Review

The redox chemistry of the Alzheimer's disease amyloid β peptide

Danielle G. Smith [a,c,d], Roberto Cappai [a,b,c,d], Kevin J. Barnham [a,c,d,*]

[a] *Department of Pathology, The University of Melbourne, Parkville, Victoria 3010, Australia*
[b] *Centre for Neuroscience, The University of Melbourne, Parkville, Victoria 3010, Australia*
[c] *Bio21 Institute, The University of Melbourne, Parkville, Victoria 3010, Australia*
[d] *The Mental Health Research Institute of Victoria, Parkville, Victoria 3052, Australia*

Abstract

There is a growing body of evidence to support a role for oxidative stress in Alzheimer's disease (AD), with increased levels of lipid peroxidation, DNA and protein oxidation products (HNE, 8-HO-guanidine and protein carbonyls respectively) in AD brains. The brain is a highly oxidative organ consuming 20% of the body's oxygen despite accounting for only 2% of the total body weight. With normal ageing the brain accumulates metals ions such iron (Fe), zinc (Zn) and copper (Cu). Consequently the brain is abundant in antioxidants to control and prevent the detrimental formation of reactive oxygen species (ROS) generated via Fenton chemistry involving redox active metal ion reduction and activation of molecular oxygen. In AD there is an over accumulation of the Amyloid β peptide (Aβ), this is the result of either an elevated generation from amyloid precursor protein (APP) or inefficient clearance of Aβ from the brain. Aβ can efficiently generate reactive oxygen species in the presence of the transition metals copper and iron in vitro. Under oxidative conditions Aβ will form stable dityrosine cross-linked dimers which are generated from free radical attack on the tyrosine residue at position 10. There are elevated levels of urea and SDS resistant stable linked Aβ oligomers as well as dityrosine cross-linked peptides and proteins in AD brain. Since soluble Aβ levels correlate best with the degree of degeneration [C.A. McLean, R.A. Cherny, F.W. Fraser, S.J. Fuller, M.J. Smith, K. Beyreuther, A.I. Bush, C.L. Masters, Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease, Ann. Neurol. 46 (1999) 860–866] we suggest that the toxic Aβ species corresponds to a soluble dityrosine cross-linked oligomer. Current therapeutic strategies using metal chelators such as clioquinol and desferrioxamine have had some success in altering the progression of AD symptoms. Similarly, natural antioxidants curcumin and ginkgo extract have modest but positive effects in slowing AD development. Therefore, drugs that target the oxidative pathways in AD could have genuine therapeutic efficacy.
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Keywords: Amyloid beta; Metal; Oxidative stress; Redox chemistry; Alzheimer's disease

Contents

* Corresponding author. Department of Pathology, The University of Melbourne, Parkville, Victoria 3010, Australia. Tel.: +61 3 8344 2555; fax: +61 3 9347 6750.
E-mail address: kbarnham@unimelb.edu.au (K.J. Barnham).

1. Amyloid beta peptide (Aβ) generation

The Aβ peptide was first purified and sequenced from amyloid plaques found in AD and Down's syndrome brain [2,3]. It is typically a 39–42 residue polypeptide derived from the proteolytic processing of the APP molecule and consists of a largely hydrophillic N-terminal domain (1–28) and a C-terminal hydrophobic domain (29–39/43) derived from the APP transmembrane domain (Fig. 1). APP can be processed via two proteolytic pathways, each resulting in distinct cleavage products. Cleavage of APP by β-secretase generates a membrane bound C-terminal fragment which is the substrate for γ-secretase, which cleaves within the transmembrane domain of the C-terminal APP fragment to generate full length Aβ (Fig. 1). The alternative pathway involves initial cleavage of APP by α-secretase, followed by γ-secretase resulting in the truncated P3 fragment (Fig. 1).

APP is a ubiquitously expressed type 1 transmembrane protein and is a member of a multigene family which includes amyloid precursor-like proteins 1 and 2 (APLP1 and APLP2) [4–8]. The biological function of the APP family remains unknown. APP exhibits a variety of potential biological functions including regulation of intracellular calcium [9,10], cell growth [11–13], cell adhesion [14–17], axonal transport of vesicles [18] and metal ion homeostasis [19–21]. APP contains a metal binding domain in the N-terminal cysteine-rich region adjacent to the growth factor-like domain [11,19–21]. Structural studies indicate the metal binding domain possesses structural similarities to known copper chaperones such as the Menkes copper-transporting ATPase fragment, metallochaperone Atx1, and SOD1 copper chaperone [21]. The APP copper binding domain exhibits a strong affinity for Cu^{2+} ions ($K_d \approx 10$ nM) and can reduce Cu^{2+} ions in vitro [19,20,22]. APP and APLP2 knockout mice have elevated Cu levels in the brain cortex and liver [23] while APP overexpressing mice exhibit a reduction in metals including copper ions [24–26].

2. Oxidative stress in AD

The brain is the most aerobically active organ in the body due its high metabolic requirements. The brain accounts for 2% of total body mass yet consumes 20% of total oxygen in a resting individual. Therefore it is imperative to maintain oxidative balance and control in the brain, and this is tightly regulated by antioxidants that are present in vastly higher amounts than in any other organ. Therefore modifications in normal oxidative metabolism as observed in AD brain provide strong evidence that oxidative stress plays an important role in AD pathogenesis (Fig. 2).

As a general principle, the chemical origin of the majority of ROS is the reaction of molecular oxygen with the redox
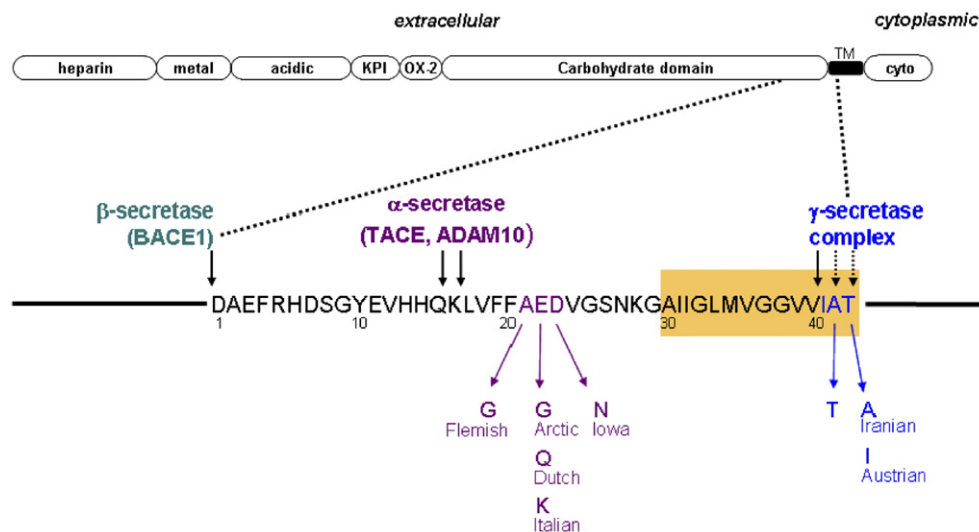
Fig. 1. Amyloid beta peptide (Aβ), a 39–43 amino acid transmembrane peptide is generated from APP cleavage by β- and γ-secretase. α-secretase cleavage of APP initiates the non-amyloidogenic processing pathway of APP. APP in a multi-domain protein consisting of eight domains: heparin binding (heparin), metal binding (metal), acidic, Kunitz-type serine protease inhibitor (KPI), OX-2 homology sequence (OX-2), carbohydrate, transmembrane (TM) and the cytoplasmic (cyto) domains [217]. The cleavage sites of the secretases are indicated by the arrows above the Aβ sequence. The transmembrane domain of Aβ is highlighted in orange and the familial mutations of Aβ are indicated below the sequence.

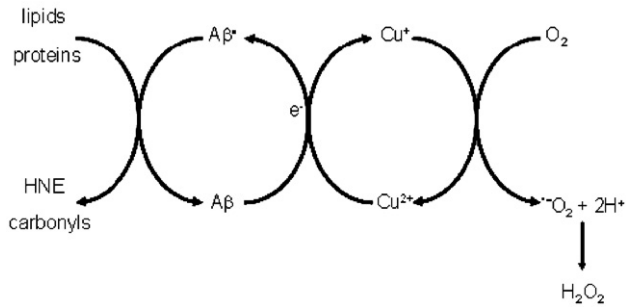Fig. 2. Aβ reduction of Cu^{2+} ions generates Aβ radicals (Aβ•) that extract protons from surrounding lipids and proteins generating hydroxy-2-nonenal (HNE) and carbonyls respectively. Cu^+ reacts with molecular oxygen (O_2) eventuating in H_2O_2 formation.

active metals Cu and Fe [27]. The ability of these metal ions to occupy multiple valence states and undertake facile redox cycling, thereby activating molecular oxygen, has been utilized by a variety of enzymes including ceruloplasmin [28], cytochrome *c* oxidase [28] and amine oxidases [29]. However, unregulated redox-active metals will inappropriately react with oxygen to generate ROS. Moreover, there is increased glucose-6-phosphate dehydrogenase activity [30,31], increased heme oxygenase levels in diseased brain [32] and the presence of oxygen radical-mediated chemical attack resulting in increased free carbonyls, lipid peroxidation adducts, protein nitration and mitochondrial and nuclear DNA oxidation adducts.

A broad range of lipid peroxidation products can evolve from the cascade of events described above. High levels of chemically reactive electrophillic aldehydes malondialdehyde and free 4-hydroxy-2-nonenal (HNE) have been found in AD brain tissue [33–35]. These aldehydes readily react with cellular nucleophiles such as DNA, proteins and other lipids and elevated HNE-protein adducts have been detected in human AD brain [34,36,37]. They are potentially more perilous than free radicals because of their longer half-lives which allow them to diffuse away from their site of formation [38]. Polyunsaturated fatty acids, such as arachidonic acid and docosohexanoic acid, found in high abundance in brain [39] are highly oxidisable and hence vulnerable constituents of membrane phospholipids. Taken together, the above studies suggest that in an AD brain free radicals could induce lipid peroxidation leading to highly reactive aldehydes that initiate a cascade of oxidation events resulting in cellular dysfunction and ultimately death. There is significant evidence to indicate that Aβ can generate these free radicals (see later section).

Protein carbonyls are a major marker of protein oxidation and can be generated from direct free radical attack on amino acid side chains, glycation, glycoxidation or from lipid oxidation products as discussed above. Elevated levels of protein carbonyl exist in the frontal pole, hippocampus and superior middle temporal gyrus of AD patients and correlate well with AD histopathology [40–45]. Nitrotyrosine and dityrosine cross-linked protein are elevated 8-fold and 3-fold respectively in hippocampus and neocortical regions of AD brain compared to age matched controls [26,46,47]. Both of

these oxidation products in mammals generally correlate with oxidative stress [48–50]. Furthermore, neuritic and cored amyloid plaques show evidence of oxidatively modified Aβ [51] with much of the Aβ isolated from plaque containing methionine sulfoxide [52].

Further evidence of oxidative stress in AD is the modification of antioxidant activity in the brain. Thioredoxin is an antioxidant protein which is decreased in AD amygdala and hippocampus/parahippocampal gyrus. In contrast, the thioredoxin protein reductase shows increased activity in the amygdala and cerebellum of AD patients [53]. The antioxidant enzyme glutathione transferase, which is primarily responsible for HNE clearance, is decreased in several regions in AD brain including the hippocampus [54]. Furthermore, conjugated glutathione and HNE are elevated in the substantia innominata and the hippocampus in severe AD cases [55]. These two regions of the brain are known to be preferentially affected by the disease. HNE conjugation to glutathione by glutathione transferase is a cellular mechanism to inactivate toxic HNE. Therefore, increased levels would indicate an elevated cellular defence against lipid peroxidation.

The antioxidant enzyme superoxidase dimutase (SOD) displays elevated expression levels in AD brains compared to age matched controls [56]. It is also elevated in red blood cells and lymphoblasts [57–60] which suggests that the normal compensatory mechanisms against free radical damage may be insufficient in AD patients. Apolipoprotein allele ε4 increases the risk of developing AD and it has been shown that AD patients homozygous for this allele have an increase in brain catalase activity [61] suggesting a genetic predisposition for increased ROS generation in some AD suffers. Furthermore, the administration of the antioxidant vitamins ascorbic acid (vitamin C) and α-tocopherol (vitamin E) in tandem caused a decrease in the risk of AD in elderly subjects [62]. Taken together these observations suggest that prophylactic intake of antioxidants may be beneficial for those with a genetic risk of AD.

3. Metals in AD

The brain tightly regulates metal ion homeostasis as a part of normal physiological processes that play an important role in neural activity. Various lines of evidence have implicated metal ions, in particular Cu, Zn and Fe in the pathogenesis of AD (reviewed in [63]).

Transition metal ions Cu and Zn co-purify with Aβ extracted from AD plaques [64]. Cu ions are normally bound to Cu enzymes or proteins like cytochrome *c* oxidase, ceruloplasmin and superoxidase dismutase during normal physiological circulation and are released into the synapse upon presynaptic excitation reaching up 15 μM in the synaptic cleft [65]. Although found in high concentrations in amyloid plaques ($\sim$400 μM) [66] compared to the normal brain extracellular concentration of 0.2–1.7 μM [67–72], most studies of bulk tissue have found either no statistical difference [73,74] or a decrease [75,76] in total Cu concentration in AD brain compared to age matched controls.

Similarly, Zn ion levels can be as high as ~1 mM in plaques [66]. Interestingly, only mature cored senile plaques, not diffuse plaques, showed histochemical reactivity for Zn [77]. Using inductively coupled plasma mass spectrometry (ICP-MS), increased Zn levels were detected in cerebrospinal fluid (CSF) of AD patients [78]. There is conflicting data on brain Zn in AD levels with one report of bulk brain analysis indicating decreased levels of Zn in post mortem AD hippocampus [79], while a separate study observed an increase in the same region as well as in the amygdala and inferior parietal lobule [76,80]. Histological staining of normal brain indicates the distribution of Zn resembles the areas of the brain most prone to amyloid deposition and neuropathy in AD which includes the aforementioned hippocampus, amygdala and parietal lobe [81]. The primary source of labile Zn in the brain is from Zn released into the synapse during transmission [82] at ~200–300 μM [83,84]. Zn ions can promote Aβ aggregation and plaque formation, and this activity may not be as a neurotoxic modulator but rather as a neuroprotective agent since Zn can attenuate Aβ toxicity in cortical cultures [85–89]. The precise mechanism of cytoprotection is not clear, though possible mechanisms include inherent blockading of membrane channels formed [90–96], Zn enhancement of Na+/K+-ATPase [86] or competing with Cu for Aβ binding and thereby inhibiting Aβ initiated redox chemistry [85,97,98]. Oral Zn supplements significantly delay cognitive decline in AD patients [99–101].

The third transition metal found localized in human amyloid plaques is Fe. Bulk analysis of post mortem AD brain indicates that Fe is predominantly localized in neocortical grey matter [102], amygdala [103] and olfactory tract [73]. Despite having a high concentration in AD plaques (~1 μM) [66], Fe ions are not likely to interact directly with Aβ in vivo. Although in vitro studies indicate that Fe is able to interact with Aβ [104–106], unlike the other two transition metals Fe does not co-purify with Aβ extracted from plaque[64] and is predominantly located in neuritic processes within the plaque itself associated with ferritin [107]. A number of studies suggest that Fe homeostasis is altered in AD [66,107–111], however this is likely to be a secondary effect via another process such as increased heme oxygenase activity in response to cellular oxidative stress [32] or a decrease in functional heme from Aβ binding to heme increasing free Fe levels [112].

APP knockout mice have an increase in brain Cu levels [23]. In contrast, the Tg2576 APP transgenic mouse model which overexpresses the human APP Swedish mutation and forms amyloid plaques [113], display significantly reduced brain Cu levels prior to the appearance of amyloid neuropathology [24]. Two other APP transgenic mouse models, the APP23 and TgCRND8 mice also had reduced Cu levels. Zn [77] and Fe [114] levels were also dysregulated in the Tg2576 mice with the Tg2576 plaques containing high levels of both ions. The formation of amyloid plaques is associated with Zn-mediated Aβ aggregation since crossing the Tg2576 mice with mice where the synaptic Zinc transport 3 protein was knocked out reduced the cerebral amyloid burden by 50% compared to Tg2576 control mice [115].

Oxidative stress markers have been detected in 12 month old senescence accelerated prone (SAMP8) mice which accumulate brain Aβ and display learning and memory impairments, and have elevated levels of oxidative stress markers compared to 4 month old mice [116]. Treating the aged SAMP8 mice with antisense oligonucleotide targeted to the Aβ sequence in APP showed decreased levels of oxidative stress markers including protein carbonyls, 3-nitrotyrosine adducts and HNE in the brain [117]. These results support a link between Aβ and oxidative stress.

4. Aβ is a metalloprotein

Multivalent metal ions are fundamental to redox chemistry as they facilitate electron transfers during the redox process. Aβ is a metalloprotein that displays high affinity binding of Cu^{2+}, Zn^{2+} and Fe^{3+} ions [118]. The metal binding site was initially mapped to the region between positions 6 and 28. Solution state NMR implicated three histidine residues (His_6, His_{13} and His_{14}) in binding Cu^{2+}. This is supported by EPR data which suggests a square planar configuration in a 3N1O co-ordination sphere [119]. The nitrogen atoms involved in this co-ordination are from the imidazole rings of the three histidines (Fig. 3). The identity of the oxygen is unclear and it may be the hydroxyl from Tyr_{10}. Other options include the oxygen atoms of the sidechain carboxylate of Glu_5 [120], N-terminal aspartate [120], the amino terminus itself [120,121] or possibly a water molecule [122].

Aβ possesses a strong positive formal reduction potential and rapidly reduces Cu^{2+} and Fe^{3+} to Cu^+ and Fe^{2+}, respectively [98]. Molecular oxygen is then trapped generating free radical and peroxide species via Fenton chemistry with the Aβ42 species exhibiting the greatest activity [98] (Fig. 2). The chemistry of these reactions is discussed in more detail later in this review. The free radicals generated are suggested to be involved in the oxidation of the methionine 35 residue (Met_{35}) [123–129]. The replacement of Met_{35} with norleucine (sulphur of methionine is replaced with a methylene group) abolishes the

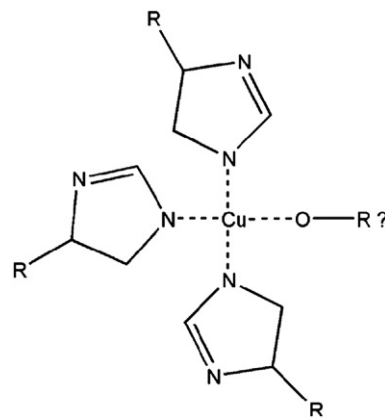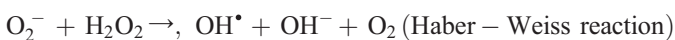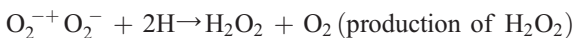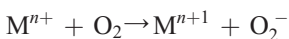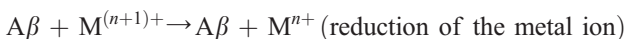Fig. 3. Model of Cu^{2+} coordination with Aβ in a 3N1O coordination sphere via His_6, His_{13}, His_{14}through the imidazole nitrogens. The fourth oxygen donor ligand putatively is the hydroxyl of Tyr_{10}, side chain carboxylate or a backbone carbonyl.

oxidative chemistry and neurotoxic activity as compared to the wildtype peptide [130]. The free radicals could possibly initiate the oxidation of the sulfhydryl to a sulfuranyl radical cation, which can then abstract protons from other lipids (causing lipid peroxidation) or proteins (initiating protein oxidation).

5. Aβ, metals and redox chemistry

There is good evidence that metal ions mediate the oxidative stress mechanism of Aβ toxicity. Metal ions modulate Aβ toxicity in neuronal cultures, with Cu^{2+} ions increasing Aβ toxicity whilst Zn^{2+} ions attenuate it [85]. Likewise, Aβ dissolved in Fe^{3+} containing media is toxic to neurons whilst Aβ in Fe^{3+} free media is not toxic [131]. The difference in the activity of the metal ions was attributed to the redox capabilities of Cu^{2+} and Fe^{3+}. Aβ/Cu(Fe) complexes are capable of generating reactive oxygen species such as H_2O_2 which mediates toxicity [98,132]. Synthetic Aβ incubated in the presence of the chelator desferrioxamine (Fe^{3+} chelator) exhibited decreased toxicity which was restored by the addition of 0.1 mM Fe^{3+} [104]. Synthetic Aβ contains significant concentrations of Cu and Fe both of which can mediate Aβ production of H_2O_2 [133]. The addition of the H_2O_2 scavenging enzyme catalase to the cell cultures inhibits Aβ toxicity and suggests that Aβ neurotoxicity is mediated by ROS generation [98,132,134–137]. A caveat is that catalase can bind to Aβ and thus exert a static effect independent of the H_2O_2 production [138]. Co-incubation of Aβ with metal chelators can also attenuates Aβ toxicity [134,139] (Fig. 4).

The production of H_2O_2 by Aβ is dependant on the presence of specific redox active metal ions [132,140]. The generated peroxide may degrade forming a highly reactive hydroxyl radical (ROS) via Fenton chemistry or a Haber–Weiss reaction outlined as outlined below [141,142].

$$A\beta + M^{(n+1)+} \rightarrow A\beta + M^{n+} \text{ (reduction of the metal ion)}$$

$$M^{n+} + O_2 \rightarrow M^{n+1} + O_2^-$$

$$O_2^- + O_2^- + 2H \rightarrow H_2O_2 + O_2 \text{ (production of } H_2O_2)$$

$$M^{n+} + H_2O_2 \rightarrow M^{(n+1)+} + OH^\bullet + OH^- \text{ (Fenton Chemistry)}$$

$$O_2^- + H_2O_2 \rightarrow, OH^\bullet + OH^- + O_2 \text{ (Haber} - \text{Weiss reaction)}$$

Aβ generation of ROS requires reduction of metal ions (Cu or Fe) thus inducing oxidation of another moiety. Mass spectrometry has shown that Cu^{2+} ions are able to oxygenate Aβ with the most likely candidate being the sulphur atom of methionine 35 (Met_{35}) [143,144]. It has been proposed that
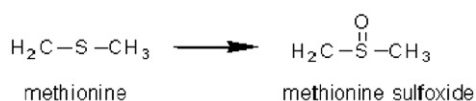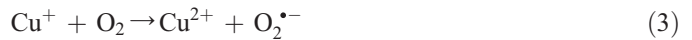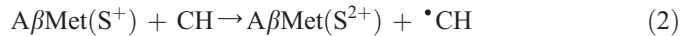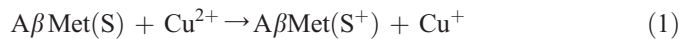

Fig. 4. Oxidation of methionine residue leads to the formation of methionine sulfoxide.

Met_{35} serves as a source of electrons for the reduction of molecular oxygen to hydrogen peroxide [119,127,130,145–148] (Eqs. (1)–(5)).

$$A\beta Met(S) + Cu^{2+} \rightarrow A\beta Met(S^+) + Cu^+ \tag{1}$$

$$A\beta Met(S^+) + CH \rightarrow A\beta Met(S^{2+}) + {}^\bullet CH \tag{2}$$

$$Cu^+ + O_2 \rightarrow Cu^{2+} + O_2^{\bullet -} \tag{3}$$

$$A\beta Met(S^{2+}) + H_2O \rightarrow A\beta Met(S = O) + 2H^+ \tag{4}$$

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 \tag{5}$$

consistent with the in vitro data, oxidised methionine Aβ (AβMet-ox) has been isolated from AD brain amyloid deposits [149,150] and furthermore is bound to Cu [52].

The redox properties of Aβ indicate it could function as both an antioxidant and a pro-oxidant under specific conditions. Aβ has been observed at low concentration (nM range) to have a neuroprotective and neurotrophic effect on neonatal cell cultures [151–156]. However, the condition(s) under which Aβ ceases to act as an anti-oxidant and function as a pro-oxidant are not clearly understood, although several lines of evidence indicate the activity is dependant on the concentration of the peptide. The concentrations of Aβ required to induce toxicity in vitro is in the micromolar range [89,134,139,157–162]. In cerebrospinal fluid where Aβ has been reported to act as an anti-oxidant, the concentration of the peptide is between 0.1 and 1 nM, while at higher concentrations this activity was ablated [163]. Similarly, anti-apoptotic activity was only observed at nanomolar levels and conversely at higher concentrations it was toxic [164]. There is a strong negative correlation between oxidative damage and Aβ deposition in AD brain [1,165]. The accumulation of Aβ is either from excessive production of Aβ or alternatively an impairment of Aβ clearance pathways. Zn displacement of Cu binding to Aβ could be a detoxification mechanism followed by deposition into plaques which may not be toxic. Neuronal cells exposed to Aβ42 in the presence of both Cu and Zn had lower toxicity than Aβ plus Cu, suggesting Zn suppressed the Cu-dependent formation of H_2O_2 and rescued the cells [85]. Therefore, localized excessive levels of Aβ and Cu may generate ROS concentrations exceeding the capacity of the normal oxidation defence system. Moreover, synaptosomes treated with Aβ results in the release of free fatty acids and this effect was inhibited by the antioxidant vitamin E [166].

5.1. The role of methionine 35 in Aβ toxicity

There is conflicting data regarding the role of Met_{35} in Aβ neurotoxicity. Met_{35} is located in the C-terminal portion of the Aβ peptide, within the putative transmembrane domain and is the most susceptible side chain to oxidation [167,168]. Investigation into the role of Met_{35} in Aβ toxicity suggests the methionine is paramount to the redox chemistry of the peptide [127]. The sulfur containing methionine could

potentially donate electrons for metal ion reduction and thus initiate a redox chemistry cycle as discussed previously.

An amino acid substitution of Met_{35} to valine increases Aβ toxicity compared to the WT peptide [139] whilst nor-isoleucine or cysteine amino acid substitution ablated it [169]. The oxidised methionine Aβ peptide (AβMet-ox) displayed no toxicity after a 24 h exposure [130], however longer incubations of AβMet-ox exerted similar toxicity to wildtype Aβ [134]. These results support the concept that Aβ mediated generation of ROS is initiated by the Met_{35} residue. In order for Met_{35} to facilitate ROS generation by reducing metal ions, Met_{35} must come within ~19 Å distance of the acceptor atoms for the electron transfer to occur [170]. This might be achieved by either the folding of the N and C-terminal ends or via the fibrillization of the peptide resulting in the metal bound N-terminal end of one Aβ being stacked adjacent to the Met_{35} in the C-terminal end of another Aβ. Under these conditions, Cu^{2+} induced radicalization of Met_{35} could also induce the generation of a stable carbon centred radical on the peptide backbone (most likely glycine residues), which can subsequently participate in lipid peroxidation reactions [171]. This is consistent with elevated post mortem 4-hydroxynonenal (HNE) levels in AD brain [33,172].

An alternative mechanism to the aforementioned process for the generation of HNE is based on full length Aβ inserting into the lipid membrane via its hydrophobic C-terminus wherein the sulfuranyl radical abstracts a proton from an unsaturated bond in the membrane phospholipid generating a carbon centred radical on the lipid. The carbon centred radical can readily react with molecular oxygen (O˙) forming a peroxyl free radical (HO˙) which in turn initiates a cascade of events that amplify the original Aβ peptide free radical. The lipid peroxidation products, such as HNE, arise from the systematic breakdown of the lipids subsequent to the free radical attack [127,173,174].

The oxidation state of the peptide can also affect aggregation as synthetic AβMet-ox has a slower rate of fibrillization, presumably by disrupting the switch from small soluble oligomers to larger insoluble oligomers. By arresting the oligomerisation to dimers, Aβ is able to be cleared from the brain before any toxic effect is exerted [175]. Interestingly, AβMet-ox is more soluble than wildtype Aβ and was unable to

insert into membranes and cause pore formation. However, the AβMet-ox was still neurotoxic [134] indicating membrane insertion is not a pre-requisite for Aβ toxicity.

5.2. A model for Aβ redox chemistry

The origin of the electron(s) that cause a reduction in the oxidation state of the metal ion could be the peptide itself, from Met_{35} or from biological reducing agents such as dopamine and ascorbate [64]. The presence of an external reductant would permit the catalytic cycling of Cu or Fe without any net oxidation of the peptide. We used density function theory (DFT) to propose a detailed reaction scheme of the catalytic cycle that generates H_2O_2 (Fig. 5) [135]. The model proposed that as a consequence of the formation of a reactive tyrosyl radical on Tyr_{10}, that dityrosine cross-linked Aβ would be generated giving rise to covalently linked soluble oligomers. The formation of tyrosyl radicals is known to induce lipid peroxidation, a feature well characterized in AD.

The DFT model was supported by experimental data for the catalytic production of H_2O_2 by Aβ/Cu^{2+} and was shown to resemble the catalytic activity of galactose oxidase (GO). The role of the tyrosine residue was tested by using Aβ with alanine substituted for Tyr_{10} (AβY10A) [135]. The AβY10A readily formed oligomers but was not toxic to cortical cultures whereas the wildtype peptide containing dityrosine linked oligomers was neurotoxic. Moreover AβY10A can catalytically produce H_2O_2 at half the rate observed for the WT peptide consistent the pivotal role of the tyrosine residue in the redox chemistry as predicted by the model [135].

Opazo and colleagues have addressed other aspects of the model experimentally, specifically the ability of biologically significant reductants to initiate the redox chemistry [64]. Aβ42, coordinated with up to two equivalents of Cu^{2+}, can generate H_2O_2 catalytically by utilizing biological reducing agents as substrates under conditions where the Cu^{2+} or reductant will not form H_2O_2 themselves. The redox activity observed was inhibited by the presence of anti-Aβ antibody, Zn^{2+} or chelators targeting Cu [64]. Moreover, the toxicity of Aβ to neuronal cell cultures was accentuated with the addition of one such reductant dopamine [64].
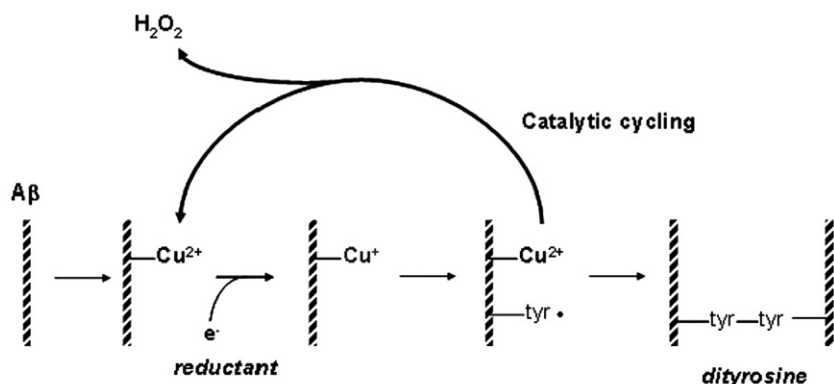

Fig. 5. A simplified model of the redox chemistry events of Aβ catalytic production of H_2O_2 with dityrosine Aβ generated as a side product to the reaction.

5.3. Oxidatively modified stable oligomeric Aβ products

In addition to Aβ methionine sulfoxide, a number of other adducts resulting from redox reactions can be generated. Synthetically oxidatively modified Aβ products include tyrosine modified with adducts such as DOPA, dopamine, dopamine quinine, dihydroxyindol and isodityrosine (shown in Fig. 6) [176]. Aβ extracted from AD plaques contains oxidative modifications such as oxidised Met_{35} [51,149], modification of the histidines to 2-oxo-histidine [177] and oxidatively modified tyrosine adducts including 3,4-dihydroxyphenylalanine (DOPA), dopamine and dopamine quinine [46,177,178]. Tyrosine is particularly susceptible to free radical attack due the conjugated aromatic ring. Evidence of other dityrosine linked peptides/proteins and 3-nitrotyrosine (Fig. 6) within the neuronal lesions in AD brain has been reported [51]. In vitro Aβ42 in the presence of Cu^{2+} and H_2O_2 forms dityrosine cross-linked oligomers [179]. In a separate study, the formation of dityrosine linkage in Aβ facilitated further peptide aggregation, leading to the formation of higher order oligomers [143].

The formation of dityrosine adducts in vivo is a generalized sign of oxidative stress and is a result of free radical reactions [180,181]. The formation of dityrosine arises from the reaction of two tyrosine residues, which form carbon centered radicals in the aromatic ring (Fig. 7). The formation of dityrosine results in the creation of a very stable, irreversible covalent bond [182]. Stable Aβ oligomers resistant to SDS, urea and formic acid treatment have been extracted from AD brain tissue [3,183,184] suggesting that the Aβ peptides are covalently linked. Furthermore, naturally occurring stable Aβ oligomers but not monomers or fibrils inhibit hippocampal LTP [185]. We hypothesize that the Aβ toxic species requires the oxidative modification of Aβ resulting from redox chemistry that leads to the formation of covalently linked (dityrosine crosslinked) soluble Aβ oligomers.

6. Therapeutic inventions

6.1. Antioxidants

The use of antioxidants has proven to be a promising approach for slowing progression of AD by inhibiting oxidative stress damage linked to cognitive and functional decline. A large amount of literature exits in relation to the potential benefits of vitamin supplements as AD prophylactics. Extensive in vitro studies are being conducted and some appear promising, however tandem administration of α-tocopherol (Vitamin E) (Fig. 8a) and ascorbic acid (Vitamin C) (Fig. 8b) only had modest benefits in elderly subjects [62]. Gossypin (3,3′,4′,5,7,8-hexahydroxyflavone 8-glucoside) (Fig. 8c), a bioflavonoid from the yellow petals of hibiscus flowers, is a radical scavenger and can protect cortical cell cultures [186] from Aβ induced toxicity. Melatonin (Fig. 8d) is neuroprotective against Aβ toxicity in vitro [187] and displayed beneficial effects in experimental mouse models of AD, including improvement of cognitive function [188], anti-oxidative injury [188,189], anti-apoptosis [188], inhibition of β-amyloid (Aβ) deposition and Aβ fibril formation [190]. Ginkgo biloba-extract EGb 761 also exhibited neuroprotective effects in several mouse models [191] as well as maintaining and improving cognitive function in AD patients [192,193]. In contrast, a double-blind placebo controlled study found no effect on dementia in AD patients [194]. In vitro studies indicate that the ginkgo extract activity is due to inhibition of Aβ induced free radical generation and occurs in a dose dependant manner [195].

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6 heptadiene-2,5 dione) (Fig. 8e), is the polyphenolic yellow pigment
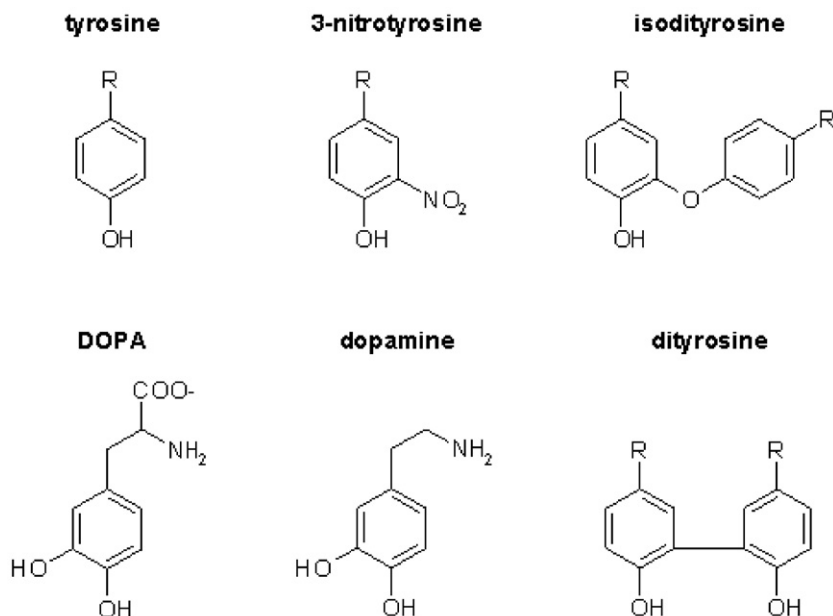

Fig. 6. Tyrosine adducts formed under oxidative conditions.

Fig. 7. Dityrosine formation under oxidative conditions.

in the commonly used turmeric spice in Indian curries and food preservation [196]. Interestingly the prevalence of AD between the ages of 70–79 years in India is 4.4-fold less than in the USA [197]. The compound is neuroprotective against Aβ toxicity in vitro [198] while also being anti-amyloidogenic [199,200]. Furthermore aged transgenic Tg2576 mice with high amyloid plaque load either fed or injected with curcumin had less brain amyloid load and plaque burden; and curcumin labelled plaques [201]. Spectrophotometric studies indicate that curcumin is able to bind the more readily redox reactive metals Cu and Fe but does not bind Zn and therefore acts as an antioxidant by chelating the redox active metal ions [202].

While the details of the mode of pathogenesis remains elusive antioxidants that act to soak up the dangerous free radicals probably have some protective value. However, since they do not address the underlying cause of radical generation (metal-induced redox chemistry) they may have limited value as therapeutics once the disease has started to progress. This is because the reactions that define oxidative stress are chain reactions and once initiated give rise to a growing cascade of

events and therefore it is unlikely that sufficiently high concentrations of drug can reach the active sites to effectively soak up the radicals being generated. Therefore, antioxidants may find their most beneficial role when used as prophylactics.

6.2. Metal coordinating compounds

An alternative approach is to target the initiating event in the generation of free radicals: that is to employ metal complexing agents to prevent the metal ions from participating in the redox chemistry of Aβ that leads to the oxidative stress in AD. An important property of a potential AD therapeutic is its ability to cross the blood brain barrier (BBB). This excludes a large number of common metal chelators as possibilities due to their hydrophilic nature.

Desferrioxamine (N'-[5-(acetyl-hydroxy-amino)pentyl]-N-[5-[3-(5-aminoopentyl-hydroxy-carbamoyl0propanoylamino]pentyl]-N-hydroxy-butane diamide) (Fig. 9b), is a specific Fe chelator with high affinity also for Cu, Zn and aluminium (Al) [203,204]. In one trial desferrioxamine slowed the progression

Fig. 8. The molecular structure of antioxidants that show potential as Alzheimer's disease therapeutic treatments. (a) α-tocopherol, (b) ascorbic acid, (c) gossypin, (d) melatonin and (e) curcumin.
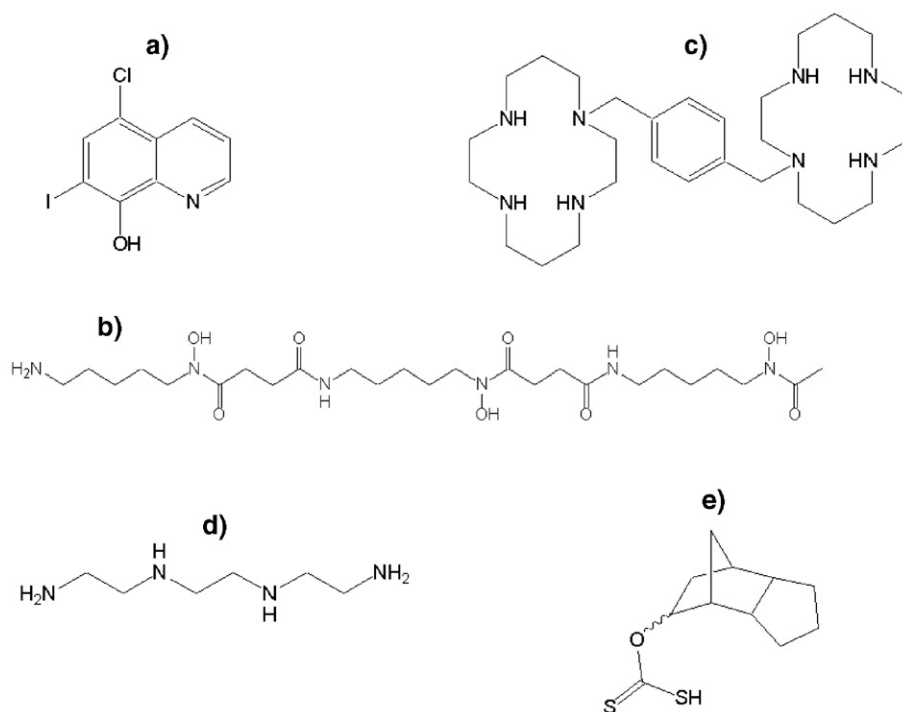
Fig. 9. The molecular structure of metal binding compounds that show potential as Alzheimer's disease therapeutic treatments. (a) clioquinol, (b) desferrioxamine, (c) bicyclam analogue JKL169, (d) triene and (e) tricyclodecan-9-yl-xanthogenate.

of AD. As a part of this study, the Al levels were monitored. Although Al has received some interest due to high concentrations being found in amyloid plaques and intracellular neurofibrillary tangles of AD brain [205–208], no convincing evidence has been published to suggest a role of the metal in the development of the disease although it remains a controversial issue [209]. Unfortunately no other metals were examined so it is unclear as to whether the therapeutic effect of desferrioxamine was actually due to chelation of other metals such as Cu and Zn. Another drawback for desferrioxamine is that it is not orally bioavailable.

Derivatives of a 14-membered saturated tetramine have attracted some recent interest. Bicyclam analogue JKL169 (1,1′-xylyl bis-1,4,8,11 tetraaza cyclotetradecane) (Fig. 9c) was effective in reducing Cu levels in brain cortex and is able to maintain normal Cu levels in the blood, CSF and corpus callossum in rats [210]. A number of other chelators have been studied such as the lipophillic chelator DP109 that reduced the level of aggregated insoluble Aβ and conversely increased soluble forms [211]. The chelator triene (triethylenetetramine) (Fig. 9d), which is used to treat Wilson's disease (intracellular Cu accumulation disease) was ineffective in reducing cerebral plaque concentration. This is attributed to the compound's inability to cross the blood brain barrier [212]. Brain tissue pre-exposed to tricyclodecan-9-yl-xanthogenate (Fig. 9e) showed significant reduction in oxidative stress markers after exposure to Aβ [213] however further work is required to evaluate whether the compound has promise as potential therapeutic for AD treatment.

Oral treatment with the chelator clioquinol (CQ, 5-chloro-7-iodo-8-hydroxyquinoline) (a former antibiotic) (Fig. 9a) in Tg2576 mice resulted in a reduction of cortical deposition of amyloid (49%) with an improvement in general health and weight parameters remaining stable compared to untreated mice [212]. This quinoline compound is able to cross BBB and actually increases the brain Cu and Zn levels in treated mice. The increase in metal ions is suggested to be a result of CQ-metal complexes forming in the intestinal tract and crossing the BBB [210]. Clioquinol was used as an antibiotic, but was removed from the market in 1971 as it was implicated in the development of subacute myleo-optico-neuropathy (SMON) in some patients in Japan. However the link between CQ administration and the development of SMON in these patients is circumstantial as 25% of the diagnosed patients did not receive CQ [214] and was likely due to a vitamin B-12 deficiency within that population exacerbated by CQ [212,215]. The positive animal data lead to CQ being trialled clinically to treated AD, the drug was administered at much lower concentrations than was used to treat bacterial infections [216]. CQ showed significant promise for the treatment of AD, with a small phase II clinical trial revealing positive effects of the drug administered orally in moderately severe AD patients with no signs of SMON [216].

7. Conclusions

The brain requires high metal ion concentrations to carry out its numerous functions. However the brain has a poor capacity to cope with oxidative stress. Aberrant interactions between Aβ and redox active metals could initiate a cascade of events resulting in oxidative stress and chemically modified forms of Aβ. These chemical modifications include the generation of soluble covalently-linked oligomers of Aβ that have been

linked to the toxicity of the peptide. A number of therapeutic strategies aimed at ameliorating or inhibiting Aβ induced redox chemistry, including the use of antioxidants and metal complexing agents are currently being investigated for their utility in treating AD. Therefore, defining the primary trigger for the oxidative stress in AD and the role of Aβ in this process should be critical to our understanding of AD pathogenesis.

Acknowledgements

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