Metal binding to the amyloid- β peptides in the presence of biomembranes: potential mechanisms of cell toxicity

Sebastian K. T. S. Wärmländer¹ · Nicklas Österlund¹ · Cecilia Wallin¹ · Jinming Wu² · Jinghui Luo² · Ann Tiiman³ · Jüri Jarvet^{1,4} · Astrid Gräslund¹

Received: 4 June 2019 / Accepted: 10 September 2019 / Published online: 27 September 2019
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Abstract

The amyloid- β (A β) peptides are key molecules in Alzheimer's disease (AD) pathology. They interact with cellular membranes, and can bind metal ions outside the membrane. Certain oligomeric A β aggregates are known to induce membrane perturbations and the structure of these oligomers—and their membrane-perturbing effects—can be modulated by metal ion binding. If the bound metal ions are redox active, as e.g., Cu and Fe ions are, they will generate harmful reactive oxygen species (ROS) just outside the membrane surface. Thus, the membrane damage incurred by toxic A β oligomers is likely aggravated when redox-active metal ions are present. The combined interactions between A β oligomers, metal ions, and biomembranes may be responsible for at least some of the neuronal death in AD patients.

Keywords Amyloid-beta peptide · Phospholipid membrane · Redox-active metal ion · Cytotoxicity · Amyloid disease · Membrane pore · Reactive oxygen species

The amyloid- β peptides and Alzheimer's disease

The amyloid β peptides (A β) are considered to be key players in the molecular mechanism(s) behind Alzheimer's disease (AD) [1–3]. The peptides are 39–43 residues long and derived by proteolytic cleavage from the transmembrane protein amyloid- β precursor protein (A β PP). The amino acid sequence of the 42 residues long peptide [i.e., A β (1–42)] is shown in Fig. 1. The N-terminal segment 1–16 is relatively hydrophilic with six charged residues. After residue 16, the peptide is more hydrophobic, particularly the segments

comprising residues 17–21 and 30–42, where the last segment belongs to the transmembrane part of A β PP. Although the A β peptides are generally unstructured in aqueous solution, the two hydrophobic segments may form a transient hairpin structure with residues 22–29 as a turn [1]. The A β peptides form amyloid material in aqueous solution in a self-aggregation process that ends in formation of insoluble fibrils with a well-defined amyloid structure. The hairpin conformation of the monomers is likely an important structural motif in this aggregation process [1]. Heterogeneous intermediate stages involve so-called oligomeric forms that typically consist of 2–60 A β monomers. The structures of these oligomers are different from fibrils and less well understood [3–5]. The oligomeric aggregates are generally considered to be the most cell-toxic A β species, especially those formed by the A β (1–42) peptide, and to be the major initiators of the AD disease mechanism(s) [4–6].

The structural and other properties of possibly toxic A β oligomers will depend on the surrounding environment [7]. Here, we discuss two important environmental parameters: metal ions and membranes. Membrane is a wide term that in this context ranges from biomimetic model membranes to biomembranes in general, including cellular plasma and organellar membranes. The lipid composition of biomembranes varies between different organelles, cell types, and

✉ Astrid Gräslund
astrid.graslund@dbb.su.se

¹ Arrhenius Laboratories, Department of Biochemistry and Biophysics, Stockholm University, 106 91 Stockholm, Sweden

² Department of Biology and Chemistry, Paul Scherrer Institut, 5232 Villigen, Switzerland

³ Department of Clinical Neuroscience (CNS), Center for Molecular Medicine CMM L8:01, Karolinska Institutet, Stockholm, Sweden

⁴ The National Institute of Chemical Physics and Biophysics, Tallinn, Estonia

Fig. 1 Amino acid sequence of the A β (1–42) peptide. Residues that may coordinate metal ions are marked in blue (negatively charged) or orange. Positively charged residues are marked in red. Neutral pH is assumed

even organisms. It is very challenging to mimic the complex and dynamic features of a living membrane *in vitro* or *in silico*, including regulation of the lipid phase and membrane asymmetry. Thus, model membranes with reduced complexity are often used in biophysical and molecular studies [8]. Most of the studies on interactions with metal ions and model membranes have been carried out for monomeric A β , but oligomeric aggregates likely have similar (although perhaps not identical) interactions. Here, we discuss mainly how A β could interact in parallel with both metal ions and membranes, and how such parallel interactions could be relevant for the AD disease mechanism(s). It should be pointed out that these interactions may be important also for the A β antimicrobial activity, which appears to benefit the host organisms (including humans) [9–11].

A β metal binding

AD patients are known to display altered metal homeostasis [12], and the insoluble plaques found in the brains of AD patients—which are a hallmark of AD pathology and which to a large part consist of aggregated A β peptides [3, 13]—are known to contain high levels of elements such as Ca, Cu, Fe, and Zn [14, 15]. The A β monomers are known to bind metal ions [2, 16] and the N-terminal A β segment (i.e., aa 1–16) displays several residues with a high propensity for metal ion binding (i.e., three His, one Tyr, and four negatively charged amino acids; cf. Figure 1) [2]. At neutral pH, Zn(II) and Cu(II) ions have been shown to bind to the His6, His13, and His14 residues as well as to the N-terminus of the monomeric A β peptide [17–19], displaying K_d values around 1–50 nM for A β /Cu(II) and 0.1–1 μ M for A β /Zn(II) interactions [20, 21]. At lower pH, alternative binding ligands have been observed [20]. Metal ions such as Fe(II) [22], Mn(II) [23], and Pb(IV) [24] also display binding sites that mainly involve the A β histidines, possibly in combination with the negatively charged Asp and Glu residues [23] or the Tyr10 residue [24]. Different interactions have been observed for different ions of the same metal, e.g., Cu(I)/Cu(II) and Pb(II)/Pb(IV) ions [24, 25]. As many different metal ions can bind the A β N-terminal part with similar coordination ligands and binding affinities, binding competition between different metal ions with different properties—such as

redox-active and non-redox-active ions—might be of biological relevance in AD pathology.

While binding of metal ions is known to affect A β aggregation [16, 26–29], for example, by reducing the net charge of the complex and thereby reducing the electrostatic repulsion between two A β peptides, this aggregation will in turn affect the peptides' metal-binding properties: bringing several A β peptides together in aggregates arguably increase their capacity for metal coordination, as several A β N-termini then may wrap around a single metal ion. For example, Ca(II) ions display no specific binding to A β monomers, but exhibit profound interaction with A β aggregates [30]. The brain contains ions of several d-block transition metals, and the *in vivo* concentrations of Cu, Fe, and Zn ions are in the range for relevant A β interactions [31]. Metal ions are, however, tightly regulated in biological systems and the freely available metal ion pool is consequently limited. Metal–A β interactions therefore most likely occur in certain locations, such as the synaptic cleft, where Cu and Zn ions are secreted in close proximity to the A β PP and A β proteins. In addition to metal ions, cationic molecules such as polyamines may interact with the N-terminal part of A β and induce similar effects as bound metal ions [32, 33].

Redox reactions and A β toxicity

There is strong evidence that oxidative stress is involved at an early stage in AD development [34]. The brain has a high consumption of dioxygen and is a vulnerable target for deleterious oxidative stress reactions. Redox-active metal ions like those of Cu and Fe are known to interact with molecular oxygen to form so-called reactive oxygen species (ROS), typically transient free radicals like the superoxide anion ($O_2^{\cdot-}$) and hydroxyl radicals ($HO^{\cdot}$) that are harmful to living cells. By redox cycling in the presence of a reducing agent, Cu and Fe ions can catalyze the formation of the free radicals from dioxygen and hydrogen peroxide [35]. Manganese ions are also redox active, but the concentration of Mn in the brain is much lower than the Cu and Fe concentrations. When ROS are not readily detoxified by antioxidants, they can react with almost all biomolecules in living cells, including lipids in the cell membrane. Some *in vitro* studies indicate that ROS species formed under controlled chemical conditions can penetrate through bilayer vesicle membranes [36, 37].

Compared to free available Cu ions in aqueous solution, A β /Cu(II) complexes decrease the ROS formation [35]. Notably, it has been reported that the murine A β peptide, where three N-terminal residues are substituted, binds Cu ions with a higher affinity and generates even less ROS compared to the human A β peptide [38]. The transient A β /Cu(II) and A β /Cu(I) coordination modes facilitate electron transfer

and redox cycling via Fenton and Haber–Weiss reactions involving an in-between state [35]. In addition to various biomolecules in the cell, the A β peptide itself is also a target for molecular modifications induced by oxidative stress. The N-terminal residues in close proximity to the metal-binding site are exposed to hydroxyl radicals that can oxidize in particular Asp and the aromatic side-chains of Tyr, Phe, and His residues.

As the amyloid plaques in AD brains contain increased concentrations of Cu and Fe ions, i.e., in the millimolar range [15], it stands to reason that ROS are generated around these plaques. Cells and molecules around these plaques will therefore suffer oxidative damage, and this is one possible mechanism for AD cell toxicity. The aggregated A β peptides in these plaques have been shown to contain dityrosine cross-links [39, 40], which typically are formed as a result of oxidative stress. Whether these cross-links mostly form before, after, or during the A β aggregation process in AD brains is still unknown [39]. In vitro studies using Cu ions and hydrogen peroxide to induce dityrosine have shown that cross-linked A β dimers display slower self-aggregation [41], and thus spend longer time in the oligomeric and likely neurotoxic states. It is, however, unclear if dityrosine-linked A β peptides are more or less toxic than native A β . Nevertheless, chemical modifications of the A β peptides, induced by ROS that has been generated by redox-active metal ions, are a possible factor in AD neuronal death.

A β interactions with membranes and membrane mimetics: pores and leakage

Biomembranes are another factor known to affect A β aggregation and amyloid formation [42], and both increased and decreased fibrillation rates have been observed [43, 44]. Interactions between A β peptides and membranes, by various mechanisms, have been suggested to be important for AD pathology [45, 46]. Interactions with membrane models typically induce changes in the A β secondary structure, as demonstrated with, e.g., large unilamellar vesicles (LUVs), bicelles, planar lipid membranes, and micelles of detergents such as SDS [8, 47–50]. The local A β concentration can increase when the peptides bind to a membrane, which might facilitate A β self-interactions on the membrane surface [9, 10]. A balance seems to exist between peptide insertion into the membrane, which stabilizes the secondary structure of the hydrophobic parts of A β , and surface-catalyzed aggregation [51]. This balance likely varies with the lipid composition and other factors in the local environment.

In many molecular studies of A β aggregation, micelle-forming surfactants/detergents have been used in otherwise aqueous solvents, to achieve a simple membrane-mimetic environment [8, 52]. For example, in the presence of sodium

dodecyl sulfate (SDS) micelles, the more hydrophobic parts of the peptide insert into the micelle as two α -helical regions 16–24 and 29–35, held together by the unstructured 25–28 loop [49]. The N-terminal segment (residues 1–15) is left free outside the micelle, and is able to bind metal ions like in an aqueous solution and with similar binding affinity [53, 54]. Figure 2 shows a schematic picture of how the A β peptide is located partly inside and partly outside of the surface of an SDS micelle, and how it is able to bind a metal ion with ligands in the N-terminal segment of the peptide.

Several models of membrane-induced toxicity have been proposed for A β , including leakage through pore structures, by membrane thinning, or by mechanical disruption of the membrane by growing A β aggregates [51, 55, 56]. Aggregated A β species appear to be more capable of reacting with the membrane than monomeric A β . A two-step model for membrane disruption has been presented, where an initial formation of cationic-specific pores is followed by general membrane damage by a detergent-like mechanism [57]. As the A β concentration reaches the critical limit required to damage the membrane, the overall membrane conductance increases in the presence of the oligomers, possibly due to pores being formed across the membrane. This is called the “barrel-stave model” or general membrane leakage [58, 59]. Although the formation of cell-toxic oligomeric

Fig. 2 Superimposed model for A β (1–40) positioned in a sodium dodecyl sulfate (SDS) micelle [49] interacting with a Zn(II) ion [23]. The two α -helices involve residues 15–24 and 29–35. The metal-binding site is represented by a top-ranked cluster representation from a molecular dynamics simulation of A β (1–16) in complex with Zn(II) [23]. Model building and equilibration of a 62-molecule SDS micelle in water was done with the Micelle Maker [80]. The N-terminal region of A β is positioned outside the micelle surface, where it is free to bind metal ions. Redox-active metal ions such as Cu(II), Fe(II) and Mn(II) bound to this region would generate oxygen radicals that could damage the membrane surface or induce modifications of the A β peptides themselves

states is influenced by the presence of membranes, the oligomers that are formed conversely affect the properties of nearby membranes. It has, for example, been proposed that A β can recruit cholesterol and ganglioside lipids as these specifically bind to the peptide [60, 61], effectively forming a lipid–peptide microdomain with altered membrane properties.

Membrane pore formation and metal ions

A specific type of membrane disturbance would be formation of membrane pores allowing passage of certain molecules (e.g., small molecules, ions of calcium and other metals) in and out of the cell. Such pores, which could be rather heterogeneous in terms of size and properties, have been identified by a number of methods, including single-channel electrical recording, atomic force microscopy and molecular dynamic modeling, using both biological and artificial model membranes [56, 62–64]. Protocols for preparation of relatively homogenous β -barrel pores, formed by A β (1–42) but not A β (1–40), have recently been presented [4, 5]. It has been shown that certain metal ions, such as Zn(II), may bind to the A β aggregates and block pore leakage [57, 65]. One *in vivo* study showed that addition of Zn(II) or removal of Ca(II) ions inhibited A β toxicity, while addition of Cd(II) ions (which are known inhibitors of native calcium channels) or antioxidants did not [66, 67]. This might be related to Zn(II) but not Cd(II) ions displaying specific binding to the A β N-terminal region [18, 24], as another study showed that various ions and molecules that interact specifically with the A β His13 and His14 residues could block leakage in planar lipid bilayers, and stop cytotoxicity in rat neurons [68], thus indicating similar mechanisms in artificial and biological membranes. Zinc ions have interestingly also been shown to block the leakage caused by truncated A β peptides that lack the metal-binding domain, such as A β (17–42) [69]. This indicates different ion specificity for the channel, in addition to the suggested histidine gating. Thus, Ser26 has been proposed as an important cation-binding residue in the internal channel opening [61]. Zn(II) or Cu(II) ion binding has been shown to structurally convert the C-terminal part of A β into a helical conformation inside the membrane, thus preventing formation of the β -sheet structures considered important for aggregation [70]. Binding to other metal ions has also been shown to affect the structure of A β species in membranes and their related cytotoxicity [71]. Metal ion bridges between oligomeric species

might bring together and stabilize pore structures in the membrane environment [70, 72]. Binding of redox-active metal ions capable of generating oxygen radicals could lead to stabilization of pore structures—cell toxic or not—e.g., via formation of ROS-induced covalent dityrosine cross-links (see discussion above) [41, 73]. Thus, in addition to modulating the toxic activity of oligomeric pore structures, Cu and Fe ions bound to A β aggregates located halfway into a membrane may add additional damage by generating high concentration of ROS species just outside the membrane surface. Even though these ions will generate less ROS when bound to A β species than in their free form, the local concentration of Cu and Fe ions could be rather high around A β aggregates, as a large number of A β N-termini—ready to attract metal ions—would be positioned outside the membrane/aggregate surface (Fig. 2).

Possible pathways for toxic mechanisms related to A β interactions with membranes and metal ions are shown in Fig. 3. The metal-related effects are: promoting aggregation by coordinating more than one A β peptide (A, E), generating ROS that is harmful in general and in particular creates dityrosine-linked A β dimers (B), and blocking calcium leakage by binding to the A β pore (H). The membrane-related effects are: inducing changes in A β secondary structure (D), promoting general A β aggregation (D, E) and in particular formation of calcium-leaking A β pores (F). These effects may occur simultaneously or in sequence according to various pathways. It should be stressed that in our opinion, there are likely multiple toxic/harmful factors involved in the neuronal death observed in AD brains.

Concluding remarks

Recent research has shown many similarities between the amyloid formation and cell toxic mechanisms of different amyloidogenic peptides and proteins, such as A β , α -synuclein, and the islet amylin polypeptide (IAPP/amylin) [7, 74–79]. Common concepts may be membrane-perturbing oligomeric states and harmful oxidative stress caused by binding of redox-active Cu and Fe ions. For the A β peptides, the membrane activity may be related to a potential physiological role as an antimicrobial agent [9, 10]. Further studies are needed to more precisely pinpoint the role(s) of membranes and metals in the mechanisms of AD and other amyloid diseases.

Fig. 3 Hypothetical overview of toxicity-related interactions between the Aβ peptide, biological membranes, and metal ions. Small red arrows indicate the effect of metal ions on the illustrated processes. Skull symbols indicate possibly toxic events. **a** Metal ions such as Cu(II) and Zn(II) are transiently coordinated by the Aβ N-terminal segment via histidine ligands. The hairpin NMR structure of an Aβ monomer (pdb 2OTK) is shown in a blue ribbon representation. An NMR structure of Aβ(1–16) in complex to Zn(II) (pdb 1ZE9) is included, where metal-coordinating His residue side chains are shown in stick representation. Metal binding likely increases peptide–peptide interactions in small Aβ oligomers [28, 29], which are considered particularly neurotoxic [6]. **b** Binding of redox-active metal ions (such as Cu(II)) can generate reactive oxygen species (ROS) in close proximity to the peptide [53]. ROS-induced oxidation of Aβ residue Tyr-10 (shown in purple stick representation) facilitates formation of covalent di-tyrosine cross-links [39]. **c** Di-tyrosine cross-linking stabilizes toxic oligomers and decreases the propensity for formation of

larger fibrillar amyloid aggregates [81]. An amyloid fibril is shown in blue (pdb 2MXU). **d** The hydrophobic C-terminal segment of Aβ is known to bind to phospholipid membranes [8], where an α-helical structure may be induced at low peptide concentrations [47]. Aβ is considered to oligomerize in the membrane environment, and this process may be affected by the specific membrane composition. **e** Membrane-embedded oligomers, now in hairpin β-structure, may be coordinated and stabilized by interactions with metal ions. **f** β-barrel-shaped oligomers composed of 4–6 peptides are large enough to form pore-like structures facilitating cation-specific leakage. This pore formation disrupts the calcium regulation of the cell, and results in toxic effects [4, 5, 46]. **g** Formation of di-tyrosine cross-links in the pore structures (by interaction with, e.g., Cu(II) ions) may further stabilize the toxic structures in the membrane environment. **h** Addition of Zn(II) ions are known to block the membrane leakage induced by Aβ pores, thereby inhibiting the disruption of calcium homeostasis [57, 65]

Acknowledgments Open access funding provided by Stockholm University. This work was supported by grants from the Magnus Bergvall Foundation to SW, from the Lawski foundation to NÖ, and from the Olle Engkvist Foundation and the Swedish Research Council to AG.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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