]}\)

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**Coordination site.** ATCUN containing peptides can bind Cu(II) avidly because the binding site is made of three adjacent metallacycles of 5, 5 and 6 membered rings, the Cu(II) being bound by the N-terminal amine, the imidazole ring from the His and the two deprotonated peptidic functions in between.\(^{[77, 97, 98]}\) The ATCUN coordination mode of Cu(II) to Aβ\(_{4,a}\) and to Aβ\(_{11,a}\) is predominant from pH 5, while deprotonation of outer-sphere and non-binding side chains occurs when the pH is increased.\(^{[103-105]}\) A weak fifth ligand, a water molecule, can be present as apical ligand. This coordination is analogous to the one proposed for the DAHK tetrapeptide, the N-terminal sequence of human serum albumin, the structure of which was resolved by X-ray diffraction.\(^{[77]}\) The spectroscopic signatures of Cu(Aβ\(_{4,16}\)) and Cu(Aβ\(_{11,15}\)) used as models of the Aβ\(_{4,42}\) and Aβ\(_{11,42}\) peptide, are fully reminiscent of those of the Cu(II) parent complexes, such as Cu(DAHK) and Cu(AAH) (See Table 1). The formation of such ATCUN motif would preclude the formation of ternary species between Cu(Aβ\(_{4/11-a}\)) and a chelating drug candidate,\(^{[106]}\) in contrast to what has been reported in case of the Aβ\(_{1,a}\) peptide.\(^{[106-108]}\)

**Affinity.** While Cu(II) binds to Aβ\(_{1-16}\) with a relatively high affinity (Ka≈10\(^{10}\) M\(^{-1}\) at pH 7.4, see table 1), Aβ\(_{4-16}\) and Aβ\(_{11-15}\), bind Cu(II) with a very high affinity (Ka≈10\(^{13.5}\) M\(^{-1}\) at pH 7.4) through their ATCUN motif (Scheme 5D).\(^{[103, 104]}\) Such affinity is perfectly in line with those of other ATCUN
peptides.\textsuperscript{109, 110} Note that a secondary Cu(II) site is present in Aβ\textsubscript{4-16} and is relatively weak (K\textsubscript{a} = 10\textsuperscript{6.7} M\textsuperscript{-1} at pH = 7.4) and separated from the N-terminal site.\textsuperscript{103} The more than three orders of magnitude higher affinity for Cu(II) compared to the full-length peptides make these peptides key biological partners in the trafficking of Cu(II) within the synaptic cleft.

**ROS production.** Up to now, the ROS production has only been studied with the Aβ\textsubscript{4-16}. It has been shown that Cu(Aβ\textsubscript{4-x}) can only be oxidized and at relatively high cathodic potential (0.8 V vs. SCE) and that, in line with this redox properties, it doesn’t yield ROS under biologically relevant conditions.\textsuperscript{111} The other truncated ATCUN-type Aβ peptides Aβ\textsubscript{11-x} might have similar properties to those of Aβ\textsubscript{4-42}. However it remains to be determined if the ATCUN site of Aβ\textsubscript{11-x} peptides can undergo redox cycling and generate ROS.

**Aggregation.** As non-truncated Aβ, Aβ\textsubscript{4-40/42} is also able to aggregate and cause cell toxicity and memory impairment in mice. In vitro experiments have also shown that in ThT fluorescence assay, the maximal ThT intensities reached by Aβ\textsubscript{38/40/42} were similar to the one of Aβ\textsubscript{1-42}, but decreased faster. This could be explained by a burial of ThT-binding site as aggregates evolve. TEM images showed an important difference in the morphology at the aggregation end-point: the N-truncated peptide showed clumps of fibrillar aggregates, while Aβ\textsubscript{1-42} show longer individual fibrils.\textsuperscript{112} Aβ\textsubscript{11-40} forms fibrils faster than Aβ\textsubscript{1-40} and Aβ\textsubscript{1-42} and it does co-assemble with Aβ\textsubscript{1-40} but not Aβ\textsubscript{1-42}.\textsuperscript{104, 113} Cu(II) addition to Aβ\textsubscript{11-28/40/42} seems to preclude fibrillary aggregation,\textsuperscript{104, 114, 115} likely due to the strong 1:1 Cu(II) - ATCUN coordination that destabilize peptide-peptide interactions required for the nucleation of the aggregation process.\textsuperscript{44}

### 4.2 Other motifs

N-terminal truncated peptides in position 3 and their pyroglutamate counterparts have been found in high amount in the brain of Alzheimer’s patients (up to 25\% of the plaques content).\textsuperscript{116, 117} These peptides are considered to be more toxic than the full-length ones,\textsuperscript{118-131} in link with modified plaque morphology, hampered degradation of the pyroglutamate forms, higher propensity to form β-sheets and impact on full-length peptides aggregation process. Formation of pyroglutamate at position 11 (see previous paragraph) is also possible but its effect has been less studied.\textsuperscript{124, 132, 133}

**Coordination site.** Despite the similarity between the sequences of Aβ\textsubscript{1-16} and Aβ\textsubscript{3-16}, the Cu(II) binding sites to the Aβ\textsubscript{3-16} peptide are significantly different to those of Aβ\textsubscript{1-16}. The main form of Cu(Aβ\textsubscript{3-16}) at pH 7.4 is a component II-like and is shown in Scheme 5C. The equatorial Cu(II) coordination is made of the N-terminal amine, an amidyl function from the Glu3-Phe4 peptide bond,
the imidazole ring of His6 and the carbonyl function from the His6-Asp7 bond.\textsuperscript{[134]} Such differences has been explained by second sphere interactions, such as the size of cycle due to the H-bond between the side chain of Asp1 or Glu3 and the adjacent amide function.

The formation of the pyroglutamate in Aβ\textsubscript{[3-16]} precludes the binding of the N-terminal amine (Scheme 5D) and induces subsequent changes. In the main form present at neutral pH, the Cu(II) is thus bound by two imidazole rings from His 13 and 14, the amidyl function of His13-His14 bond and either carboxylate function or a N atom form the imidazole ring of His6.\textsuperscript{[134]}

**Affinity.** The affinity values of Cu(II) for the Aβ\textsubscript{[3-16]} and the Aβ\textsubscript{[3-16]} are three and hundred times weaker than the one of Aβ\textsubscript{1-16} respectively. The strong decrease in the affinity of the Aβ\textsubscript{[3-16]} is due to the preclusion of the N-terminal binding and has also been reported for the N-terminally acetylated Ac-Aβ peptide.\textsuperscript{[134, 135]}

**ROS.** It has been shown recently that N-terminal modification at the third position significantly slow down the ROS production rate while keeping the amount of ROS escaping the Cu(Aβ\textsubscript{3-16}) or Cu(Aβ\textsubscript{3-16}) complex unchanged. This was attributed to the importance of the N-terminal Asp1 residues in the catalytically active Cu(Aβ\textsubscript{1-16}) species.\textsuperscript{[74]}

**Aggregation.** To the best of our knowledge, there is no study on the impact of Cu(II) on the aggregation of Aβ\textsubscript{3-40/42}, Aβ\textsubscript{3-40/42} and Aβ\textsubscript{11-40/42}.

**Table 1.** Spectroscopic parameters and conditional affinity of Cu(II) bound to a selection of Aβ peptides and ROS production of the corresponding complex.

<table>
<thead>
<tr>
<th>peptide</th>
<th>spectroscopic signatures</th>
<th>affinity</th>
<th>ROS production\textsuperscript{[c]}</th>
<th>refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ\textsubscript{[1-16]}</td>
<td>2.27 ± 0.01</td>
<td>183 ± 5</td>
<td>620</td>
<td>60</td>
</tr>
<tr>
<td>Aβ\textsubscript{[3-16]}</td>
<td>2.22 ± 0.01</td>
<td>160 ± 5</td>
<td>ND\textsuperscript{[b]}</td>
<td>ND</td>
</tr>
<tr>
<td>Aβ\textsubscript{[4-16]}</td>
<td>2.18 ± 0.01</td>
<td>210 ± 5</td>
<td>525</td>
<td>100</td>
</tr>
<tr>
<td>Aβ\textsubscript{[11-15/28]}</td>
<td>2.17 ± 0.01</td>
<td>205 ± 5</td>
<td>520</td>
<td>90</td>
</tr>
<tr>
<td>Aβ\textsubscript{[3-16]}</td>
<td>2.26 ± 0.01</td>
<td>180 ± 5</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

\textsuperscript{[a]}A\textsubscript{g} parameters refer to \textsuperscript{63}Cu. \textsuperscript{[b]}ND = not determined. \textsuperscript{[c]}measured as rate of hydroxylation of CCA (in percentage of hydroxylation of CCA by Cu in buffer only). \textsuperscript{[c]}K\textsubscript{s} corresponds to the conditional affinity value, i.e. the absolute affinity value at a given pH avlue.

5. Concluding remarks
As reminded in Table 1, N-terminal modifications strongly impact the Cu(II) binding site, both from a structural view or a thermodynamic one. The most striking difference might well be the Cu(II) affinity of the Aβ_{4/11-16} peptides compared to the one of Aβ_{1-16}. Such more than three orders of magnitude higher affinity will thus impact Cu(II) trafficking within the synaptic cleft and between peptides. Another key point is that all N-terminally truncated peptides has a weaker (up to a negligible) ability to produce ROS compared to the full-length peptide.

However, from the results and data described above, it appears that some studies are still missing to better decipher the importance of N-terminally truncated Aβ peptides in AD. In particular and as recently studied for Aβ_{1-16},\textsuperscript{[137]} the interference of Zn in Cu(II) binding should be addressed. Aggregation studies of N-terminally truncated Aβ peptides in presence of Cu(II) are also too scarce. It would also be very interesting to study the co-aggregation of several forms of Aβ peptides as recently done for Aβ_{11-40}, Aβ_{1-40} and Aβ_{1-42}\textsuperscript{[113]} but in presence of Cu(II).

As stated above, the difference in affinity and ROS production ability between the full-length and N-terminally truncated peptides is of importance. Indeed, because Aβ_{4/11-16/40} can (i) remove Cu(II) from Aβ and (ii) redox silence it, those peptides appear as extremely beneficial in the context of AD in link with oxidative stress, to which the ROS produced by the Cu(II)-Aβ system may contribute.

As a direct consequence, modifying the production of the Aβ peptide to favor cleavage at position 4 or 11 appears as a new therapeutic approach, at least with regards to the interaction with Cu(II). More generally, N-terminally peptides might also be considered as new therapeutic targets.\textsuperscript{[9]}

To briefly conclude and as previously pointed out in seminal works,\textsuperscript{[103, 104, 111, 134, 136]} the interaction of Cu(II) with N-terminally truncated peptides might reshape the current vision of a deleterious impact of Cu(II) binding to amyloid-β peptides.

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References


Cu(II) binding sites and affinity in the Cu(Aβ) complexes involved in Alzheimer’s are described in link with Cu(II) induced ROS formation and Aβ altered aggregation. The peptides under focus are the N-truncated Aβ at positions 3, 4 and 11. This review highlights the paradigm shift from a toxic role of Cu(II) / Aβ interaction to a possible protective when Aβ is a N-terminal truncated peptide.