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To cite this version:
Melisa Del Barrio, Valentina Borghesani, Christelle Hureau, Peter Faller. Metal-binding to Amyloid-β Peptide: Coordination, Aggregation and Reactive Oxygen Species Production. Anthony White; Michael Aschner; Lucio Costa; Ashley Bush. Biometals in Neurodegenerative Diseases 1st Edition - Mechanisms and Therapeutics, Elsevier, 2017, 9780128045626. <https://www.elsevier.com/books/biometals-in-neurodegenerative-diseases/white/978-0-12-804562-6>. <hal-01635566>

HAL Id: hal-01635566
https://hal.archives-ouvertes.fr/hal-01635566
Submitted on 15 Nov 2017

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Metal-binding to Amyloid-β Peptide: Coordination, Aggregation and Reactive Oxygen Species Production

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Abstract

Amyloid-plaques are a hallmark of Alzheimer’s disease. They consist mainly of an aggregated peptide called amyloid-β. These plaques also accumulate high concentrations of metal ions, mainly Cu, Zn and Fe. Cu and Zn directly bind to amyloid-β in vivo. This triggered a strong research activity in order to understand the biology and chemistry of the interaction between metal ions and amyloid-β. This includes also the chemical and biophysical aspects of the metal-amyloid-β complexes. The present article describes the coordination chemistry of the Cu(II), Cu(I), Zn(II) and Fe(II) complexes in terms of ligand spheres and affinities. Moreover, metal ions modulate the self-assembly of amyloid-β into amyloids, a central event in Alzheimer’s disease. Attention was paid on the catalytic role of Cu-bound to amyloid-β, in particular in terms of the production of reactive oxygen species, which have been proposed to be involved in the disease progression. Recent mechanistic insights are described.

Keywords: amyloid, bioinorganic chemistry, copper, zinc, aggregation, ROS, coordination chemistry, Alzheimer’s disease, redox reaction
1. Introduction

1.1. Interest in chemistry of metal-amyloid-β complexes

Amyloid-β (Aβ) is a peptide of around 40-42 amino acids and is the major constituent of the amyloid plaques, a hallmark of Alzheimer’s disease. The amyloid plaques also contain a high amount of metal ions, in particular Cu, Zn and Fe.\(^1\) Zn and Cu co-purify with Aβ from brain extract\(^2\) and Raman studies suggested that Cu and Zn ions bind directly to the Aβ in the plaques.\(^3\) Based on these results, chemists and biophysicists became interested in the interaction of metal ions, in particular Cu and Zn with the peptide Aβ in the test tube.

The main form of Aβ is 40 amino acids long (Aβ1-40). A longer form, 42 amino acids long (Aβ1-42, 2 additional amino acids at the C-terminus), is more prone to aggregation and hence enriched in amyloid plaques (Figure 1). Aβ1-42 is also the more toxic form, in line with the idea that aggregation is an important step in the toxicity of Aβ. Aβ is produced by cleavage of precursor protein APP (amyloid-precursor protein) a transmembrane protein (Figure 2). Aβ is generated under healthy conditions and its role is ill defined. Also the role of APP is not clear and might be multiple.\(^4\)

Zn and Cu metabolism is tightly controlled and they are mainly bound to metalloproteins, where they have catalytic and/or structural functions. Generally, there is little free Cu or Zn in cells due to their tight binding. Free or loosely bound Cu is dangerous due to its capacity to produce reactive oxygen species. However, there are some particular tissues, clearly established for Zn, where higher concentrations of less strongly bound Zn are available. This is the case of certain glutamatergic neurons, in which Zn is ejected into the synaptic cleft upon neuron activation and then taken up again (Figure 2). Local Zn concentration in and around the synaptic cleft is estimated...
to rise up to hundreds of μM. This Zn pool is not strongly bound (thermodynamically and kinetically) as fluorophores that sense Zn are able to compete.\textsuperscript{5}

It has also been suggested, although less established yet, that Cu ions can also be ejected in certain synaptic clefts by the ATPase\textsubscript{7a}. Estimations go up to tenth of μM. The oxidation state and type of complex they are present in is not known.\textsuperscript{6}

This makes certain synapses and their surroundings a potential place where complexes between Cu/Zn and Aβ could occur. When, and under which conditions, is not known, apart from the well-established complex formation in the amyloid plaques.

**Figure 1:** Amino acid sequence (1-letter code) of the two most abundant forms of Aβ, Aβ1-40 and Aβ1-42 (the color represents the amino acids involved in metal binding, with red as the main amino acids).

**Figure 2:** Metal ions and Aβ at the synapse: Aβ is cleaved from the amyloid precursor protein (APP) under healthy conditions. Amyloid aggregates (oligomers and plaques) are found in Alzheimer’s disease. In certain synapses Zn can be released from vesicles upon excitation of the presynapses. The transporter ZnT3 is pumping Zn into these vesicles. In certain synapses, upon postsynaptic activation, Cu can be released in the synapse upon translocation of the Cu-transporter CuATP7a to the plasma membrane.
With this background, chemists and biophysicists were interested in the following points among others:

i) Coordination chemistry of metal ions with Aβ: where and how do the metals bind and with what affinity.

ii) What is the impact of metal-binding on the structure of Aβ and on Aβ aggregation. Latter is considered an event linked to AD.

iii) What is the chemical reactivity of the complex of metals with Aβ. Particular attention was paid on the catalysis of reaction that could be linked to the toxicity of Aβ, like the production of reactive oxygen species in the case of Cu (and Fe).

iv) Metal transfer reactions of metal-Aβ with other biological metal-binding compounds (like metalloproteins) and with synthetic chelators. Latter can be considered as a therapeutic approach, based on the idea that metals ions (or one specific) bind only to Aβ under AD conditions and have detrimental effects when they are bound to Aβ.

The present chapter deals with the first 3 points and reports on the current knowledge from in vitro studies.
2. Structure of the metal-Aβ complexes

2.1 Coordination of metal ions Cu, Zn and Fe to soluble, monomeric amyloid-β

It is well established that for soluble, monomeric Aβ, Cu(I), Cu(II), Zn(II) and Fe(II) bind to the N-terminal domain, i.e. between amino acids 1-16. This means that the main binding site is in this domain. As the metal-binding is very dynamic, this does not exclude that amino acids outside of this domain could not transiently bind to the metals, although not as major ligands. Moreover, amino acids from the 17-40/42 could also play a modulating role via second (or further) sphere interaction. In other words, the Aβ1-16 provides the main ligands and mimics well the main binding site of full length Aβ. However, the rest (17-40/2) has likely subtle influence on the metal-binding, like for instance on the affinity (see below).

Thus a lot of work on the coordination sphere of metals with Aβ used the soluble peptide Aβ1-16, which can be considered as a good (but not perfect) model of the monomeric full length Aβ (i.e. Aβ1-40/42). Aβ1-16 does not form fibrils and hence is a limited model for the aggregated Aβ.

2.1.1. Cu(II)- β-amyloid

The high-affinity Cu(II) binding site is located in the N-terminal residues spanning from 1 to 16. Further binding can be observed, with a second relatively well-defined binding site. The affinity of this second site is at least two order of magnitude weaker than the high-affinity binding site and hence this second binding site might not be biologically relevant. Thus, most effort concerned the study of the complex Cu(II)₁ Aβ₁₁ (noted further Cu(II)-Aβ).

Two species of Cu(II)Aβ coexist around neutral pH, noted as components I (predominant at lower pH) and II (predominant at higher pH) (Figure 3A and G). These two components have different spectroscopic signatures in EPR, CD, UV–vis, XAS and NMR. The pH value corresponding with transition between I and II (pKₐ (I/II)) is near pH 7.7 and II is obtained from I by one deprotonation
of an amide of the peptide backbone upon Cu(II)-binding. In both forms the Cu(II) ion lies in a distorted square planar geometry. In particular, the species predominant at lower pH is constituted of two equivalent sets of equatorial ligands (noted \( \text{Ia} \) and \( \text{Ib} \)), where the terminal NH\(_2\) (from Asp1), the CO from the Asp1–Ala2 peptide bond, a N atom from imidazole ring of His6 and from His13 (component \( \text{Ia} \)) or from His14 (component \( \text{Ib} \)) are involved (Figure 3A). The two components (\( \text{Ia} \) and \( \text{Ib} \)) are in equilibrium in 1:1 ratio\(^8,11\) and are thus noted component \( \text{I} \).

Regarding the second component (\( \text{II} \)) (Figure 3G), predominant at higher pH, the NH\(_2\) terminal, the amidyl function N– from the Asp1–Ala2 peptide bond, the CO from the Ala2–Glu3 peptide bond, and one N atom from one of the three imidazole rings of His are the groups predominantly involved in equatorial Cu(II) binding.\(^7\) Regarding the apical positions, involvement of water and carboxylate groups has been proposed in the literature.\(^7,11,12\)

2.1.2. \( \text{Cu(I)} \)-\( \beta \)-amyloid

The Cu(I) is bound predominantly via two His (His13–His14) residues in a linear fashion\(^7,13\) (Fig. 3H). The Cu(I) environment has been revealed by XAS and IR spectroscopy.\(^13,14\) The NMR and XAS studies also indicated that His6 is involved in the Cu(I) coordination,\(^7\) by replacing His13 and/or His14 and keeping a digonal geometry.

2.1.3. \( \text{Zn(II)} \)-\( \beta \)-amyloid

The first metal-binding site for the Zn(II) ion is in the N-terminal hydrophilic region A\(\beta\)1-16 of the A\(\beta\) peptide. The most often proposed Zn(II):A\(\beta\) stoichiometry is 1:1 with a mononuclear binding site. Further Zn-binding is possible, but is likely less defined and there is no strong indication for a biological relevance.\(^7,15,16\) The Zn(II) coordination sphere is less well known than for Cu(II) and Cu(I), as Zn is not EPR and UV-Vis active. The most involved ligands are the imidazoles of the
three His residues and the carboxylate of Glu11\textsuperscript{17} and to a lesser extent Asp1 and Glu3.\textsuperscript{18} The most recent propositions based on a multimethod approach are shown in Fig. 3K (unpublished results).

2.1.4. \textit{Fe(II)-}\textit{amyloid}

In contrast to Cu(II) and Zn(II), Fe(II) has not been detected in Aβ brain extracts.\textsuperscript{10} Fe(II) is air-sensitive and can oxidize into Fe(III) under aerobic conditions. Fe(II) binding to Aβ was investigated under anaerobic conditions and a coordination model was suggested (Figure 3J).\textsuperscript{9,19}

\textbf{Figure 3.} Proposed coordination sites of major components at physiological pH for Cu(II)-hAβ (A), Cu(I)-mAβ (B), Cu(II)-Aβ(His6Arg) (C), Cu(II)-3-16Aβ (D), Cu(II)-p3-16Aβ (E), and Cu(II)-4-16Aβ (F). The minor binding modes of Cu(II) to hAβ and mAβ are also proposed (G and I respectively). Proposed binding modes of Cu(I) (H), Fe(II) (J) and Zn(II) (K) to human Aβ and Zn(II) to murine Aβ (L). K represents the apparent dissociation constant of the metal ion in monomeric Aβ form and in aggregate form at pH 7.4 with 0.1 M salt but in the absence of a competing buffer. Affinities taken from 20-26

2.1.5 \textit{Cu(II) coordination to Aβ with disease relevant mutations or other derivatives}

The coordination of Cu(II) to different derivatives of Aβ have been studies in the past (for recent review see reference 27). This includes several biologically relevant mutations such as the one occurring in familial AD cases (e.g. H6R, D7N and D7H) and truncations.\textsuperscript{9,28-30} Some examples are given in Figure 3. In general, for all the Aβ modifications, the Cu(II) coordination sphere is pH dependent. Thus the binding can differ in the coordination’s sphere and also in the pH dependence (at which pH one form changes into another).
It is important to notice that mutations of amino acids that are not coordinated to Cu(II) can change the first coordination sphere dramatically via second sphere interactions.\textsuperscript{28} This is for instance the case for the mutation of Arg5 to Gly that occurs in the murine Aβ (Figure 3I). In contrast, mutations of a coordinating amino acid might change less. This is for instance the case for the mutation of His14, as it can be substituted by His13.

2.2 Coordination of metal ions Cu and Zn to aggregated β-amyloid.

Knowledge about the coordination chemistry of Zn and Cu ions in the aggregates might be important, because this could contribute to the explanation why the aggregates are more toxic than monomeric Aβ. The structures of Aβ fibrils derived from solid state NMR indicate that the N-terminal part (residues 1 to about 10-14) is flexible and does not take part of the rigid β-sheet structure typical for amyloids. Thus, it is possible that the metal-binding is quite similar to the monomeric Aβ.

2.2.1. Cu(II) coordination to aggregated Aβ peptide

Comparison of EPR signature of fibers and oligomeric forms with those of corresponding soluble forms did not show significant difference in the EPR parameters. This suggests that the coordination is similar and that the same type of ligands are involved. However, if in this case the ligands come all from the same peptide as in the monomer or from different peptides is not clear yet. There are also indications that subtle difference, which might be important for activity, are present. For instance solid state NMR indicated binding of the carboxylate of the C-terminus and of Glu22.\textsuperscript{31}

2.2.2. Cu(I) coordination to aggregated Aβ peptide
The Cu(I)-coordination in aggregated Aβ has been little studied and is not well described. Only one study reports a tetrahedral geometry for Cu(I), thus different from the monomeric diagonal geometry.\textsuperscript{7,32}

2.2.3. Zn(II) coordination to aggregated Aβ peptide

Very little is known about the detailed Zn-coordination in the aggregated Aβ, but likely the same ligands are mainly involved. There are some propositions based on experimental results and more detailed models from theoretical studies. It has also been suggested that the peptide aggregates through intermolecular His(Nτ)-Zn(II)-His(Nτ) bridges.\textsuperscript{33}

3. Affinity of metals to Aβ

3.1. Affinity of metal ions Cu(I/II) and Zn(II) to soluble, monomeric amyloid-β

There are a lot of reports on the affinity of metal ions, mostly Cu(II) to Aβ in the literature.\textsuperscript{34} The results seem to be very divergent. However, one has to consider the different type of affinities. They can be i) apparent, i.e. at a given pH and without considering competition with buffer or ii) conditional, i.e. at a given pH but with considering contribution of buffers. This reflects the affinity at a given pH (often 7.4 is used) in water at 0.1M ionic strength or iii) thermodynamical, (a theoretical affinity extrapolated at an infinite pH). Here we refer to the conditional dissociation constant at pH 7.4. There are also other reasons for the discrepancies (see \textsuperscript{10}). However, recently, at least for Cu(II) and Zn(II) converging values have been obtained (see Figure 2).

3.2. Affinity of metal ions Cu(I/II) and Zn(II) to aggregated β-amyloid.
The conditional affinities were only determined for Cu(II) and Zn(II), but not for Cu(I) nor Fe(II).

In general, the affinities are a little stronger for both metals. For Zn(II) represent about a 3-fold increase.\textsuperscript{10} In contrast, for Cu(II) values of at least an order of magnitude have been proposed in some reports,\textsuperscript{22} but not in others.\textsuperscript{23} However, one has to keep in mind that several types and structures of aggregates are known and hence the affinities could be aggregation dependent.

3.3. Cu(II) vs. Zn(II) binding

In terms of the Kd, Cu(II) binding to A\textbeta is about 4-5 orders of magnitude stronger than Zn(II) binding. It is also clear that the two metal ions are at least partially bound to the same ligands. This means that Cu(II) is principally able to displace Zn(II) from its main binding-site in the Zn(A\textbeta) complex. In the plaque, the concentration of A\textbeta seems to be higher than those of Cu plus Zn, implying that the two metal ions are likely bound in their respective site. However, A\textbeta, Cu and Zn concentrations are not well defined elsewhere in the brain and thus the binding of the two metal ions might be of biological relevance.\textsuperscript{35}
4. Aggregation

4.1 General consideration for Aβ

The term amyloid is a structural definition, in which an amyloidogenic peptide or protein is in a cross β-structure (where the secondary structure is a β-sheet). Macroscopically, they often form fibrils, tubes or ribbons. Folded proteins have first to undergo a partial unfolding, before being able to convert to an amyloid. Monomeric Aβ belongs to the class of intrinsically disordered proteins, meaning that in its isolated state it does not acquire a well-defined 3D structure. No unfolding is needed to acquire the β-sheet conformation. It is proposed that aggregation of Aβ into amyloids is an autocatalytic self-assembly process, in which aggregates can serve as template to catalyze the growth of amyloids. Hence, it resembles the better known crystal growth. Although amyloids are ordered structures they are not crystalline. Aβ can also form amorphous aggregates. There also several smaller Aβ aggregates described, but it is not clear if these are amyloid off-pathways or intermediates. Small and soluble aggregates, often called oligomers, are supposed to be the most toxic species of Aβ (and hence a prime therapeutic target). However, little is known about their exact identity, their structure and their biological action.\(^{36}\)

**Scheme 1.** Schematic representation of amyloid aggregation paths of the Aβ peptide (top section) and the typical sigmoid curve of fibril formation often revealed by thioflavin T (ThT) fluorescence (bottom section). Nucleation–polymerization reaction causes a sigmoidal growth curve with a lag-phase and elongation. Fiber formation is initially very slow until nucleating seeds are generated; this initiates rapid fiber generation until equilibrium with low amounts of protein monomer is reached. Adapted from \(^{37}\).

4.2. Impact of metal ions on Aβ aggregation
A multitude of articles report on the impact of metal ions on Aβ aggregations. It is very difficult to obtain a general tendency, as the results are very divergent, like inhibiting or promoting amyloid formation, generation of amyloid or amorphous aggregates. As an autocatalytic process, the event is very sensitive to various conditions and in particular to the presence of aggregation nuclei (seeds). The latter might be responsible for the strong batch dependence observed, including batches that do not form amyloids. Considering that already aggregation results are difficult to reproduce without metal-ions, with metal ions it seems worse. Never mind, let’s start with some robust results:

i) Metal ions (at least Cu(II) and Zn(II)) always impact the aggregation behavior (in terms of kinetics or obtained structure). This is not very surprising as the process is very sensitive and metal-binding can change the structure and/or charge of the peptide.

ii) The impact is metal-specific: Cu(II) and Zn(II) do not impact the Aβ in the same way.

That’s about all the good news there is. In a recent review, we tried to distill some more tendencies from all the literature. It seems that depending on the conditions, metal-ions can promote the formation of amyloids or the formation of amorphous aggregates (could be larger or smaller including oligomeric type). What might be a crucial factor is the proneness of the system to aggregate. Under conditions where Aβ already aggregates fast into amyloids (e.g. high Aβ concentrations, longer peptide Aβ1−42, pH close to pI, etc.), Cu(II) or Zn(II) binding (in particular at high concentrations or ratios) promotes aggregation, but aggregation becomes too fast to properly align the Aβ into an ordered β-sheet structure and hence amorphous aggregates are formed. Under conditions with a low propensity to aggregate (higher pH, low concentrations of Aβ, etc.) metal ion-binding can promote amyloid-type aggregates.
In summary, in the absence of batch and research group independent results for Aβ aggregation in absence of metal ions, and so far little systematic studies on this problem, one can hardly say anything about the effect of metal ions on the aggregation behavior in the test tube.
5. Reactive Oxygen Species induced oxidative stress

Oxidative stress, which can be defined as the consequence of an imbalance between pro-oxidants and defence systems (antioxidants, enzymes), resulting in (often irreversible) cell damage, is implicated in numerous neurodegenerative disorders, including AD. Increased levels of reactive oxygen species (ROS), such as superoxide (O$_2^-$) and hydroxyl (HO') radicals and hydrogen peroxide (H$_2$O$_2$) lead to oxidative stress.

There is considerable evidence of the toxicity of ROS in the context of AD. Post-mortem brains from AD patients clearly show indications of ROS in affected tissues. However, it is still debated if ROS is a cause or a consequence of the disease.$^{38}$

The toxicity of Aβ to neurons is related to the production of ROS.$^{39}$ In this sense, the addition of the H$_2$O$_2$ scavenging catalase$^{40-42}$ or synthetic catalytic free radical scavenger$^{43}$ inhibits the Aβ toxicity towards cells in culture.

Free or loosely bound Cu and Fe ions efficiently catalyze the production of ROS in the presence of a reducing agent (ascorbate, glutathione, etc.) and molecular oxygen.$^{44}$ Recently, it was shown that an increased labile Cu pool and oxidative stress markers correlated well with brain regions most affected in AD victims.$^{45}$ Given the scarce understanding of the role of Fe-Aβ in ROS production, we will uniquely concentrate here on Cu-Aβ complexes and their study of redox reactions in vitro.

5.1. Reactive Oxygen Species production by Cu-Aβ complexes

In terms of redox activity of Cu-Aβ, most activity in vitro was about the reaction of the production of ROS from dioxygen with physiological relevant reducing agents. The following questions are of interest:

i) what kind of reaction can Cu-Aβ catalyze?
ii) what are the mechanisms of these reactions?

iii) what is the efficiency of its catalytic activity? This is an issue in order to evaluate the in vivo relevance. A low activity might be negligible due to other competing reactions in vivo, sometimes with very high efficiency (like SOD or catalase).

5.1.1. Cu-Aβ and ROS production

In principle, the catalytic cycle of ROS production by Cu(II)-Aβ in the presence of a reductant and dioxygen can involve six one-electron reaction steps that successively generate $\text{O}_2^-$, $\text{H}_2\text{O}_2$ and $\text{HO}'$ (Figure 4):

**Figure 4.** Scheme of potential mechanisms of ROS production by Cu-Aβ in the presence of a reductant (e.g. ascorbate) and $\text{O}_2$ (a) and redox potentials of the involved species (b). $\text{DH}_2$ represents cellular reductants such as ascorbate, glutathione, quinol, etc., and 'DH represents the single oxidized species (i.e. ascorbyl radical).

In the late 90s it has been shown that Cu-Aβ in the presence of a reducing agent and $\text{O}_2$ can produce $\text{H}_2\text{O}_2$ and $\text{HO}'$. Different reductants were able to drive the production of ROS. Mostly ascorbate was studied. Other reductants were also tested, but sometimes, contradictory results were obtained, such as for dopamine and cholesterol.\(^{46-48}\)

Early studies proposed that no superoxide is produced, i.e. that the reaction 1b occurs, rather than reactions 2-4 (Figure 4). This seemed to make sense as the reaction from $\text{O}_2$ to $\text{O}_2^-$ (step 2) is an uphill process but reaction 1b (from $\text{O}_2$ to $\text{H}_2\text{O}_2$ is downhill (see redox potentials, Figure 4). This last reaction would require the presence of two reduced metal ions in close vicinity (likely a dimeric form Cu$_2$Aβ$_2$). Theoretical studies supported a direct reaction from $\text{O}_2$ to $\text{H}_2\text{O}_2$ without a release of
O$_2^-$ (But O$_2^-$ bound to Cu-Aβ was proposed as a reaction intermediate).$^{49,50}$ Thus, O$_2^-$ would not be released during the H$_2$O$_2$ production. Recently, it has been shown that H$_2$O$_2$ production by Cu-Aβ in the presence of ascorbate occurs mainly via a free O$_2^-$ intermediate.$^{51}$ This finding could have not only mechanistic consequences, but also biological relevance. First, it has been shown that the formation of H$_2$O$_2$ (steps 1-4) requires only one Cu center to cycle between Cu(I) and Cu(II) and hence the transfer of one electron at a time. Thus, there would be no need for a dimeric Cu center and monomeric Cu-Aβ would be sufficient to catalyze these reactions.$^{2,38}$ Regarding the biological consequences, the potential role of Cu-Aβ as a catalyst in the context of oxidative stress have been extended, given that Cu-Aβ would not be only able to generate H$_2$O$_2$ and HO’, but also O$_2^-$. Additionally, the production of O$_2^-$ can lead to very deleterious species, in particular peroxynitrite whose role in neurodegenerative diseases has been reviewed.$^{52}$

In summary, the in vitro experiments show that Cu-Aβ is able to catalyse the reactions 2, 4 and 6 in Figure 4, i.e. Cu(I)-Aβ reacts with O$_2$, O$_2^-$ and H$_2$O$_2$ to form O$_2^-$, H$_2$O$_2$ and HO’, respectively. The next question is what is the exact reaction mechanism, i.e. structure of the Cu-Aβ, substrate binding, electron transfer etc.

5.1.2. *Redox chemistry of Cu-Aβ complexes by cyclic voltammetry*

The production of ROS by Cu-Aβ species requires the redox cycling of the metallic center and thus determining the electron transfer reactivity of the Cu-Aβ complex is a key issue. Several electrochemical studies using cyclic voltammetry have been reported with divergent results.$^{9,39,53}$ As previously described, the coordination spheres of Cu(I) and Cu(II) in Aβ differ quite a lot and, as a consequence, a large structural rearrangement in the Cu coordination environment is expected during the electron transfer.$^{54}$ This was confirmed by electrochemical studies on the model Cu-Aβ1-16.$^{55}$ Actually, the reduction or oxidation process of the Cu(II)-Aβ or Cu(I)-Aβ states (resting
states) proceeds via an intermediate species (“in-between” state), which is in equilibrium with the resting states and undergoes a fast redox reaction (Figure 5). This “in-between” state represents only 1/1000 of the species in solution but leads to the redox conversion of the totality of the Cu-Aβ species. The standard potential of the redox couple is 0.300 V vs. normal hydrogen electrode (NHE).

The identity of the “in-between” state or if there are an assembly of different states is not known yet. It is likely that the electrochemically “in-between” state is different from the redox active state(s) in the presence of a substrate, such as ascorbate and O₂, as discussed below.

**Figure 5.** Mechanism involved in Cu²⁺-Aβ / Cu¹⁺-Aβ redox process. Top: resting states, that is, the most populated states. The redox reaction between these states is sluggish owing to a large reorganization energy. Bottom: ROS production through the in-between state, which can undergo fast electron transfer (adapted from ⁵⁶).

5.1.3 Mechanism of ROS production and the reactive state of Cu-Aβ complexes

a) The Fenton type reaction

Information about the coordination chemistry of the reactive state of Cu-Aβ, which catalyzes the reaction of H₂O₂ -> HO’, has been provided by experiments and calculations. ⁵⁶,⁵⁷ This reactive state has to be different from the redox-silent resting state and is only little populated. Since the highly reactive HO’ obtained from the Cu-Aβ/ascorbate/O₂ or H₂O₂ systems is only produced by the “in-between” state, the resulting oxidative damages should occur predominantly on the Cu ligands. Based on this assumption, the potential ligands involved in the “in between state” have been identified: Asp1, His13, and His14. Thus, the coordination sphere differs from those of the resting states of Cu(II)-Aβ and Cu(I)-Aβ. ⁵⁶ Further theoretical studies, suggest that the
reactive state able to form HO’ from H₂O₂ consists of Cu(I) bound by two peptide ligands with an angle far from 180° (Figure 6).

**Figure 6.** Structures of the Cu states catalysing the formation of HO’ from H₂O₂

b) Dioxygen reduction

Recently, theoretical studies investigated the reactive structures of Cu(I)-Aβ most prone to activate dioxygen to superoxide and Cu(II)-Aβ. The study indicates that this active state is different from the one able to reduce H₂O₂ and likely from the state being redox-competent in electrochemistry. The active state included a tetra-coordinated Cu(I) with the substrate O₂ bound and three peptide coordination, most favorable was 1His, carboxylate and amine from Asp1.

5.1.4 Efficiency and biological relevance of the ROS production by Cu-Aβ complexes

To the best of our knowledge, the rate constants of the formation of O₂•−, H₂O₂ and HO’ are still undetermined. This makes it difficult to comment on the biological relevance for each individual reaction. However, several studies measured the reaction rates of ascorbate oxidation (Figure 4: reactions 1, 3 and 5) and superoxide dismutation (Figure 4: reactions 2 and 4). The rate constants determined for the reaction of O₂•− with Cu-Aβ1-12/16/42 are 2.0/1.1/0.3 10⁵ M⁻¹ s⁻¹, respectively. These values are in the range of spontaneous dismutation (~10⁵ M⁻¹ s⁻¹ at pH 7) and at least 10³ times slower than that of superoxide dismutase, and hence likely of little biological relevance.
At low ascorbate concentrations (<100 μM), the rate of ascorbate oxidation reaction by O₂ mediated by Cu-Aβ is 117 M⁻¹ s⁻¹ [Asc][Cu]₀ and is independent of [O₂]. At higher ascorbate concentrations, the rate is 16 M⁻¹ s⁻¹ [Cu]₀[O₂]. It is worth pointing out that the rate of ascorbate oxidation catalyzed by the enzyme ascorbate oxidase is 1.7 10⁴ M⁻¹ s⁻¹. Thus, the reaction rates obtained for the ascorbate oxidation by O₂ mediated by Cu-Aβ are moderately high. In general, the activity of Cu-Aβ seems to be intermediate as the efficiency is below enzymes that catalyze ascorbate oxidation or H₂O₂ production, but Cu-Aβ produces more than biologically relevant Cu-peptides/proteins tested, such as metallothionein and human serum albumin. Nevertheless, anti-oxidant properties were also proposed for the monomeric form of Aβ, as well as dual role.

In vitro studies support the pro-oxidant role of Cu-Aβ complex. H₂O₂, which seems to be the major ROS observed, was generated in the (necessary) presence of a reductant, and the production of HO• in presence of ascorbate was described by several groups. The argument that Aβ is an anti-oxidant since Cu-Aβ produces less H₂O₂ and/or HO• than free Cu ions might be not valid, as free Cu does not exist in a biological environment (its metabolism is tightly controlled and Cu is always bound to a ligand). If Cu binding is involved in AD by catalyzing ROS production, Cu-Aβ should generate more ROS than Cu bound to other physiologically relevant peptides/proteins under normal (healthy) conditions, and it generally occurs indeed (see above).

Thus, taking into account the time scale in AD development, the contribution of ROS produced by Cu-Aβ might be significant. If aggregates are more or less active in ROS production is not clear, and might depend on the type of ROS and/or type of aggregate. However, this does not exclude that Aβ may have antioxidant activity via other mechanisms or through an indirect interaction with Cu.
6. Conclusions

In conclusion, the interaction of the metal ions Cu and Zn with Aβ is very versatile and dynamic. On the one side this is a very interesting feature for a chemist, as the versatility is also mirrored in the structure (indeed several structures are present in fast equilibrium) and in the reactivity. On the other side, it makes the system difficult to study, as one has to consider always a multitude of structures and reactions. At least for Cu-Aβ one could also wonder if such a versatile and difficult to control reactivity, can be physiological useful or if Cu-Aβ is not rather a potential danger.

Acknowledgements:

The authors gratefully acknowledge the support from ERC aLzINK - Contract n° 638712 and of the University of Strasbourg Institute for Advanced Study (USIAS).

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