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Chapter X

Role of metal ions in Alzheimer's disease: Mechanistic aspects contributing to neurotoxicity

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ABSTRACT/WEB SUMMARY

Metal ions have long been linked to the etiology of Alzheimer's disease (AD) via a variety of mechanisms that mainly contribute to a worsening of the pathology. The present chapter proposes a state-of-the-art perspective about the toxic paths related to metal ions, mainly copper and zinc. Copper and zinc that are exchanged within the synaptic cleft can bind to the amyloid- β ($A\beta$) peptides, a key player in AD. This interaction has two main fallouts: the modulation of the self-assembly ability of the $A\beta$ peptide leading to the formation of amyloid deposits and the production of reactive oxygen species contributing to the overall oxidative stress detected in AD. The molecular basis of these two main toxicity pathways will be described as well as their interrelation and their link with other factors.

Liminary note: Alzheimer's disease is a complex and multifactorial pathology. In the present chapter, the focus is on the role of metal ions in relation with neurotoxic mechanisms. The view is that of a coordination chemist and since the field is fast-evolving only recent articles will be quoted except for the "seminal ones".

X.1 Introduction

Alzheimer's disease (AD) was first reported by Alois Alzheimer in 1906 when he described the presence of extracellular deposits in the brain of a 50 years-old woman patient suffering from main memory and behavioral disturbance.¹

Since then, two hypothesis which will be at the core of this chapter have been reported: the amyloid cascade^{2, 3} and the metal hypothesis.⁴ They were firstly described in the 90's and are still used as valuable working bases for both the understanding of the etiology of the disease and the search for therapeutic lines.⁴

X.1.1 The amyloid cascade hypothesis

The amyloid cascade assumes that the self-assembly of the amyloid- β ($A\beta$) peptides is a key and early event in the development of AD.² The process takes place extracellularly in the synaptic cleft. The $A\beta$ peptides are crucial biomolecules, originating from the cleavage of the transmembrane amyloid precursor protein (APP) by dedicated proteases.⁵ The cleavage is heterogenous both at the N- and C-termini and thus several isoforms exist,^{6, 7} the longer one being the $A\beta_{1-42}$ (See sequence in

Table 1). Main isoforms at the N-termini are $A\beta_{3/4-y}$ and at the C-termini $A\beta_{x-40/42}$ while some pyroglutamate derivatives $A\beta_{p3/p11-y}$ are also present.⁸ The importance of the $A\beta$ self-assembly in the pathology is in line with (i) early forms of AD (early-onset (EOAD), counting for about 5% of the total cases) being associated with $A\beta$ mutations^{9, 10} or post-translational modifications¹⁰⁻¹² that increase the peptide self-assembly propensity and (ii) the longer form $A\beta_{1-42}$ being more prone to self-assemble and more neurotoxic.⁷

Although it is well accepted that accumulation of $A\beta$ is tightly linked to AD, its exact role is not clear. Suggestions go from a very central causative role, a risk factor up to a consequence of AD. Accumulation of $A\beta$ originates from a disturbed equilibrium between its production by secretases and its degradation by several proteases. Increased extracellular content of $A\beta$ is, at least in part, responsible for the formation of $A\beta$ assemblies gathered into deposits, which would instigate further pathological events, including formation of intracellular neurofibrillary Tau tangles, disruption of synaptic connections and neuronal cells death and dementia.^{7, 13, 14}

X.1.2 The metal hypothesis

The crucial role of metal ions appeared in the same period and is based on the detection of (i) huge concentration of d-metal ions in the senile plaques (up to mM) while level of such ions are in the μM range in CSF (Cerebro-Spinal Fluid)¹⁵ and (ii) a disturbed metal homeostatis.^{16, 17} In addition, spectroscopic evidence suggests that Zn and Cu are bound to $A\beta$ in the amyloid plaques. In certain neurons Cu or Zn are expelled into the synaptic cleft, where they can reach concentrations of 1-10 and 10-

100 μ M, respectively. Hence in some synaptic clefts, high concentrations of loosely-bound Cu and Zn can be present and they can interact with the A β peptides. The interaction between A β and metal ions will be detailed below as the first central event leading to neurotoxicity.

Further examples of the involvement of metal ions in AD include (i) the binding and regulation of the APP, (ii) a correlation with single nucleotide polymorphism of the Cu transporter ATP7B with sporadic AD and (iii) the decrease of amyloid plaque load in AD mice after disruption of the Zn transporter ZnT-3.^{18, 19} In addition, studies on post-mortem AD brains showed increased of tissue oxidative damages.^{20, 21} These elevated levels are correlated with the well-known capacity of loosely bound Cu to catalyze the production of reactive oxygen species (ROS). These findings were also confirmed in AD model mice.²² Hence, redox metal ions can participate to the overall oxidative stress detected in the pathology, by catalyzing the incomplete reduction of dioxygen to ROS.^{20, 21}

X.1.3 Link between the two hypothesis

Metal ions and A β self-assembly are related. In vitro, Zn and Cu ions can bind to A β ^{23,}²⁴ and modulate its aggregation leading to more toxic species.²⁵ The increased toxicity could be due to enhanced resistance of aggregated peptides to degradation by protease²⁶ and/or to formation of oligomeric species able to interact with neuronal membranes (detailed below).²⁵ In addition, in familial mutants developing EOAD, amino-acid residues able to bind metal ions (histidine (His), aspartate (Asp) are mutated while the self-assembly propensity of the peptides is modified.²⁷⁻²⁹

X.1.4 Concluding notes.

Metal ions mediated toxicity of the A β peptide is widely studied on its own but also as a model of other amyloid-related diseases, which share many features with AD: ^{23, 30,} ³¹ (i) involvement of intrinsically disordered peptides/proteins (IDP) that have the capacity to self-assemble and form β -sheet rich fibrils (defining the amyloid-related diseases) and to bind metal ions; (ii) the detection of amyloid deposits in the vicinity of cells (synaptic cleft in AD, Langherans Islets in Type-2 Diabetes....), (iii) mutations in the peptide sequence of murine animals leading to absence of pathology, and (iv) most importantly for the purpose of the present chapter, the co-presence of abnormally high levels of metal ions in the peptides deposits.

In the present chapter, the ability of metal ions to intervene at several places of toxic pathways will be described (Figure X.1). This mainly includes their intervention in the self-assembly of A β and ROS production. A first data to know for assessing their role in such processes is how the metal ions do interact with the A β , id est how they bind to the peptides from structural, thermodynamic and kinetic point of views.

[Insert Figure X.1 here]

X.2 Metal ions, A β peptides and their interactions

Note: In plaques, mainly Copper (Cu), Zinc (Zn) but also Iron (Fe) are found at high levels. Because the molecular interactions of Fe and A β and their biological relevance are still unclear with only very scarce reports on a possible Fe(II) binding to A β ,³²⁻³⁴ Fe will not be discussed here. The interested readers can find insights into the putative roles of Fe in AD, in the following recent reports (refs. ³⁵⁻⁴²). For similar reasons, the role of Aluminium (Al) in AD is not under focus here, but the interested readers can refer to recent reviews (refs. ⁴³⁻⁴⁵).

In contrast to Fe and Al, the binding ability of Cu and Zn to the A β peptides have been studied in depth for more than a decade, but there is no structural data by X-ray crystallography for such metal-complexes of IDP and hence all the binding sites have been proposed from spectroscopic investigations while and the affinity for metal ions were evaluated by complementary methods. The metal binding sites are located in the N-terminal part of the peptides (Table X.1) and to ease the spectroscopic studies A β _{x-16} is often used as short and soluble surrogates of the corresponding full-length forms.

X.2.1 Cu(II)

While main studies have focused on the A β _{1-x} peptides including the murine variant (Table X.1) and more recently proposed structures have been reported for N-terminally truncated peptides (NT-A β). As general and common features the Cu(II) ion lies in an equatorial binding site involving the N-terminal amine, His side-chains, carbonyl function from the peptide bond and, depending on the pH and/or the NT-A β isoforms, deprotonated amide bond(s). The apical coordination of water or carboxylate side

chains have also been reported. Because Cu(II) is a d^9 ion, several (including advanced) characterization methods (Electron Paramagnetic Resonance (EPR), circular dichroism...) are available and a fair consensus is now shared in the field for Cu(II) binding to $A\beta_{1-x}$.^{23, 25, 46-48} The main sites of Cu(II) in the key $A\beta$ isoforms are shown in Figure X.2, panel A, while the Cu(II) affinity can range from 10^9 ($A\beta_{1-x}$) to 10^{13} M^{-1} ($A\beta_{4-x}$) depending on the nature of the Cu(II) binding site.⁴⁹⁻⁵¹

Apart from the structural and thermodynamic parameters, the kinetic of Cu(II) binding to various forms of $A\beta$ have been scarcely studied, with binding (k_{on}) rates that depend on the sequence. For $A\beta_{1-x}$ values reported range $k_{\text{on}} = 2.6 \cdot 10^6 \text{ M}^{-1}\text{s}^{-1}$ to $5.0 \cdot 10^8 \text{ M}^{-1}\text{s}^{-1}$ where the discrepancy may originate from different investigation methods and working conditions.^{52, 53} For $A\beta_{4-x}$ (or analogues): reported k_{on} value is $2.0 \cdot 10^8 \text{ M}^{-1}\text{s}^{-1}$, which corresponds to the anchoring of the Cu(II) forming a 2N intermediate species,⁵⁴ but not to its insertion in the ATCUN (Amino-terminal Copper and Nickel Binding) motif (slower step in the milliseconds to seconds time scale).^{55, 56}

[Insert Table X.1 here]

[Insert Figure X.2 here]

X.2.2 Cu(I)

Conversely to Cu(II), the Cu(I) binding site is almost conserved between the various $A\beta$ isoforms provided that at least two His are present in the sequence, since the Cu(I)

is linearly bonded between two imidazole rings from His.⁵⁹⁻⁶² The His pair involved is still under debate and depends on the peptides. While the nature of the binding site is no more under discussion (Figure X.2, panel B), the affinity is still one main point to solve in the future. Values ranging from 10^6 to 10^{10} M^{-1} are reported,^{56, 71, 75-77} due to different indirect evaluation methods.

X.2.3 Zn(II)

Zn(II) is likely the most difficult ion to study due to its d^{10} nature and hence mainly X-ray Absorption Spectroscopy (XAS, looking at the Zn) and Zn-induced perturbation of the NMR spectra of the peptides are available techniques. Currently proposed Zn binding sites involved mainly the His side-chains, carboxylates, and N-terminal amine, in a tetrahedral geometry (Figure X.2, panel C).^{63, 72} The affinity is the low 10^6 range.⁷⁸⁻⁸⁰ It is also worth noting that with most of the A β isoforms, mainly those bearing either EOAD mutations or post-translational alterations, the formation of dimers (Zn:A β = 1:2 or Zn: A β = 2:2) is enhanced, an event that can trigger the A β self-assembly.⁸¹⁻⁸⁷

With respect to Zn(II), most of the kinetic data reported have been obtained by SPR (Surface Plasmon Resonance) using Acetylated-A β counterparts. The k_{on} and k_{off} rates are in the $10^2 \text{ M}^{-1} \text{ s}^{-1}$ and 10^{-5} s^{-1} range, respectively.⁸⁸

X.2.4 Concluding notes

Taken into account the biological concentration of Zn and Cu within the synaptic cleft upon neurotransmission and their respective affinity for A β , the A β peptide is able to bind Cu and Zn in vivo.

Another key point to note here is that when bound to A β , the metal ions impose their preferred binding sites. This is a main difference with 3D-folded protein where the site is preformed to welcome the cation. In addition, because similar anchors (for instance three imidazoles from His, or 4 carboxylates for Asp and Glu, See Table X.1) are present on A β_{1-x} and because of the peptide flexibility, the binding of metal is highly dynamic involving exchange between interchangeable groups for one binding position.^{58, 89}

X.3 Copper mediated ROS production

Oxidative stress is a main component of AD etiology⁹⁰⁻⁹² and many markers have been reported: lipid peroxidation, protein oxidative modifications, RNA and DNA damages.^{20, 21, 93, 94} Oxidative stress results from imbalance of ROS produced over ROS detoxified by dedicated enzymes such as catalase.⁹⁵ Because redox ions such as Cu in the present case can catalyze the incomplete reduction of dioxygen, they can be involved in the (over)production of ROS that contribute to the overall oxidative stress detected in the pathology,^{20, 21} in which mitochondria dysfunction also participates.^{91, 96}

Cu in contrast to Zn has the ability to redox cycle between Cu(I) / Cu(II) / Cu(III), while the two main relevant redox states in a biological environment are Cu(I) and Cu(II).⁹⁷⁻

⁹⁹ It will thus be the main ion involved in ROS formation. Zn doesn't significantly alter

the A β -mediated Cu ROS production,¹⁰⁰ but indirect pathways linked to Zn dyshomeostasis has been reported to contributed to oxidative stress (for recent reviews, see refs. ¹⁰¹⁻¹⁰³).

X.3.1 Redox properties of Cu(A β) species

Redox properties of Cu(A β _{1-x}) complex have been studied in details. Because Cu(II) and Cu(I) lie in very different binding sites, the direct electron transfer was precluded and it was proposed that the redox process goes through a chemical - electrochemical – chemical path (Figure X.3). The two chemical reactions correspond to pre-equilibria between the Cu(II) (or Cu(I)) species predominantly detected in solution and elusive species in a geometry prone to fast electron transfer, the latter one(s) being known as in-between state(s) (IBS).¹⁰⁴⁻¹⁰⁶ The IBS nomenclature originates from the fact that the geometry is common for the two redox state and in-between each geometry of the individual Cu(I) and Cu(II) ions. The IBS has been firstly observed by cyclic voltammetry, hence the electron transfer considered was purely outer-sphere. Reaching the IBS, the population of which is about 1/1000 is made possible by the highly dynamic of ligand exchange around the Cu ion, as discussed below. The redox signature of Cu(A β _{4-x}) have been also reported several times and show a very stable Cu(II) species that can undergo reduction process at very low potentials (< - 1 V vs. SCE) while oxidation to Cu(III) is possible but beyond the biological potential range.^{66, 107, 108} When starting from Cu(I) the species can get oxidized at approx. 0.3 V vs. SCE.⁵⁶

[Insert Figure X.3 here]

In vitro studies of the ROS production ability of Cu(A β _{1-x}), thus performed in presence of physiological substrates, *id est* dioxygen and ascorbate as the reductant have also been reported.^{77, 105, 106, 109} The peptide-bound Cu is less efficient in ROS formation than loosely buffer-bound Cu in line with a sluggish electron transfer observed by cyclic voltammetry.¹⁰⁴ Recent in-depth characterizations of the influence of peptide modifications on the rate of ROS production^{105, 106} and in silico modelling¹¹⁰ reveal that in the IBS, the Cu ions lie in environment involving the terminal amine and/or the carboxylate side chain of Asp1, and one or two imidazole rings. The intervention of an IBS is anticipated for Cu(A β _{4-x}) as well as for most IDP able to bind Cu in the two redox states.

In brief, the mechanism of ROS production by Cu(A β) species depends the coordination site of Cu(II) and hence of the sequence of the A β (the Cu(I) site being similar for the various isoforms).

X.3.2 Oxidative damages.

The oxidation of surroundings biomolecules and of the peptide itself is one main biological fallout of the ROS produced by Cu(A β) species and one main origin of metal-related toxicity. The nature of the oxidative damages undergone by the Cu(A β)-induced ROS are strongly impacted by the level of external reductant in the medium with a double-faced role: from fuel of the ROS production reaction to sacrificial anti-

oxidants.^{111, 112} ROS can also further evolve to RNS (Reactive Nitrogen Species), including peroxynitrite that is formed via the reaction between $O_2^{\circ-}$ ¹¹³ and NO° .²⁰

X.3.2.1 General targets

Nucleic acids damages include the formation of oxidized nucleic bases, such as formation of 8-oxo-2-dehydroguanine, 8-hydroxyguanine, 8-hydroxyguanine, 5-hydroxyuracyl.^{114, 115} Protein damages are characterized by increased levels of protein carbonylation while membrane proteins are more touched than cytoplasmic ones⁹⁴ and mitochondrial proteins are also a main target.¹¹⁶ Another key target is the lipids from membranes:^{91, 117, 118} oxidative damages include formation of 4-NHE (4-hydroxynonenal), which can further react with lipoic acid or membrane proteins. Alteration of membrane integrity via membrane proteins and lipids damages further trigger other events such as Ca^{2+} homeostasis that would in turn increase the ROS level,¹¹⁹ leading to a vicious circle.

X.3.2.2 A β oxidative damages

Cu(A β_{1-x}) can produce ROS, mainly $O_2^{\circ-}$, H_2O_2 and HO° . Upon attack of HO° , the peptide can undergo several kind of oxidative damages. They have been recently reviewed (in ref. ^{20, 120} and refs. therein). In brief, Asp1 can undergo hydroxylation, oxidative fragmentation and decarboxylation-deamination, His (His6, His13 and

His14) can be oxidized into 2-oxo-His, Phe into hydroxy-phenylalanine, Tyr (Tyr10) can evolved to dityrosine, and Met (Met35) to methionine sulfoxide.

X.3.3 Influence of A β self-assembly

One interesting point is that oxidation of the peptide could influence the (metal-modulated) self-assembly properties of the A β peptide because it touches residues involved either in metal binding or important for the self-assembly (Tyr, Met, Phe within the hydrophobic core, Table X.1). For instance, several recent studies reported on the effect of di-tyrosine formation on the self-assembly of A β _{1-40/42} and a general consensus for a strong slow-down of the assembly process and the formation of shorter species compared to the non-oxidized counterpart.¹²¹⁻¹²³ Another key residues scrutinized was Met35. Recent reports agree on the inhibition of fibrils formation with shorter fragments while the kinetic of the self-assembly can either be fastened or slowed-down.¹²⁴⁻¹²⁷ Met35-oxidized peptide assemblies are reported to be less toxic than the unaltered counterparts.

It is worth noting that only the effect of site-specific oxidations has been reported until now, while the effect of a global oxidation process touching all the possible residues (Asp1, His, Tyr, Phe and Met)^{128, 129} has not been reported so far, although it would be more biologically relevant.

X.3.4 Concluding remark.

A β self-assembly is altered by oxidative damages, in link with Cu(A β) ROS production. Hence Cu ions, ROS formation and A β self-assembly process are related (Figure X.1).

X.4 Modulation of A β self-assembly by Cu and Zn

X.4.1 General features

The other important event in the amyloid cascade is the self-assembly of the A β peptides, since the propensity to self-assemble is linked to toxicity.

The self-assembly process is a nucleation-elongation type polymerization process involving various supramolecular interactions between monomers. The peptides exist in various supramolecular polymeric forms, id est monomeric: 1 repeat, oligomeric: number of repeats form 2-20, and protofibrillary and fibrillary states; the latest two being found in the deposits also known as senile or amyloid plaques in AD patients brains. According to the amyloid cascade hypothesis the monomeric soluble form are not toxic conversely to species present during the self-assembly process. The self-assembly process lead to the thermodynamically stable fibrils made of β -sheets, but metastable species such as oligomers and also amorphous aggregates (meaning aggregates where the polymers involved are less structured than in the fibrils, so with a weaker β -sheet content) do exist as well. A schematic view of the self-assembly process is shown in Figure X.4. While dead-end fibrillary products had long been regarded as the toxic species, the paradigm has recently shifted towards the intermediate size species, mainly the oligomers.^{14, 25, 130-134} The gold standard in the

study of self-assembly relies on the use of probes that acquire fluorescence upon interaction (ANS, Bis-ANS, Congo-Red¹³⁵...) and intercalation (thioflavin-T (ThT)) with the various aggregates,^{136, 137} while ThT is specific of β -sheets-containing species.¹³⁸ The kinetic of the self-assembly is mathematically described by a s-shape curve ($F(t) = F_0 + \frac{F_{max}-F_0}{1+e^{-k(t-t_{1/2})}}$; $t_{lag} = t_{1/2} - \frac{2}{k}$), where the three phases (nucleation, elongation and plateau) are described by the t_{lag} , k and F_{max} parameters, respectively (Figure X.4).

[Insert Figure X.4 here]

X.4.2 Mechanisms of toxicity.

In the following, we focus only on toxicity mechanisms in link with the intervention of metal ions in the self-assembly.

Firstly, the importance of Cu and Zn (to a lesser extent) has been evidenced in many studies by the use of ligands able to remove metal ions from A β thus preventing their influence on its self-assembly and restoring cell viability.^{25, 139} In a few reports an enhancement of the toxicity is described after the use of a Cu(II) chelator, that although being not the searched effect, are of crucial importance to document on the mechanism(s) of metal-associated toxicity.¹⁴⁰

At the molecular level, several hypothesis explain the role of metal ions in link with peptides self-assembly:

(i) Metal-assisted formation of oligomers and their stabilization. Oligomers are currently regarded as the most toxic species among those present during the self-

assembly process. Several modes of action have been reported. They include: formation of pores into the cellular membranes thus modifying the metal (mainly Ca^{2+}) homeostasis; strong interactions with membranes thus perturbing the membrane integrity.^{14, 25, 141-144} The identification of the exact role of metal ion in assisting the formation of oligomers and in their stabilization is still scarce,^{145, 146} with the possible role of inducing covalent cross-linked peptides via oxidative damages.¹⁴⁷⁻¹⁴⁹

(ii) Triggering of the self-assembly, consuming the non-toxic monomers species.¹⁵⁰ Possible modes of action include the bridging of two monomers, this has been more particularly been proposed for Zn ;^{151, 152} modifying the folding of the monomers to more aggregation prone species.¹⁴⁸

(iii) Preclusion of degradation pathways. Two possible mechanisms are reported. First, in link with oxidative damages of the A β peptide formation of covalent dimers (dityrosine bridged peptides for instance) can lead to less degradable peptides deposits by A β degrading proteases.^{153, 154} Besides, in link with metal homeostasis perturbation (see below) some biological cascades are down-regulated leading to a weaker expression of degrading enzymes.

(iv) Modification of the self-assembly kinetic¹⁵⁵ and paths and of the morphology of the assemblies formed. While metal ions alter the self-assembly process of the A β peptides, the role of Cu(II) and Zn(II) in this process is still highly under debate with sometimes deeply divergent reports. The more general trend reported for Cu(II) is the slow-down of the aggregation with a role on the elongation phase, where Cu(II) prevents the additions of monomer at the extremities of the filaments.^{25, 156} For Zn a chaperon-like effect is observed as low ratio (< 0.1)¹⁵⁷ while amorphous assemblies are formed at superstoichiometry.⁴⁶

Two main consensual results are: (i) under the very same self-assembly conditions, Cu and Zn influence differently the process and (ii) the stoichiometry between metal and peptide is crucial, with substoichiometric (< 0.1) ratio of metal : peptide impacting the kinetic without strong change in the morphology of the aggregates formed, while superstoichiometric ratio (>1) induce changes in the morphology with more amorphous aggregates formed.^{23, 46, 150, 156}

The discrepancies between the various reports originate from the various conditions used in the in-vitro self-assembly studies. Main parameters that can change the influence of metal ions are : environmental parameters (pH, buffer, ionic strength, agitation, vessels ...), intrinsic conditions (peptide concentration, metal concentration), and the main issue is the perfect monomerization of the peptide, any kind of pre-formed oligomers will indeed deeply impact the kinetic of the self-assembly by seeding effect. It is thus important to note here that a key bottleneck to the better understanding of the role of metal ions in the self-assembly process is the difficulty to get reproducible and relevant data in vitro.¹⁵⁸ This is one main challenge the chemist will have to keep on facing in the near future.

For Cu(I), its impact on A β self-assembly has been poorly investigated due to the difficulty to keep the reduced state intact during the whole process. A recent study reports on the possible mild triggering of the self-assembly via formation of Cu(I)-bridged dimer.¹⁵⁹ When Ag(I) is used as a redox stable Cu(I) surrogate, it retards A β fibrils elongation.¹⁶⁰

X.4.3 Influence of the aggregation state on the Cu(A β)-induced ROS production

The influence of nature of polymers (monomers, oligomers and fibrils) on the ROS production have been shown to follow the order of monomers > oligomers >> fibrils for A β _{1-x} but also for other IDP.¹⁶¹ The reason why the Cu ion is less efficient in promoting ROS production once inserted in fibrils can origin from the less accessibility of substrates and/or to the impossibility of making an IBS once the flexibility of the peptide is hampered by the fibrils structures.¹⁰⁵ Here, an apparent contradiction appears, which is why the oligomers are more toxic while they do produce less ROS. A reconciliation is found if one considers that because oligomers do interact with membranes (more than monomers), the ROS are then produced in a place where they are highly noxious, thus disrupting the membranes integrity by oxidation of the lipid bilayer, as recently reviewed in ref. ¹⁶².

X.4.4 Other important points to consider

While most of in vitro studies on the Cu(II) and Zn(II) effects on the self-assembly of A β have been performed with the A β _{1-x}, there is a few reports with other A β isoforms. Mainly, EOAD variants as well as NT forms have been studied. As a general trend and in line with the amyloid cascade hypothesis, the EOAD variants show higher propensity to self-assemble in presence of Cu(II)²⁹ or Zn(II)²⁸ with respect to the A β _{1-x} counterparts. For A β ₄₋₄₀ isoform, Cu(II) has a similar effect than on the A β ₁₋₄₀.⁶⁷

In addition to metal ions, many other biomolecules can influence A β self-assembly¹⁶³ and can also modulate the metal-altered A β self-assembly by external partner. The most significant case is that of membranes, which can help promoting metal-induced oligomerization as reviewed in ref. ¹⁶².

Metal ions can also affect Tau aggregation,¹⁶⁴ where Tau is an intracellular microtubule associated protein responsible for maintaining the stability of microtubule in axons.¹⁶⁵ Mainly an acceleration of the self-assembly and an increase of the toxicity in presence of Zn¹⁶⁶⁻¹⁷⁰ or Cu(II)¹⁷¹ was reported. Because (i) Tau is mainly an intracellular protein, (ii) because the level of intracellular metal ions is tightly controlled, thus questioning the relevance of the metal ions – Tau interaction and (iii) because the coordination between Tau and metal ions is still poorly investigated,¹⁷²⁻¹⁷⁴ the present chapter doesn't focus on the possible toxicity induced by metal ions – Tau interactions.

X.4.5 Future lines of researches

The field of amyloid-forming peptides in link with AD but also other diseases is fast expanding. In this context, future research lines could include investigations of several modulators of A β self-assembly at the same time, of A β relevant mixtures,⁶⁷ of cross-talk between different amyloid-forming peptides (such as Tau and A β).^{175, 176}

X.5 Metal targeting compounds

Because metal ions coordination to A β peptides is linked to toxic fallouts, mainly alteration of the A β self-assembly and production of ROS, targeting metal ions is currently regarded as an interesting way to alleviate metal ions –related toxicity. Due to its redox ability, Cu is the main target. Many approaches are currently developed and the interested reader can refer to recent reviews on this topic (refs. ^{46, 139, 177-185}).

They include prochelator, multifunctional ligands, ligands selective for Cu, and metallophores.

As briefly mentioned above and in link with peptide degradation, another metal mediated toxicity mechanism is link to the down-regulation of A β degradation pathways due to a too weak intracellular Cu content. It has been proposed that increasing the intracellular Cu content would trigger the extracellular A β degradation via Matrix Metalloproteases (MMP).^{26, 186} In this context, metallophores are compounds able to shuttle metal ions through biological membranes, mainly as importers from extra- to intra-cellular compartment. Compounds with metallophore activity have been studied in the AD context. The most developed metallophore-type compound, called PBT2, a derivative of 8-hydroxyquinoline, showed very promising effect in AD animal models.¹⁸⁷ Clinical phase 2 studies have shown only modest effects (see ref.¹⁸⁸ and alzforum.org). Another large family of metallophores are based on a bis(thiosemicarbazonato) scaffold.¹⁸⁹⁻¹⁹¹

Other research lines for therapeutics could include fighting the effect of metal-related toxicity, mainly by the use of suicide anti-oxidants^{120, 192-194} or of (bio)molecules able to overcome the effect of metal ions on the A β self-assembly.^{163, 195}

X.6 Concluding remarks

In this chapter, the main toxic pathways linked to metal ions have been described in tight connection with the coordination chemistry of copper or zinc and the A β peptides and its consequences on the ROS production and the peptide self-assembly. The role of metal ions is extremely difficult to delineate while AD is multifactorial, but it seems

that this topic is more than ever a timely line of research. The better description of how the metal ions influence the pathology is mandatory to discover curative therapeutics that are still severely missing.

Abbreviations

4-NHE : 4-hydroxynonenal

A β : Amyloid- β

AD : Alzheimer's disease

ANS : 8-anilino-1-naphthalenesulfonic acid

APP : Amyloid Precursor Protein

ATCUN : Amino-Terminal Copper and Nickel Binding

Bis-ANS : 4,4'-Dianilino-1,1'-Binaphthyl-5,5'-Disulfonic Acid

EOAD : Early Onset of Alzheimer's Disease

EPR : Electron Paramagnetic Resonance

IBS : in-between state(s)

IDP : Intrinsically Disordered Peptides/Proteins

MMP : Matrix MetalloProteases

NT-A β : N-terminally Truncated Amyloid- β

ROS : Reactive Oxygen Species

SPR : Surface Plasmon Resonance

ThT : Thioflavin-T

XAS : X-ray Absorption Spectroscopy

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FIGURE AND TABLE CAPTIONS

Figure Captions

Figure X.1 Toxic pathways related to metal coordination to the A β peptides and interrelation between key processes for the progress of the disease.

Figure X.2 Coordination sites of Cu(II) (A), Cu(I) (B) and Zn(II) (C) to various A β isoforms.

Figure X.3. IBS species and ROS production.

Figure X.4 Formation of A β fibrils.

Table Captions

Table X.1 Sequences of significant isoforms. Binding residues are highlighted in blue (Cu(II) binding, in thin line = from peptide backbone), orange (Cu(I) binding) and green (Zn(II) binding) for 1:1 metal ions : A β complexes, residues involved in self-assembly are underlined (solid lines correspond to β -strand and dotted line to the turn), residues undergoing oxidative damages are underlined with double red lines.

Table X.1

	1	5	10	15	20	25	30	35	40	42	refs.
A β ₁₋₄₂	<u>DA</u> EFR DAEFR <u>D</u> AEFR	<u>H</u> DSGY <u>H</u> DSGY <u>H</u> DSGY	<u>E</u> V <u>HH</u> Q <u>E</u> V <u>HH</u> Q <u>E</u> V <u>HH</u> Q	<u>K</u> L <u>V</u> <u>F</u> <u>F</u> <u>E</u> K— K—	<u>A</u> E <u>D</u> <u>V</u> <u>G</u> — —	<u>S</u> <u>N</u> <u>K</u> <u>G</u> <u>A</u> — —	<u>I</u> I <u>G</u> <u>L</u> <u>M</u> — —	<u>V</u> <u>G</u> <u>G</u> <u>V</u> <u>V</u> — —	<u>I</u> <u>A</u> — —		<u>57, 58</u> 59-62 63
A β ₃₋₄₂		<u>E</u> FR	<u>H</u> DSGY	<u>E</u> V <u>HH</u> Q	K—	—	—	—	—	—	50, 64, 65
A β ₄₋₄₂		<u>F</u> R FR	<u>H</u> DSGY <u>H</u> DSGY	<u>E</u> V <u>HH</u> Q <u>E</u> V <u>HH</u> Q	K— K—	— —	— —	— —	— —	— —	50, 51, 62, 66, 67 62
A β ₅₋₄₂		<u>R</u>	<u>H</u> DSGY	<u>E</u> V <u>HH</u> Q	K—	—	—	—	—	—	68
A β ₁₁₋₄₂				<u>E</u> V <u>HH</u> Q	K—	—	—	—	—	—	69
A β _{p3-42}		pEFR	<u>H</u> DSGY	<u>E</u> V <u>HH</u> Q	K—	—	—	—	—	—	64, 65
mA β ₁₋₄₂	<u>D</u> A <u>E</u> <u>F</u> <u>G</u> DAEFG <u>D</u> AEFG	<u>H</u> DSGF <u>H</u> DSGF <u>H</u> DSGF	<u>E</u> V <u>R</u> <u>H</u> Q <u>E</u> V <u>R</u> <u>H</u> Q <u>E</u> V <u>R</u> <u>H</u> Q	K— K— K—	— — —	— — —	— — —	— — —	— — —	— — —	70 71 72
A β ₁₋₄₂ (A2V, A2T)	<u>D</u> V <u>E</u> FR <u>D</u> T <u>E</u> FR	<u>H</u> DSGY <u>H</u> DSGY	<u>E</u> V <u>HH</u> Q <u>E</u> V <u>HH</u> Q	K— K—	— —	— —	— —	— —	— —	— —	29, 65
A β ₁₋₄₂ (H6R)	<u>D</u> A <u>E</u> FR	<u>R</u> DSGY	<u>E</u> V <u>HH</u> Q	K—	—	—	—	—	—	—	73
A β ₁₋₄₂ (D7H)	DAEFR	<u>H</u> DSGY	<u>E</u> V <u>HH</u> Q	K—	—	—	—	—	—	—	74
A β ₁₋₄₂ (D7N)	<u>D</u> A <u>E</u> FR	<u>H</u> NSGY	<u>E</u> V <u>HH</u> Q	K—	—	—	—	—	—	—	73

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Figure X.1

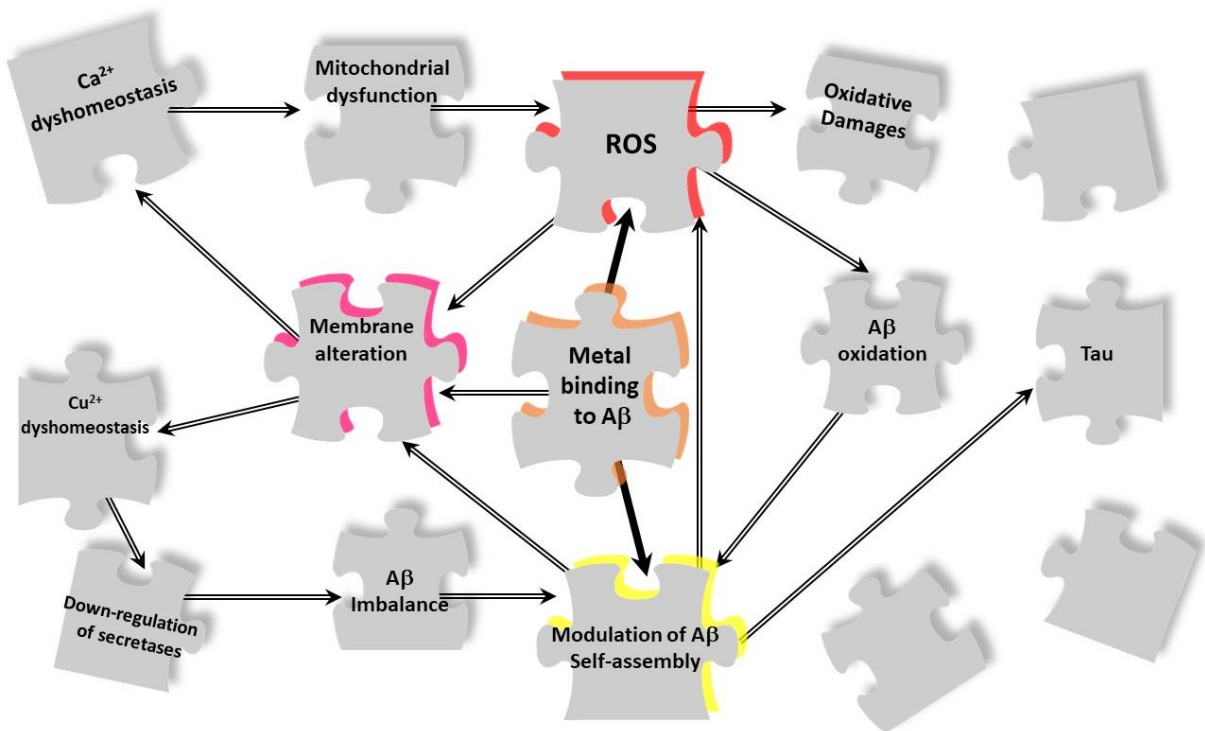


Figure X.2

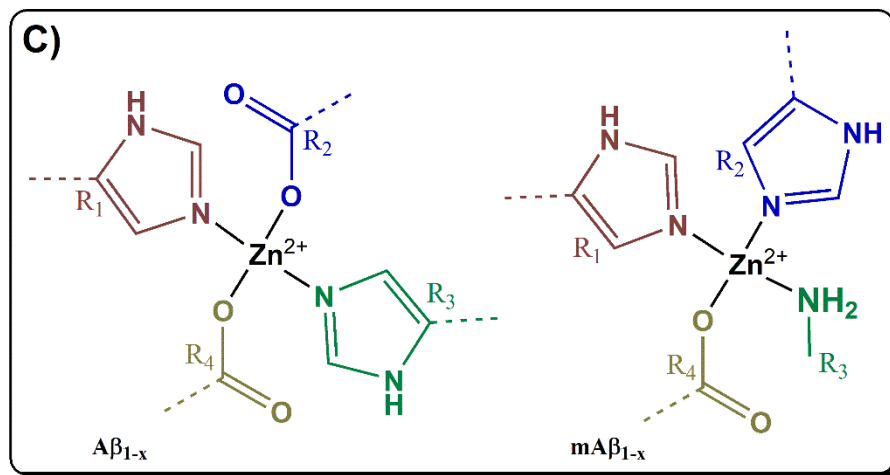
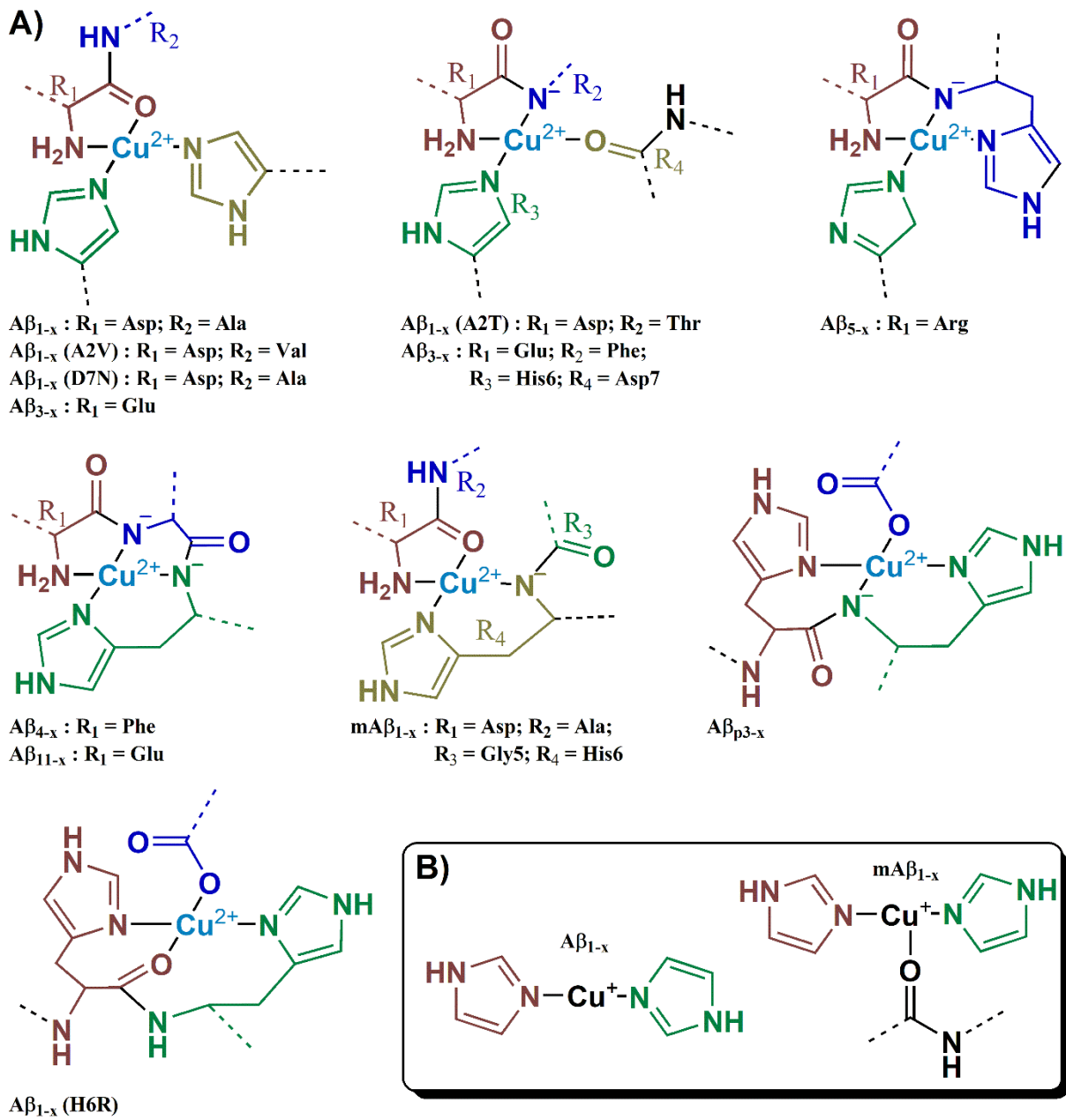


Figure X.3

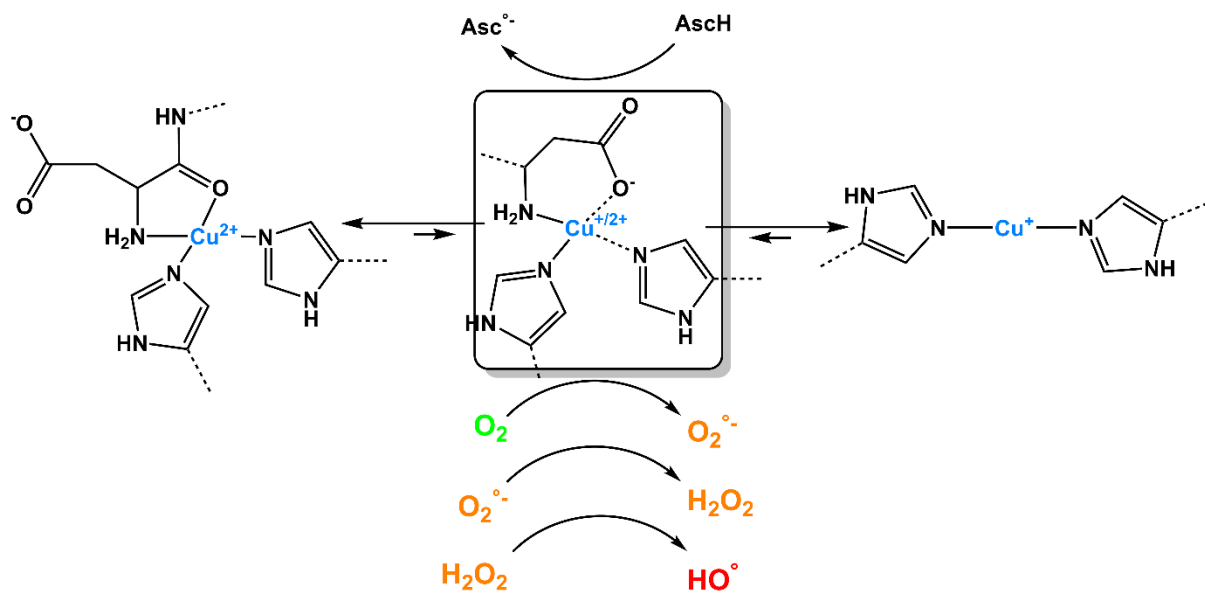


Figure X.4

