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Amyloid β -Peptide(1-42), Oxidative Stress, and Alzheimer's Disease

D. Allan Butterfield

5.1 Introduction

Alzheimer's disease (AD) a progressive, age-related neurodegenerative disorder that affects memory, cognition, and speech, is present in more than 4 million persons. The number of cases of AD will significantly elevate, because the mean population in the United States is increasing [1]. AD is characterized pathologically by the presence of extracellular senile plaques, intracellular neurofibrillary tangles, and synapse loss. Senile plaques are composed of an amyloid beta-peptide ($A\beta$) core surrounded by dystrophic neurites.

Amyloid precursor protein (APP) is a transmembrane glycoprotein of unknown function that is present in many cells. The protease α -secretase cleaves APP between residues 16 and 17 of $A\beta$ (1-42) to release soluble APP and form a C-terminal fragment of APP. β -secretase proteolytically cleaves APP at the N-terminal side of $A\beta$ (1-42), while γ -secretase cleaves APP on the carboxy-terminus of this sequence. γ -Secretase cleavage takes place at different residues near the carboxy terminus of $A\beta$ resulting principally in the 40-mer and 42-mer, $A\beta$ (1-40) and $A\beta$ (1-42), respectively. These two peptides comprise most of the brain-resident peptide. The more toxic of the two peptides, $A\beta$ (1-42), aggregates more quickly than $A\beta$ (1-40). $A\beta$ (1-42) plays a central role in the pathogenesis of AD, mostly evidenced by the observation of mutations in the genes for APP or presenilin-1 and presenilin-2, all of which result in familial AD and increased production of $A\beta$ (1-42) [2]. We and others have also demonstrated that the AD brain is under extensive oxidative stress as indexed by protein oxidation and

lipid peroxidation [3–7]. Moreover, $A\beta$ (1-42) induces protein oxidation and lipid peroxidation both in vitro and in vivo [3–6, 8–11]. Thus, $A\beta$ (1-42), central to the pathogenesis of AD, is likely also to be central to the oxidative stress under which the AD brain exists.

We developed a unifying model for the pathogenesis of AD based on the central role of $A\beta$ (1-42) as a mediator of free radical-induced oxidative stress in AD brain [4, 12–14]. In this model, $A\beta$ (1-42) inserts into the lipid bilayer as a small aggregate resulting in lipid peroxidation and oxidative modification of proteins [3, 15], both of which are inhibited by vitamin E [16]. In addition, the AD-related peptide $A\beta$ (1-42) causes an influx of Ca^{2+} into the neuron, resulting in loss of intracellular Ca^{2+} homeostasis, mitochondrial dysfunction, and ultimately cell death [17, 18].

In this review, the role of $A\beta$ (1-42)-induced lipid peroxidation and protein oxidation in the pathogenesis of AD is discussed. Additionally, we point out the importance of the single methionine of $A\beta$ (1-42) (residue 35 of this 42-mer) to the oxidative stress and neurotoxic properties of $A\beta$ (1-42).

5.2 $A\beta$ (1-42)-Mediated Lipid Peroxidation and Protein Oxidation

The 1300-g normal brain, though small, consumes more than 30% of inspired oxygen. Unfortunately, the brain is especially vulnerable to lipid peroxidation due to the relatively high abundance of polyunsaturated fatty acids (PUFAs), such as

arachidonic acid and docosahexenoic acid, the presence of redox metal ions that can take part in free-radical reactions, and the relatively low abundance of brain-resident antioxidants. These factors, coupled to the high rate of oxygen respiration in the brain, lead to lipid peroxidation, which is initiated by a free radical-mediated hydrogen atom abstraction from an unsaturated carbon on a lipid-resident acyl chain, resulting in the formation of a carbon-centered lipid radical ($L\cdot$). Because oxygen is both paramagnetic and of zero dipole moment, the lipid radical can readily react with lipid-soluble molecular oxygen to form a peroxy radical ($LOO\cdot$). This latter reactive free radical subsequently expropriates a hydrogen atom from a neighboring unsaturated lipid acyl chain, forming a lipid hydroperoxide ($LOOH$) and another carbon-centered lipid radical ($L\cdot$). Thus, the free-radical chain reaction is propagated. If chain-breaking antioxidants, such as vitamin E are present, the chain reaction is terminated (Fig. 5.1).

Lipid peroxidation leads to the production of reactive alkenals such as 4-hydroxy-2-nonenal (HNE) and 2-propen-1-al (acrolein), both of which are increased in AD brain [15, 19, 20]. These electrophilic α,β -unsaturated aldehydes easily react with protein-bound cysteine, lysine, and histidine residues by Michael addition to form covalently bound adducts that change protein conformation and structure [21],

FIGURE 5.1. Mechanism of lipid peroxidation. The free radical $X\cdot$ abstracts a H atom from unsaturated sites on the fatty acid chains of phospholipids (LH) to produce a carbon-centered free radical ($L\cdot$) (1). The latter in turn is immediately bound by paramagnetic oxygen to form lipid peroxy free radicals ($LOO\cdot$) (2). The chain reaction is propagated by attack of $LOO\cdot$ on another fatty acid chain to form the lipid hydroperoxide and $L\cdot$ again (3). The chain reaction is terminated by radical-radical recombination (4).

resulting in loss of protein function and initiation of cell death (Fig. 5.2).

$A\beta(1-42)$ leads to oxidative stress in vivo [8, 10, 21]. Increased protein oxidation (and where measured, lipid peroxidation as well) was found in *C. elegans* that express human $A\beta(1-42)$ [8, 10] and in brains from knock-in mice with the mutated human gene for APP, PS-1, or the double mutant APP/PS-1 [11, 22, 23].

The mitochondrial enzymes pyruvate dehydrogenase and α -ketoglutarate dehydrogenase are inactivated by HNE or acrolein, presumably by covalent modification of the lipoic acid cofactors of each enzyme via Michael addition [22]. Acrolein and HNE, as well as $A\beta(1-42)$, apparently covalently modify the transmembrane aminophospholipid-translocase (flippase), an ATP-requiring enzyme that maintains phospholipid asymmetry [17, 18]. Appearance of phosphatidylserine (PS) on the outer leaflet of the lipid bilayer is an early signal of apoptosis. Flippase activity is inhibited if a critical cysteine residue in the active site is not free. Consequently, oxidative modification of flippase by HNE or acrolein at this Cys residue could result in exposure of PS on the outer leaflet of the cell membrane leading to neuronal loss [17, 18].

As noted above, the AD brain is under extensive oxidative stress, manifested by, among other indices, increased oxidation of DNA [25]. We hypothesized that one means by which DNA would be oxidized in AD brain is if the protective function of the surrounding histone proteins were altered due to their oxidative modification. To test this hypothesis, we added HNE to histones and showed that (a) the conformation of histones was markedly altered as determined by magnetic resonance methods; (b) the resulting interactions of oxidatively modified histones with DNA were significantly changed from control, consistent with the notion that the protective functions of histones would be compromised in AD brain; and (c) acetylated histones seemed even more vulnerable to oxidative modification by HNE than nonacetylated histones [26]. Thus, we found evidence to support the hypothesis that the lipid peroxidation product, HNE, known to be elevated in AD brain [15, 20], may contribute to the vulnerability of DNA to oxidation in the AD brain.

Addition of $A\beta(1-42)$ to neurons or synaptosomes resulted in increased HNE, with consequent

FIGURE 5.2. HNE adducts of cysteine and histidine formed by Michael addition, and the hemiacetal formed by HNE reaction with lysine.

covalent modification of key proteins [3, 15, 27]. Additionally, treatment of synaptosomes with A β (1-42) resulted in an increase in HNE bound to choline acetyltransferase and the glutamate transporter GLT-1 (EAAT2) [3, 15]. An increase in HNE bound to glutathione-S-transferase (GST), the multidrug resistance protein-1 (MRP1), and EAAT2 in AD brain also was found [15, 28]. The activities of GST and EAAT2 are decreased in AD brain [29, 30]. Thus, removal of HNE from neurons by the action of GST and MRP1 likely is compromised, resulting in accumulation of this harmful alkenal [28]. These findings are consistent with the notion that A β (1-42)-induced lipid peroxidation leads to HNE modification of important enzymes and transporters in AD brain, resulting in loss of function. Similar considerations might explain in part the decreased activity of choline acetyltransferase in AD brain compared with control [31].

Protein oxidation, which generally results in loss of function, is also evident in AD brain [3, 5, 15, 32]. Protein carbonyls are a marker of protein oxidation [33]. Four processes cause carbonyl moieties to be introduced to proteins: (a) free radical-induced scission of the peptide backbone;

(b) oxidation of specific amino acid side chains; (c) HNE or acrolein covalent modification of proteins by Michael addition; and (d) glycooxidation reactions [33]. Protein carbonyls are measured by derivatization of the carbonyl moiety by 2,4-dinitrophenylhydrazine to form a hydrazone product, which can be detected spectroscopically or immunochemically (Fig. 5.3). Additionally, protein oxidation can be indexed by measure of 3-nitrotyrosine (3-NT) (Fig. 5.3). Increased levels of 3-NT have been reported in AD brain [7, 34–36] and CSF [37], and A β (1–42) addition to neurons results in elevated 3-NT [38, 39]. RNS leads to 3-NT in synaptosomes, and novel antioxidants are able to prevent damage to these synaptosomes or synaptosome-resident mitochondria [38–42].

Oxidative modification of glutamine synthetase (GS) and creatine kinase (CK) are found in AD brain, and both GS and CK have significantly decreased activity in AD brain [5, 32, 43, 44]. We have used proteomics to identify brain proteins that are excessively oxidatively modified in AD brain relative to control brain (Fig. 5.4) [34, 36, 43, 45–50]. These include: CK (BB isoform), phosphoglycerate mutase, glyceraldehydes-3-phosphate

FIGURE 5.3. (Top) Derivatization of protein carbonyls by 2,4-dinitrophenylhydrazine. This reaction occurs as a consequence of the well-known Schiff base formation between a primary amine and a carbonyl functionality. (Bottom) Mechanism of formation of 3-NT. The 3-position of the aromatic ring of Tyr is attacked to form 3-NT as a consequence of the electronic structure around this aromatic site.

FIGURE 5.4. Schematic of proteomic identification of carbonylated proteins involving the parallel analysis for differences in protein expression and oxidative modification.

dehydrogenase, GS, ubiquitin carboxy-terminal hydrolyze L-1 (UCH L-1), α -enolase, triphosphate isomerase, neuropolypeptide h3, and dihydropyrimidinase related protein-2 (DRP-2), among others. A wide spectrum of cellular functions including energy metabolism, glutamate uptake and excitotoxicity, proteosomal dysfunction, tau hyperphosphorylation, mitochondrial function, and neuronal communication are affected by these oxidized proteins. As noted above, oxidative modification of protein nearly always leads to loss of protein function. Thus, several plausible mechanisms of neurodegeneration can be proposed based on each of the oxidized proteins.

CK BB, α -enolase, phosphoglycerate mutase, glyceraldehydes-3-phosphate dehydrogenase, and triphosphate isomerase are all directly or indirectly involved in the synthesis of ATP. Consistent with PET scanning findings that show decreased metabolism in AD brain [51, 52], CK and enolase activities are decreased in AD brain [5, 48]. Lack of ATP would cause dysfunction in ion pumps, electrochemical gradients, voltage-gated ion channels, and cell potential, all of which are needed to combat the oxidative stress of synaptic regions of neurons induced by A β (1-42).

The oxidative modification and dysfunction of EAAT2 in AD brain [15, 29] coupled to diminution of GS function as a result of its oxidation (as revealed by proteomics) would result in a decreased conversion of glutamate. This in turn would stimulate *N*-methyl-D-aspartate (NMDA) receptors leading to an increase in Ca²⁺ influx. Alterations in calcium homeostasis would lead to dysfunctional long-term potentiation (LTP), which, in turn, would affect learning and memory. Additionally, Ca²⁺-mediated mitochondrial swelling, resulting in reactive oxygen species (ROS) and proapoptotic cytochrome c release, ER stress, and activation of calcium-sensitive proteases such as calpain and caspases, are downstream consequences of oxidative stress-related loss of Ca²⁺ homeostasis. These insults are known to lead to neuronal death, and we have hypothesized that such processes are important in AD brain [45, 49, 50].

Accumulation of damaged, misfolded, and aggregated proteins in AD brain may be due to proteasomal dysfunction [53, 54]. One protein involved in proteasome function is UCH L-1. Dysfunction of this protein is observed in AD brain

[48]. UCH L-1 catalyzes removal of polyubiquitin from damaged proteins, and its dysfunction, as a result of its oxidation, would lead to excess protein ubiquitinylation, loss of activity of the proteasome, and accumulation of damaged or aggregated proteins, all of which are found in AD.

DRP-2, which has decreased expression in AD [55–57] and is oxidatively modified in AD brain [46], is involved in pathfinding and guidance for axonal outgrowth. Moreover, DRP-2 interacts with and modulates the function of collapsin, a protein involved in dendrite elongation and guidance to adjacent neurons. Therefore, DRP-2 is involved in forming neuronal connections and maintaining neuronal communication. Consequently, the oxidation and diminished activity of DRP-2 could result in the reported shortened dendritic lengths in AD brain [58]. Neurons with shortened neurites are predicted to communicate less well with adjacent neurons, a process that could conceivably be important in a memory and cognitive disorder like AD.

Proteomics analysis has identified neuropolypeptide h3 as specifically nitrated in AD brain [34]. Neuropolypeptide h3 is also identified as phosphatidylethanolamine-binding protein (PEBP) and hippocampal cholinergic neurostimulating peptide (HCNP). A decrease in the function of PEBP could lead to loss of phospholipid asymmetry, resulting in the exposure of phosphatidylserine on the outer leaflet of the lipid bilayer, a signal of apoptosis. As noted, both A β (1-42) and the A β (1-42)-mediated lipid peroxidation product HNE lead to loss of lipid asymmetry, which may be relevant to oxidative stress-related AD [17, 18]. Upregulation of choline acetyltransferase (CAT) in cholinergic neurons after NMDA receptor activation is one function of HCNP [59]. CAT activity is known to be decreased in AD [31], and cholinergic deficits are prominent in AD brain [1, 60]. A β (1-42) leads to elevated HNE on CAT, possibly contributing to its loss of function in AD brain [3]. Nitration of neuropolypeptide h3 could lead to diminution of neurotrophic action on cholinergic neurons of the hippocampus and basal forebrain, which may be related to the observed decline in cognitive function in AD brain.

Proteomics studies in our laboratory are ongoing to identify proteins that are oxidatively modified by A β (1-42) in model systems relevant to AD [61–65]. The results of these studies show some common proteins that are oxidized by A β (1-42) in

vivo and in AD brain, consistent with the notion that A β (1-42) significantly contributes to the oxidative stress of AD brain.

5.3 Methionine-35 of A β (1-42): Role in A β (1-42)-Induced Oxidative Stress and Neurotoxicity

Methionine 35 is a critical residue in A β (1-42)-mediated oxidative stress and neurotoxicity. Substitution of the sulfur atom of methionine 35 by a methylene group, $-\text{CH}_2-$ (norleucine), significantly modulates the oxidative stress and neurotoxicity of A β (1-42), but the fibrillar morphology of both peptides is similar [10]. Methionine 35 of A β (1-42) is also involved in the oxidative stress and neurotoxicity properties of this peptide in vivo. *C. elegans* expressing human A β (1-42) exhibited significantly increased protein oxidation, but replacement of the codon for Met by that for Cys in the DNA sequence for human A β (1-42) resulted in no increase in protein oxidation in the worm compared with *C. elegans* expressing native human A β (1-42) [10]. Additionally, studies involving a temperature inducible *C. elegans* model expressing human A β (1-42) revealed that protein oxidation precedes the deposition of fibrillar aggregates [8]. This finding is consistent with increasing evidence that small soluble aggregates of A β (1-42) are the toxic species of this peptide [66–68]. Moreover, that A β (1-42) containing the norleucine derivative of A β (1-42), which through producing fibrils, was not oxidative or neurotoxic supports our hypothesis that methionine is critically involved in the neurotoxic and oxidative properties of A β (1-42) [10, 69].

Lipid peroxidation is induced by A β (1-42) [15, 27] and is found in AD brain [15, 19, 20]. Because lipid peroxidation requires that the free radical involved must be located in the immediate vicinity of the labile H-atoms of unsaturated acyl-chains on phospholipids, this requirement suggests that the Met residue of A β (1-42) is located in the bilayer [70], a suggestion confirmed by others [71]. It has been proposed that, due to the hydrophobic carboxy terminus of A β (1-42), the peptide inserts into the lipid bilayer [70–72]. A β (1-42) adopts an α -helical conformation, similar to other proteins that insert into the lipid bilayer. A methionine sulfu-

ranyl radical (MetS $\cdot$) on A β (1-42) is formed by a one-electron oxidation [12–14, 69, 72–75]. This radical, in turn, can abstract a hydrogen atom from a neighboring unsaturated lipid resulting in the formation of a carbon-centered lipid radical (L $\cdot$). Via mechanisms described above (Fig. 5.1), the carbon-centered radical on the lipid can readily react with molecular oxygen to form a peroxy radical (LOO $\cdot$). Hydrogen abstraction from a neighboring lipid results in the formation of a lipid hydroperoxide (LOOH) and another carbon-centered lipid radical (L $\cdot$), thereby, propagating the free-radical chain reaction [69, 74, 75]. Both theoretical and experimental studies demonstrate that the α -helical secondary structure of the peptide provides stabilization of the sulfuranyl radical formed by a one-electron oxidation of methionine [72, 76]. Mutation of isoleucine 31 in A β (1-42) to proline, an α -helix breaker, attenuated the oxidative stress and neurotoxic properties of the native peptide, suggesting that the amide oxygen of isoleucine 31 in the α -helix conformation interacts with a lone pair of electrons on the sulfur atom of methionine 35, priming this atom for a one-electron oxidation [72]. Subsequently, the sulfuranyl radical of methionine can react with other moieties of methionine to form an α -(alkylthio)alkyl radical of methionine ($-\text{CH}_2-\text{CH}_2-\text{S}-\text{CH}_2$ or $-\text{CH}_2-\text{CH}-\text{S}-\text{CH}_3$) [69, 72, 74, 76]. Such carbon-centered radicals provide potential substrates for reaction with molecular oxygen leading to the formation of peroxy radicals, and consequently, potentiation of free-radical generation and HNE formation [69, 75, 77]. Recently, others have confirmed our hypothesis, directly demonstrating the existence of the sulfuranyl free radical in A β (1-40) [78]. Other researchers [79, 80] invoke Cu(II) reduction and subsequent H₂O₂ formation in the oxidative stress and neurotoxic properties of A β (1-42). Critical in this scenario are the three His residues at positions 6, 13, and 14 and the Tyr at position 10. The former are the likely binding sites for Cu(II) on A β (1-42), while Tyr 10 is proposed to be the source of the electron to reduce Cu(II) to Cu(I). However, substitution of the three His residues by asparagine (which has at least a 100-fold less binding affinity of Cu(II) than does His) or substitution of Tyr 10 by aromatic Phe (which, though still aromatic, is incapable of providing an electron to Cu(II)) leads to peptides that are similarly toxic and oxidative as

native $A\beta$ (1-42) [81, 82]. In contrast, substitution of Met by norleucine, which still has the three His residues and Tyr 10 present, is no longer toxic or oxidative [10]. Using the reverse peptide, $A\beta$ (40-1), which is nontoxic, others showed that a Tyr free radical could be formed [78]. That is, a central feature required in mechanisms that involve Cu(II) reduction as a cardinal paradigm occur only in a peptide that is nontoxic [78].

Oxidative modification of methionine 35 to methionine sulfoxide constitutes a major component of the various amyloid β -peptides isolated from AD brain [83–85], consistent with the role of methionine in the oxidative properties of $A\beta$ (1-42). In vitro oxidation of methionine to methionine sulfoxide has been shown to abolish the oxidative stress and neurotoxic properties of $A\beta$ (1-42) after a 24-h incubation with neurons. Mitochondrial dysfunction as measured by MTT reduction was also observed [73]. This finding was confirmed in a recent study [80]. However, after a 96-h treatment, the methionine sulfoxide of $A\beta$ (1-42) reportedly resulted in neuronal death as observed by phase contrast microscopy. $A\beta$ (1-42) containing methionine sulfoxide does not associate itself with the lipid bilayer due to the hydrophilic oxidized sulfur atom [80]. It is conceivable that $A\beta$ (1-42) containing methionine sulfoxide may not form fibrils readily but does so after a long enough period. Thus, toxicity of $A\beta$ (1-42) containing methionine sulfoxide may occur via a different mechanism than with native $A\beta$ (1-42), that is, fibril formation conceivably could activate the receptor for advanced glycation end products (RAGE) leading to oxidative stress and neurotoxicity [86, 87].

5.4 Conclusions

$A\beta$ (1-42) plays a critical role in the oxidative stress present in AD brain and, consequently, may play a central role in the pathogenesis of the disease. $A\beta$ (1-42) induces protein oxidation and lipid peroxidation both in vitro and in vivo. Methionine 35 has been shown to play a vital role in the oxidative stress and neurotoxic properties of $A\beta$ (1-42). Ongoing proteomic studies will lead to the identification of proteins that are specifically oxidatively modified by $A\beta$ (1-42), providing insight into mechanisms of $A\beta$ (1-42)-induced neurodegenera-

tion and, consequently, a greater insight into the role that $A\beta$ (1-42) plays in the pathogenesis of this dementing disorder.

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