Hypothesis Paper

AMYLOID-β: AN ANTIOXIDANT THAT BECOMES A PRO-OXIDANT AND CRITICALLY CONtributes TO ALZHEIMER’S DISEASE

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Abstract—Elevated production of amyloid-β (Aβ) as a preventive antioxidant for brain lipoproteins under the action of increased oxidative stress in aging is postulated to represent a major event in the development of Alzheimer’s disease (AD). Increase in Aβ production is followed by chelation of transition metal ions by Aβ, accumulation of Aβ-metal lipoprotein aggregates, production of reactive oxygen species and neurotoxicity. Chelation of copper by Aβ is proposed to be a most important part of this pathway, because Aβ binds copper stronger than other transition metals and because copper is a more efficient catalyst of oxidation than other metals. This amyloid-binds-copper (ABC) model does not remove Aβ peptide from its central place in our current thinking of AD, but rather places additional factors in the center of discussion. Most importantly, they embrace pathological mechanisms known to develop in aging (which is the major risk factor for AD), such as increased production of reactive oxygen species by mitochondria, that are positioned upstream relative to the generation of Aβ. © 2001 Elsevier Science Inc.

Keywords—Amyloid-β, Alzheimer’s disease, Antioxidant, Transition metals, Oxidation, Lipoproteins, Free radicals

INTRODUCTION

Deposition of amyloid-β (Aβ) is thought to play a central role in the development of Alzheimer’s disease (AD). Accumulation of Aβ in the form of senile plaques is a pathological hallmark of the disease. According to the widely accepted amyloid cascade hypothesis, increased production of Aβ, especially of its longer and more amyloidogenic form Aβ42, leads to the formation of amyloid plaques [1]. The extracellularly located plaques subsequently cause formation of intracellularly located neurofibrillary tangles, another essential feature of the AD brain, and neuronal death. There is good experimental evidence in support of the primary role of Aβ in this temporal sequence. Mutations that have been linked genetically to AD, i.e., those in the genes coding for amyloid-β precursor protein (APP), presenilin 1 (PS1), and presenilin 2 (PS2), bring about alterations in Aβ metabolism, resulting in the elevation in the brain of either total Aβ or Aβ42 [2]. However, at most, 5% of all cases of AD are associated with such mutations and are classified as familial AD (FAD) [3], whereas an overwhelming majority of AD cases is sporadic. It remains unexplained how amyloid plaques can be formed in the absence of any genetically determined increase in Aβ synthesis. Remarkably, the amyloid cascade hypothesis does not contain any explanation for the basal Aβ production that is known to occur in neurons and many other cells [4]. It is therefore no wonder that a physiologic role of Aβ continues to be a mystery. In contrast to mutations in APP and PS genes, normal aging represents a factor that is a clear contributor to all AD cases [5], including those of FAD (which do not develop until the brain has aged to a required stage, despite increased levels of Aβ production). It is unclear which factors are responsible for Aβ deposition in the aging brain.

One of the earliest pathological events in AD is oxidative damage to vulnerable neurons (reviewed in [6]). The damage is not limited to the AD lesions, but instead involves the neuronal cytoplasm and it precedes lesion formation. Increased intracellular production of reactive oxygen species (ROS) by abnormal mitochondria caused by damage to mitochondrial enzymes has been proposed to be responsible for the observed effects (reviewed in [7]). It is surely not a coincidence that qualitatively
similar changes are found in normal aging [8]. Importantly, the cytoplasm of vulnerable neurons shows striking increase in transition metals, which are potent catalysts of ROS production [9]. Imbalance in the metabolism of transition metals and their extra- and intracellular accumulation in AD brain have been repeatedly demonstrated (see [10] for review). It is thus feasible that accumulation of Aβ in AD is related to increased oxidative stress experienced by the brain in aging. This assumption becomes even more likely when a number of oxidation-modulating properties of Aβ, unusual for such a small molecule, are taken into account.

**Aβ AS A REDOX-ACTIVE AGENT**

Aβ, a peptide containing 39–42 amino acids, possesses a structure that implies a strong influence on oxidative processes. Aβ has two major sites that are potentially important for its activity towards oxidation (Fig. 1, A). The first site is located in the hydrophilic N-terminal part of the peptide and consists of three histidine (at positions 6, 13, and 14) and one tyrosine (at position 10) residues, all of which are known to efficiently chelate transition metal ions [11]. Chelation of transition metals in a redox-inactive form at this site may theoretically serve to inhibit metal-catalyzed oxidation of biomolecules. A second site is in the lipophilic C-terminal part of Aβ and consists of a single methionine residue at position 35. Methionine can both scavenge free radicals [12] and reduce metals to their high-active low-valency form [13], possessing thereby both anti- and pro-oxidative properties.

Recently, Aβ has been shown to be a very efficient chelator for transition metal ions [14]. Particularly strong binding is observed for copper, which is a more efficient catalyst of oxidation than other transition metals. Aβ1–42 has higher affinity to Cu(II) than Aβ1–40 (apparent stability constant of Aβ-copper complexes reaches $2.0 \cdot 10^{17}$ and $1.6 \cdot 10^{10}$ M$^{-1}$, respectively [14]), which is comparable to the affinity of best metal chelators known, such as ethylenediaminetetraacetic acids (stability constants of about $10^{10}–10^{17}$ M$^{-1}$ [14]). Compared to copper, iron is a less suitable ligand for Aβ. Aβ appears to possess two binding sites for copper (located between residues 6 and 14), which differ in their affinity. Copper can bind to nitrogen atoms of all three histidine residues of Aβ [15], to the hydroxyl group of Tyr10 [16], and to amide groups of the N-terminus [14].

Reduction of transition metals by Aβ has also been demonstrated [17]. In comparison with chelation (which occurs instantly), reduction is slow (its rate constant can be estimated at about $10^3$ M$^{-1}$ s$^{-1}$ from the data of [17]), only efficient at high (micromolar) concentrations of Aβ and presumably occurs through metal interaction with Met35 [18]. Methionine sulfoxide, which has been found in amyloid plaques in AD [19], can be expected to be formed in this reaction [20]. It is important to note that Met35 oxidation by metals is a more common process than its spontaneous metal-independent radicalization through the formation of sulfur-centered radical of Aβ, as has been recently proposed [21].

Reduced metal ions are highly active oxidants and can catalyze further oxidation of biomolecules. For instance, they produce highly reactive hydroxyl radicals from hydrogen peroxide, an important by-product of mitochondrial electron transport [22]. Another worthy example is oxidation of hydroxylamine impurities observed upon incubation of Aβ with some spin traps in laboratory buffers, which has been shown to be due to the presence of trace amounts of transition metals [23]. Reduction of the metals by Met35 residue of Aβ can provide their redox cycling essential for hydroxylamine oxidation.

It is worth mentioning that Aβ42 is a more effective reductant than Aβ40 [17], which can be related to its higher efficiency as a metal chelator [14]. Thus, the efficiency of metal reduction by Aβ can be influenced by the efficiency of metal binding to the peptide. In agreement with this, Aβ reduces Cu(II) more efficiently than Fe(III).

These data show that Aβ possesses a unique combination of redox properties. It is a lipophilic metal chelator with a metal-reducing activity. The physiologic metal chelators known to date are mainly hydrophilic proteins.
with a metal-oxidizing activity [24]. An intricate combination of metal chelation, metal reduction, and radical scavenging can thus be expected to govern the overall activity of Aβ towards oxidation, which may basically embrace the full spectrum of anti- and pro-oxidative effects.

**Aβ AS A PRO-OXIDANT**

Pro-oxidative properties of Aβ have been known for about a decade. In various biochemical systems, Aβ efficiently initiates oxidation of different biomolecules. It induces peroxidation of membrane lipids [25] and lipoproteins [26], generates H$_2$O$_2$ [27] and 4-hydroxy-2-nonenal [28] in neurons, damages DNA [29], and inactivates transport enzymes [30].

Importantly, to induce oxidation, Aβ must be present at high concentrations, typically in a micromolar range. In addition, Aβ preparations must be ‘aged’ (incubated for a relatively long time at room temperature) to become aggregated and fibrillated. Fibrillated Aβ is able to initiate free radical processes resulting in protein oxidation, lipid peroxidation, ROS formation, and cellular dysfunction, leading to calcium ion accumulation and subsequent neuronal death. Chain-breaking antioxidants, such as vitamin E or vitamin C, can abrogate these processes (reviewed in [25,31,32]). Production of H$_2$O$_2$ is thought to be central for Aβ toxicity [27]. To be toxic, Aβ must be fibrillated [33].

It has been shown that Aβ aggregation to fibrils, which is essential for its toxicity and pro-oxidative activity, is caused by traces of transition metals inevitably present in laboratory buffers [34]. In vitro, Aβ is readily aggregated by transition metal ions. Cu(II), Fe(III), Zn(II), and Al(III) all induce formation of β-sheet structure and rapid aggregation of Aβ [35]. Decrease in pH typically increases Aβ aggregation. Zn is more efficient in inducing Aβ aggregation than other transition metal ions, at both neutral and acidic pH [15]. Transition metals are such efficient precipitation agents that binding of even one atom of copper or zinc is thought to lead to Aβ precipitation [14]. In contrast, in the absence of metals Aβ is monomeric, has α-helix conformation and does not form aggregates [36]. Aβ1–42 is more prone to aggregation, probably due to its higher metal affinity, in comparison with Aβ1–40 [35].

The molecular mechanism of Aβ aggregation by zinc or copper includes formation of intermolecular cross-links between β-sheets of Aβ by the atoms of metal. The cross-links are formed between nitrogen atoms of all three histidine residues in Aβ [15]. His13 seems to be essential for Aβ aggregation [36]. As a result, Aβ1–40 aggregates formed have about 3–4 metal atoms per molecule of Aβ [35].

Of particular importance, the presence of transition metals are not only required for Aβ aggregation but also for its pro-oxidative activity. Aβ25–35 accelerates ROS generation in mitochondrial fraction from rat cortex in the presence of Fe(III) or Cu(II), but is ineffective in the absence of metals [37]. Accordingly, iron is required for the toxicity and pro-oxidative activity of aged preparations of Aβ25–35 and Aβ1–42 to neuronal cells, whereas iron chelators protect the cells from Aβ [38,39]. Copper potentiates neurotoxicity of human Aβ [18]. Incubation of Aβ1–40 and Aβ1–42 with transition metals results in the generation of H$_2$O$_2$ [17]. Therefore, Aβ toxicity is likely to be mediated by a direct interaction between Aβ and transition metals with subsequent generation of ROS [18,39].

Another factor essential for the pro-oxidative activity of Aβ seems to be the presence of Met at codon 35. Substitution of this residue by another amino acid abrogates the pro-oxidant action of Aβ25–35 towards liposome oxidation [40]. In addition, replacement of Met35 decreases protein oxidation and neurotoxicity induced by Aβ25–35, Aβ1–40, and Aβ1–42 in neurons [25]. Aβ fibril formation per se is not required for neurotoxicity, because replacement of methionine by norleucine abrogates toxicity but not fibril formation [25].

The triple requirement of fibrillation, transition metals, and presence of Met35 for the pro-oxidative activity of Aβ can be understood taking into account its unusual redox properties. In order to function as a pro-oxidant, Aβ must first bind metals to its metal-binding site(s) and then reduce them in its metal-reducing site in order to produce ROS (e.g., hydroxyl radicals from H$_2$O$_2$). However, metals are bound to the N-terminal hydrophilic part of Aβ, whereas metal reduction occurs at its C-terminal part (Fig. 1, A). Because metals must be placed in the vicinity of the reductant to be reduced, fibrillation is likely to fulfill this task by forming complexes where metal atoms bound to the N-terminal part of one molecule of Aβ, can be simultaneously available for the reductive Met35 residues belonging to other Aβ molecules (Fig. 1, B). Reduced transition metal ions formed can participate in further redox reactions, generating various free radical species. Due to the relatively slow reduction of metals by Aβ [17], this mechanism can only be operative at high (micromolar) concentrations of the peptide.

It is interesting to note that the pro-oxidant effect of Aβ (at micromolar concentrations) on lipoprotein oxidation [26] closely resembles that of vitamin E at low concentrations of transition metals [41]. Vitamin E changes its activity towards lipoprotein oxidation from anti- to pro-oxidative when concentrations of transition metals decrease, functioning through the pathway of α-tocopherol-mediated peroxidation [42]. In the absence
of vitamin C, increase in vitamin E content of lipoproteins accelerates both metal reduction [43] and lipoprotein oxidation [44] and results in oxidation kinetics similar to those recorded in the presence of Aβ [26]. This additionally argues for the central role of metal reduction in the pro-oxidative action of Aβ.

**Aβ AS AN ANTIOXIDANT**

In contrast to pro-oxidative properties, antioxidative activity of Aβ peptides has been barely studied. This is somewhat surprising, in view of the fact that strong metal chelators typically are strong antioxidants, and that the metal-binding properties of Aβ have long been known. Recently, exogenously added Aβ has been demonstrated to inhibit metal-catalyzed oxidation of lipoproteins from human cerebrospinal fluid (CSF) and plasma [26]. Importantly, the effect is observed at the peptide concentration measured in these biological fluids (0.1–1.0 nM), whereas at higher Aβ concentrations its antioxidative action is abolished. Both Aβ1–40 and Aβ1–42 are efficient antioxidants, whereas Aβ25–35 fragment is much less effective. In contrast, all Aβ peptides are unable to considerably influence metal-independent lipoprotein oxidation, suggesting that the antioxidative activity of Aβ is mainly mediated by chelating transition metal ions by its hydrophilic moiety rather than by free radical scavenging through Met35. Nevertheless, the latter mechanism contributes to the antioxidative activity of Aβ, because Aβ25–35 is able to inhibit lipid peroxidation (at low, compared to lipids, concentrations of the peptide) and since replacement of Met35 by Leu considerably weakens the effect [40].

Endogenous Aβ present in CSF can also act as an antioxidant, as is suggested by the positive correlation between CSF resistance to oxidation and its levels of Aβ [45]. Interestingly, CSF level of Aβ correlates better with CSF oxidative resistance than the level of ascorbate, a major chain-breaking antioxidant in human CSF [46], demonstrating an importance of Aβ in CSF protection from oxidation. The level of Aβ42 better correlates with CSF oxidative resistance than that of Aβ40 [45], which is in accordance with stronger binding to Aβ1–42 than Aβ1–40 [14]. CSF from AD patients has lower oxidative resistance than CSF from control subjects [47], in accordance with increased oxidative stress known to occur in AD [6]. Because Aβ at its CSF concentrations has antioxidative properties, this is in agreement with its lower CSF levels typically measured in AD [45,48,49].

An antioxidant role for Aβ in vivo is in agreement with recent data on the distribution of oxidative damage to AD neurons. 8-Hydroxyguanosine (8OHG), a major product of nucleic acid oxidation, markedly accumulates in the cytoplasm of cerebral neurons in AD. Unexpectedly, an increase in Aβ deposition in AD cortex is associated with a decrease in neuronal level of 8OHG, i.e., with decreased oxidative damage [50]. Similar negative correlation between Aβ deposition and oxidative damage is found in patients with Down syndrome (DS) [51]. Aβ deposits observed in both studies mainly consist of early diffuse plaques. These findings indicate that in brains of patients with AD and DS, early Aβ deposition is related to decreased oxidative damage. Thus, formation of diffuse amyloid plaques may be considered as a compensatory response that reduces oxidative stress [6].

It is important to emphasize that Aβ is normally produced by neurons and many other cells, such as astrocytes, neuroblastoma cells, hepatoma cells, fibroblasts, and platelets [4,52,53]. Neuronal cells are the major source of Aβ in brain. Cell-produced Aβ can be both secreted in the cell medium and stored intracellularly [54,55]. Secreted Aβ is associated with lipoprotein particles [56]. Thus, Aβ is an apolipoprotein. This can be expected, due to the fact that Aβ is highly hydrophobic [57]. More exactly, Aβ is one of several known oblique-oriented peptides that have a hydrophobicity gradient and are partially inserted into lipids by their more hydrophobic C-terminal tail [58]. This implies that a more hydrophilic metal-binding region of Aβ extends into the aqueous phase where it can bind transition metals (Fig. 2).

Accordingly, endogenous Aβ is largely associated with lipoproteins in CSF and plasma [59,60]. Neuronal cell cultures secrete a high molecular weight product, presumably a lipoprotein complex that possesses an antioxidative activity [61]. Taken together with antioxidative properties of Aβ, these data suggest that Aβ is an antioxidant secreted as a part of lipoprotein complexes (Fig. 2). Aβ can function as a preventive lipoprotein-associated antioxidant that binds transition metal ions in inactive form and prevents them from catalyzing lipoprotein oxidation. Protection from hydroxyl radicals that can be generated by transition metal ions must occur in the nearest vicinity of the protected molecules. This implies that lipoproteins must be protected in their nearest vicinity, the task that is most easily performed by a lipoprotein-associated chelator, such as Aβ. Amphiphilic properties of Aβ may allow extracellular chelation of metal ions which have avoided binding by hydrophilic chelators and reached lipoproteins.

Transition metal ions must be bound to specialized chelators in redox-inactive form, because in a free form they are exceptionally effective catalysts of oxidation. Concentrations of free copper and iron in plasma are estimated to be as low as 10⁻¹⁸ and 10⁻²³ M, respectively [62]. Close value of 10⁻¹⁸ M is obtained for intracellular free copper that corresponds to less than one
Increased oxidative stress is related to the development of AD [6], and Aβ is considered to be an important pro-oxidant in this process [32]. However, to accelerate oxidation, Aβ must be present in concentrations greatly exceeding those normally measured in biological fluids (i.e., micromolar vs. nanomolar; see [25,26,32]). In addition, Aβ must be aggregated to fibrils by transition metals. Fibrillated Aβ is highly toxic for neurons and other cells [33]. In contrast, at low-nanomolar concentrations (i.e., those circulating in CSF and plasma), Aβ is monomeric and functions as an antioxidant [26]. At these low concentrations, Aβ is nontoxic and even has beneficial effects on neuron survival, axonal length, and neurite outgrowth [64–66]. These activities may be related to antioxidative properties of the peptide. Thus, Aβ may become a pro-oxidant from an antioxidant, if its concentration increases enough to induce its substantial fibrillation and if transition metal ions are available to catalyze this process.

Various stress conditions are known to increase Aβ production. Importantly, Aβ production increases under oxidative stress induced by different ways. Both H₂O₂ and UV irradiation elevate production of Aβ peptides in monkey eye lenses [67] and neuroblastoma cells [68,69]. H₂O₂ upregulates both secretion of Aβ in the cell medium [69] and levels of Aβ in the cell [70]. Antioxidants Trolox and dimethyl sulfoxide are able to block the effect [70]. Increased production of Aβ in the presence of H₂O₂ is not related to increased synthesis of APP, but rather to increased generation of Aβ from APP [70]. The upregulation of Aβ production from APP is likely to occur through the activation of AP-1 transcription factor [67].

Other sources of oxidative stress, less common than H₂O₂, similarly lead to increased Aβ production in cell culture. Inorganic mercury decreases cellular glutathione and increases release of Aβ from neuroblastoma cells [71]. Paired helical filaments from AD patients generate superoxide radicals and increase release of Aβ from neurons [72]. Interestingly, secretion of Aβ is also increased when oxidative stress is induced by micromolar concentrations of Aβ itself [69]. This can provide a feedback loop mechanism allowing Aβ to increase its own production that can be considered as a vicious circle [68].

Aβ generation can also be increased when cells are subjected to a more general metabolic stress. For example, serum deprivation increases Aβ production by human neurons [73], and inhibition of energy metabolism results in increased amyloidogenic APP processing by β-secretase [74].
Finally, Aβ production increases in vivo after brain injury. In patients with head injury, both Aβ40 and, especially, Aβ42 increase in CSF during the first week following the trauma [75]. Fatal head injury results in the formation of diffuse parenchymal deposits of Aβ in the brain, all of which contain Aβ42 as a major component [76]. Notably, the post-traumatic deposits of Aβ do not arise as a result of passive leakage from damaged cerebral blood vessels but are similar to the early Aβ42 deposits observed in AD and DS. In addition, Aβ accumulates in the brain as a response to ischemic/hypoxic injury localized to cerebral cortex [77].

Taken together, these data strongly suggest that Aβ behaves as a stress-related protein whose synthesis is increased under stress conditions. Antioxidant metal-chelating properties of Aβ may form a rationale for this phenomenon. Indeed, an increase in Aβ production may be aimed at chelating potentially harmful transition metal ions which can be released, e.g., from metal-binding proteins, during abnormal cellular metabolism and otherwise catalyze adverse oxidation of biomolecules (Fig. 2). This mechanism has been recently proposed [78]. It is supported by the fact that increased levels of oxidative damage (measured as neuronal 8OHG immunoreactivity) occur prior to the onset of Aβ deposition in brains of patients with DS [51]. It is interesting to speculate that the basal production of Aβ observed in many cell types under normal conditions of cell culture [4], can represent a response to the presence of trace amounts of free transition metal ions in the cell medium (which is always the case, because even those cell mediums which do not contain transition metals as nutritional supplements, such as MEM, inevitably contain at least nanomolar levels of both iron and copper as impurities originating from highly dosed calcium salts). Whatever the case, increase in Aβ production may be a regulatory response which helps cells to cope with abnormal metabolism of transition metals [79]. Processing of APP to Aβ was suggested more than a decade ago to represent a release of an active peptide ligand and constitute a part of reactive plasticity response to the neuronal loss [80]. Now, when Aβ production is known to occur by a fundamental mechanism of regulated intramembrane proteolysis [81], its obligatory physiologic significance is even more highlighted.

Interestingly, increased production of Aβ observed in PS1 mutant cells is accompanied by decreased production of ROS [82] and is not associated with increased sensitivity to apoptosis [83]. Generation of hydroxyl radicals, a major product of metal-catalyzed decomposition of H₂O₂ produced by mitochondria, is especially decreased in PS1 mutant cells [82]. Similarly, overexpression of wild-type PS (which also leads to increased Aβ production) in brains of transgenic mice results in increased brain resistance to metal-induced oxidation [84]. These findings suggest that Aβ production protects cells from oxidation induced by metals.

Assuming that oxidative stress causes increase in Aβ generation in AD, a question of the source of oxidative stress becomes central to explain this pathology. Oxidative damage to neurons is one of the earliest pathological events in AD [6]. Similarly, accelerated lipid peroxidation precedes accumulation of Aβ in animal models of AD [85]. Mitochondria have long been known to be the major source of ROS in actively metabolic cells [22]. This is especially true for neurons, because the brain accounts for 20–25% of the total body oxygen consumption but for less than 2% of the total body weight. Superoxide is a major ROS produced by mitochondrial respiratory chain as a result of direct ‘electron leakage’ to oxygen. Superoxide can be further metabolized to H₂O₂ by catalase. Steady-state concentrations of superoxide and H₂O₂ in the mitochondrial matrix are of about 10⁻¹⁰ and 10⁻⁷ M, respectively [22]. H₂O₂ may diffuse from mitochondrial matrix into the cytosol and extracellular space, where it can react with redox-active transition metal ions to produce hydroxyl radicals. Importantly, superoxide leakage from the mitochondrial respiratory chain sharply increases in aging, due to accumulated damage to mitochondrial DNA and respiratory chain enzymes (reviewed in [8]). It is worth mentioning that AD most strongly affects brain regions with highest metabolic rate and highest expression of mitochondrial enzymes (cf. [86–88]).

Thus, mitochondria may represent a major source for ROS in aging and AD. Increased ROS production may lead to increased generation of Aβ as a compensatory response, similar to increased synthesis of antioxidant enzymes well documented in AD [89]. This mechanism implies that transition metal ions become abnormally sequestered and need to be chelated in a redox-inactive form by Aβ. Indeed, metabolism of transition metals is heavily impaired in AD brain [10]. The brain is a specialized organ that concentrates transition metals. For example, cerebral cortex contains the highest concentrations of zinc in the body [90]. Therefore, the brain must have efficient mechanisms to prevent abnormal distribution of metals, and Aβ production may be an important part of this pathway.

It is noteworthy that Aβ aggregation by transition metals is accelerated at acidic pH [35]. Because inflammation is well known to decrease pH in the vicinity of the inflammation site, it can facilitate formation of amyloid plaques. In addition, inflammation leads to increased secretion of ceruloplasmin, a copper-transporting protein, from the liver and accelerated transfer of copper to the inflammation site [91]. This cascade may represent an important source for redox-active copper in AD, because microglial activation and inflammation typically
accompanies this disease [92] and ceruloplasmin is increased in AD brain [93]. One out of seven copper atoms transported by ceruloplasmin is redox-active and may catalyze oxidation of biomolecules when it is reduced by a hydrophilic reductant, such as superoxide [94]. Similar pro-oxidative activity is known for iron carried by transferrin. While free copper does not exist in the cytosol [63], extracellular copper may be more readily exchangeable and increased levels of ceruloplasmin-bound copper may facilitate copper interaction with Aβ (Fig. 2). In addition, transition metals released into extracellular space from synapses upon depolarization [95], as well as iron produced from mitochondrial heme upon its degradation in the cells [79], may serve as other potential sources for metals in Aβ-metal complexes.

Aβ-metal complexes formed must be efficiently removed, which may occur through lipoprotein receptors known to be abundant in the central nervous system [96]. The balance between the synthesis and degradation is very fine, because in most early onset FAD cases, Aβ accumulation is caused by only about 50% increase in Aβ anabolism [97]. Degradation is likely to be effective in young age and in the absence of genetically linked increase in Aβ production observed in FAD. In contrast, increased oxidative stress in aged brain can lead to increased production of redox-active transition metals, which can in turn lead to increased production of Aβ-metal complexes.

At some stage, efficient removal of Aβ-metal complexes can be overtaken by their disproportionally high generation. This can result in their accumulation in a form of Aβ oligomers and/or early (diffuse) senile plaques. Transition metal ions are indeed highly enriched in the plaques [98]. Diffuse plaques do not contain fibrillated Aβ and are not toxic [99,100]. Their accumulation can thus be considered as a final nonpathological stage of this protective pathway and should inversely correlate with oxidative damage, as has been recently reported [50,51]. In contrast, Aβ oligomers have fibrillated structure, are highly neurotoxic, and may represent the toxin responsible for neurodegeneration in AD [101]. It is interesting to speculate that diffuse plaques may represent a form of detoxification of Aβ oligomers, e.g., deactivated by Zn as has been recently suggested [102].

Late (compact) amyloid plaques can be another toxic form of aggregated Aβ. They contain fibrillated Aβ and can lead to the formation of pathological structures, such as neurofibrillary tangles, in neurons [103]. Remarkably, compact plaques contain redox-active transition metal ions that can catalyze H₂O₂-dependent oxidation in vitro and may thereby exert pro-oxidant activity in vivo [104]. This finding suggests that compact amyloid plaques may represent a pathological stage of the Aβ production pathway when metal sequestration in redox-inactive form becomes ineffective. However, a negative correlation between accumulation of compact plaques and 8OHG [51] contradicts this assumption.

Whatever the nature of the toxic form of Aβ, the antioxidant activity of Aβ finally evolves into pro-oxidant representing a typical gain-of-function transformation. This can further stimulate Aβ production, providing a feedback loop mechanism to accelerate plaque growth by a “seeding” mechanism [105] and further worsen the situation. Massive accumulation of Aβ in the brain of AD patients might be accordingly considered as a hyperresponse to increased oxidative stress in aging. This model is in accordance with the recently proposed three-stage mechanism of neurodegeneration in AD that consists of protein aggregation in neural tissue, oxidation of neural tissue mediated by redox-active metal ion interaction with a target protein, and functional demise [10]. It also agrees with accumulation of early amyloid plaques in cognitively normal individuals that can be considered as a successful compensation to aging. In contrast, an unsuccessful compensation, when “the primary pathogenic force” [106] is too strong, is accompanied by an uncontrollable growth of plaques and represents AD. Finally, this mechanism allows explanation of a well-known epidemiological association between aluminum exposure and incidence of AD [107] by accelerated formation of Aβ aggregates in the presence of aluminum ions (which are well-established initiators of Aβ aggregation [108]). Similarly, increased incidence of AD in subjects having apolipoprotein E4 allele [109] can be related to its decreased, compared to apolipoprotein E3, ability to bind transition metals [110] and thereby prevent their binding to Aβ.

It is essential to emphasize that Aβ precipitation by transition metals is not irreversible and can be reversed by strong metal chelators, such as ethylenediaminetetraacetic acid (EDTA) [35]. In the absence of free transition metals, i.e., in the presence of a strong metal chelator, Aβ remains soluble [14]. This implies that the chelators can also reverse formation of amyloid plaques (at least at a particular stage of plaque development, which probably includes diffuse plaques). In accordance with this assumption, EDTA increases Aβ extraction from AD brains [111] and partly dissolves Aβ-metal complexes [34]. These data underlie therapeutic use of metal chelators in AD, which have been already shown to be effective in both AD patients [112] and animal models [113].

Another intriguing therapeutic possibility relevant to this mechanism involves usage of cholesterol-lowering drugs (statins), which have recently been shown to decrease the risk of AD [114,115]. Statins efficiently decrease synthesis of cholesterol in the cells and its secretion into the extracellular space. Thus, it is not surprising
that they also decrease secretion of Aβ [116,117], because Aβ is secreted as a part of lipoprotein complexes [56], which largely consist of cholesterol [96]. This mechanism is in agreement with direct relationship between Aβ binding to lipid membranes and their cholesterol content [118]. Lipoprotein origin of Aβ in plaques is supported by a close correspondence between the deposition of apoE, the major brain apolipoprotein [96], and Aβ in AD brains [119] as well as by the presence of apoE in amyloid plaques [120]. Accordingly, cholesterol feeding increases Aβ secretion and accumulation in the brain [121,122].

Interestingly, progression of AD can be also delayed by antioxidants, such as vitamin E [123]. In the presence of vitamin C, vitamin E is a very efficient inhibitor of oxidation of both plasma [44] and brain [124] lipoproteins. It is feasible that antioxidant supplementation is beneficial via decreasing oxidative stress in the brain and diminishing responsive production of Aβ.

Finally, immunization with Aβ1–42 of transgenic mice expressing mutant human APP causes reduction in the number and size of compact amyloid plaques and ameliorates cognitive deficits of the mice [125]. Metal chelators, cholesterol-lowering drugs, antioxidants, and immunization with Aβ can thereby aim the same target, namely precipitation of lipoprotein-located Aβ by transition metals.

**CONCLUSIONS**

Increased production of Aβ in a form of lipoprotein antioxidant under the action of increased oxidative stress in aging with subsequent chelation of transition metal ions, accumulation of Aβ-metal lipoprotein complexes, production of ROS, and neurotoxicity are postulated to form the temporal sequence of events in the development of AD (Fig. 3). Because (i) Aβ binds copper stronger than iron and other transition metals [14], and (ii) copper is a more efficient catalyst of oxidation than other transition metals [104], chelation of copper by Aβ is proposed to be a most important part of this pathway. This amyloid-binds-copper (ABC) mechanism can be expected to be central in sporadic AD, where no other cause for increased Aβ production is known. In FAD, elevated generation of Aβ related to genetic defects in APP and PS proteins can accelerate the development of the disease in comparison with sporadic cases. Whereas the proposed ABC model does not remove Aβ peptide from its central place in our current thinking of AD, it places additional factors in the center of discussion. Most importantly, they embrace pathological mechanisms known to develop in aging (which is the most important risk factor for AD), such as increased production of ROS by mitochondria, that can be positioned upstream relative to the generation of Aβ.

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Amyloid-β: an antioxidant that becomes a pro-oxidant


