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Methionine residue 35 is critical for the oxidative stress and neurotoxic properties of Alzheimer's amyloid β-peptide 1–42

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Abstract

Amyloid β-peptide 1–42 [Aβ(1–42)] is central to the pathogenesis of Alzheimer's disease (AD), and the AD brain is under intense oxidative stress. Our laboratory combined these two aspects of AD into the Aβ-associated free radical oxidative stress model for neurodegeneration in AD brain. Aβ(1–42) caused protein oxidation, lipid peroxidation, reactive oxygen species formation, and cell death in neuronal and synaptosomal systems, all of which could be inhibited by free radical antioxidants. Recent studies have been directed at discerning molecular mechanisms by which Aβ(1–42)-associated free radical oxidative stress and neurotoxicity arise. The single methionine located in residue 35 of Aβ(1–42) is critical for these properties. This review presents the evidence supporting the role of methionine in Aβ(1–42)-associated free radical oxidative stress and neurotoxicity. This work is of obvious relevance to AD and provides a coupling between the centrality of Aβ(1–42) in the pathogenesis of AD and the oxidative stress under which the AD brain exists.

Keywords: Amyloid; Methionine; Oxidative stress

1. Introduction

Alzheimer's disease (AD) is the leading cause of dementia in the elderly, and is the fourth leading cause of death in the United States, where, currently, more than 4 million persons have this neurodegenerative disorder [33]. In the absence of intervening therapies, it is estimated that 14 million Americans and more than 22 million persons worldwide will develop AD in the next few decades [32]. One principal pathological hallmark of AD is the presence of senile plaques in vulnerable brain regions. These plaques consist of a dense core of an aggregated peptide, amyloid β-peptide (Aβ), whose length varies from 39 to 43 amino acid residues, surrounded by a sheath of dying neurites and other components.

Based mostly on genetic evidence of early- and late-onset familial AD, Aβ is postulated to be central to the pathogenesis of this disorder [68]. In addition, the AD brain is under extensive oxidative stress (review [49]), with the chief biomarkers being protein oxidation [4,5,27,43,69,70,73], lipid peroxidation [23,42,45,49,50,53,58,59,74], DNA, and RNA oxidation [20,28,44,54,55], advanced glycation end products [71,72,81] and widespread occurrence of 3-nitrotyrosine, a product of peroxynitrite-induced tyrosine nitration [72].

Our laboratory has united these two aspects of AD—the importance of Aβ in the pathogenesis of this disorder and the extensive oxidative stress under which the AD brain exists—into a comprehensive model of neuronal death in AD brain. The model is based on amyloid β-peptide-associated oxidative stress induced neurotoxicity [10,12,78]. Consistent with this model, our laboratory and other laboratories have shown that, in ways that are inhibited by free radical scavengers such as Vitamin E, melatonin, estradiol, EUK-34, etc. Aβ induces: lipid peroxidation [7,8,12,18,22,35,47,48], protein oxidation [24,25,76,77,83–87], ROS formation [86], inhibition of key transport proteins [24,25,34,39,46,47,52,85,87], and enzymes [1–3,13,26,83], and other markers of oxidative stress (recently reviewed in [78]).

Cellular mechanisms associated with Aβ-induced free radical oxidative stress that may lead to neuronal dysfunction and neurotoxicity include, among others [78]: Aβ-derived lipid peroxidation products, e.g. 4-hydroxynonenal or acrolein, which upon reaction with membrane proteins changes their conformation and induces oxidative events [11,13,61,62,75]; mitochondrial dysfunction [6]; neuroinflammation secondary to Aβ fibril formation [15]; activation of certain receptors leading to Ca^{2+} accumulation with subsequent ROS formation [37]; and apoptosis [36].

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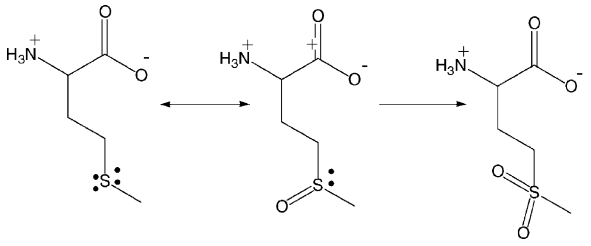


Fig. 1. Reversible oxidation of methionine to methionine sulfoxide. Methionine sulfoxide can be further irreversibly oxidized to methionine sulfone.

Aβ(1-42): H_2N-Asp[1]-Ala[2]-Glu[3]-Phe[4]-Arg[5]-His[6]-Asp[7]-Ser[8]-Gly[9]-Tyr[10]-Glu[11]-Val[12]-His[13]-His[14]-Gln[15]-Lys[16]-Leu[17]-Val[18]-Phe[19]-Phe[20]-Ala[21]-Glu[22]-Asp[23]-Val[24]-Gly[25]-Ser[26]-Asn[27]-Lys[28]-Gly[29]-Ala[30]-Ile[31]-Ile[32]-Gly[33]-Leu[34]-**Met[35]**-*Val[36]*-Gly[37]-Gly[38]-Val[39]-Val[40]-Ile[41]-Ala[42]-COOH

Aβ(1-42) Met Residue 35 Side Chain: -	-CH_2- CH_2-**S**-CH_3
Aβ(1-42M35NLE) Residue 35 Side Chain:	-CH_2-CH_2-**CH_2**-CH_3
Aβ(1-42M35Sox) Residue 35 Side Chain:	-CH_2-CH_2-**S(O)**-CH_3

Fig. 2. Top: amino acid sequence of Aβ(1–42). Note the position of Met in residue 35. Bottom: methionine residue 35 side chain structure for Aβ(1–42), and its slight modification in Aβ(1–42M35NLE) and Aβ(1–42M35Sox).

ApoE genotype is a risk factor for AD that modifies the susceptibility of synaptosomes to Aβ-induced structural and oxidative effects [38]. As outlined in the following sections, the mechanism of Aβ-associated oxidative stress must certainly involve the single methionine residue of Aβ at position 35 [14,77,84], and there is the evidence supporting this mechanism that is the subject of this review.

2. Methionine as initiator of free radical processes in peptides

Methionine is one of the most easily oxidized amino acids. Methionine, an essential amino acid, undergoes a reversible oxidation under relatively mild conditions leading to formation of methionine sulfoxide that can exist in two diastereomeric forms. In fact, oxidation of methionine in living systems might serve two purposes: (a) a regulatory function similar to that of phosphorylation of tyrosine residues; (b) an antioxidative defense: by oxidizing the presumably surface-exposed methionine residues, other important amino acids are spared. The former function is supported by occurrence of methionine in active sites of several enzymes [40,82], and the latter function is supported by the presence of peptide methionine sulfoxide reductase (pMSR) enzyme in brain and elsewhere [40,69]. Interestingly, studies have shown that pMSR seems to be specific to only one type of sulfoxide diastereomer as in the case of reduction of calmodulin in vitro, suggesting that the accumulation of methionine sulfoxide in the aged organism might be a consequence of pMSR diastereomeric selectivity [69]. In AD brain, pMSR activity is reduced [21], suggesting the possibility that surface-exposed Met residues no longer can serve their antioxidant function. Methionine sulfoxide can be further oxidized to form a corresponding sulfone (Fig. 1); however, this process requires a much stronger oxidant, and as a result it is not an easily reversible process.

Mechanistic aspects of free radical oxidation of sulfur in methionine, or methionine containing peptides have been extensively studied [56,57,63,65]. Considering free radical initiated oxidation, the sulfur atom of methionine can be easily attacked by hydroxyl radicals leading to formation of a sulfuramyl radical cation (S^+) or hydroxysulfuramyl radical cation. Several oxidative pathways can result from these sulfuramyl radicals. They can react with superoxide to produce the corresponding sulfoxide via several possible pathways [56]. Alternatively, as in case of N-terminal methionyl peptides, sulfuramyl radical cation can also undergo a proton transfer between the N-terminal nitrogen to form a nitrogen–sulfur radical cation that rearranges to give azasulfonium compounds [57]. It appears that a H-atom abstraction step is likely from the neighboring methyl group on Met, forming an α(alkylthio)alkyl radical of methionine that can subsequently react with paramagnetic oxygen to form a highly reactive peroxyl radical or other species such as formaldehyde and sulfhydryl [65]. In another model system, dipeptides containing N-terminal Ser or Thr, free radical initiated oxidation of C-terminal methionine has resulted in release of formaldehyde, or acetaldehyde for Ser and Thr, respectively, via proton transfer mechanisms [65]. This type of chemistry is not unique to dipeptides, however, provided that the larger N-terminal Ser or Thr peptide possesses enough conformational flexibility to allow close contact in space between these amino acids and C-terminal Met [63].

In the case of Aβ peptides, as discussed further, it is postulated that interaction of the S-atom of Met with the carbonyl oxygen of Ile-31 leads to an intermediate that can be oxidized by molecular oxygen to form a sulfuramyl free radical [65].

3. Importance of methionine in Aβ(1–42)-induced oxidative stress and neurotoxicity

Fig. 2 shows the structure of Aβ(1–42), which has a single methionine at residue 35. Addition of Aβ(1–42) to 9–11-day-old primary hippocampal cultures leads to protein oxidation, indexed by protein carbonyls [11], and neurotoxicity, indexed by Trypan blue exclusion [78,84–87] (Fig. 3A). Vitamin E, a chain-breaking antioxidant, significantly modulates the oxidative stress and neurotoxic effects of Aβ(1–42) (Fig. 3B and C), as would be expected

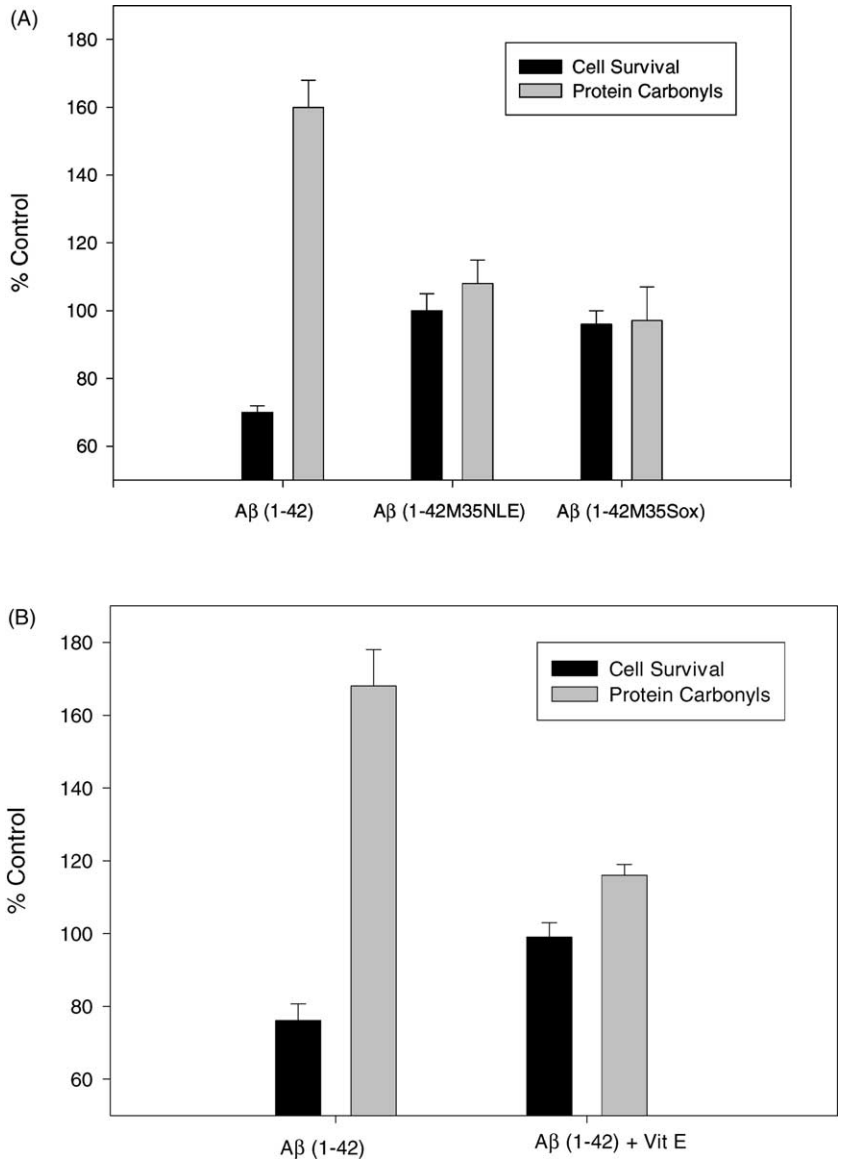


Fig. 3. (A) The relative changes in neuronal survival and protein carbonyl content in 9–11-day-old rat embryonic hippocampal neuronal cultures treated with 10 μM of each indicated peptide for 2 days. Both neuronal survival and protein carbonyl content are expressed as the mean (%) ± S.E.M. of untreated control cultures. Statistical comparisons were made using ANOVA. Only Aβ(1–42) caused statistically significant changes in both neuronal survival and protein carbonyl content ($P < 0.001$, $n = 3$–6), with each n a minimum of four replicates. (B) The relative changes in neuronal survival and protein carbonyl content in 9–11-day-old rat embryonic hippocampal neuronal cultures treated with Aβ(1–42) (10 μM) for 2 days without or with pretreatment with the antioxidant, Vitamin E (50 μM). Both neuronal survival and protein carbonyl content are expressed as the mean (%) ± S.E.M. of untreated control cultures. Statistical comparisons were made using ANOVA. Aβ(1–42) caused statistically significant changes in both neuronal survival and protein carbonyl content relative to controls ($P < 0.001$), while Vitamin E-treated cells exposed to Aβ(1–42) yielded neuronal survival and protein carbonyl content means that were statistically identical to those of untreated controls. ($n = 2$–4, with each n a minimum of four replicates). (C) Inhibition of Aβ(1–42)-induced ROS formation, detected by the conversion of 2′,7′-dichlorofluorescin to 2′,7′-dichlorofluorescein, in 9–11-day-old rat embryonic hippocampal neuronal cultures. Color images were obtained using a fluorescence confocal microscope and digitized. Quantification of the images was performed using imaging software, and mean ± S.E.M. values are presented. Aβ(1–42) induces about a four-fold increase in ROS over untreated controls ($P < 0.001$), while Vitamin E pretreatment for 1 h prior to Aβ(1–42) addition leads to a mean ROS value that is highly significantly different than that of Aβ(1–42) ($P < 0.005$) ($n = 3$, with each n the mean of 8–11 neurons). (D) EPR spectra of Aβ(1–42) after 60 h incubation at 37 °C with highly purified PBN in buffer containing 2 mM deferroxamine mesylate and prepared with water stored over chelex-100 beads. The PBN was synthesized and repeatedly recrystallized and resublimed in our laboratory. Purity was evaluated using NMR and HPLC analyses: (a) EPR spectrum of PBN after 60 h incubation. Note the absence of any resonance lines; (b) EPR spectrum of Aβ(1–42) after 60 h incubation with highly purified PBN. A prominent four-line spectrum and a weak six-line spectrum is observed; and (c) EPR spectrum of Aβ(1–42M35NLE) after 60 h incubation with highly purified PBN. Note the absence of a spectrum in this peptide in which the S-atom of methionine residue 35 of Aβ(1–42) has been replaced by a methylene (–CH_2–) group.

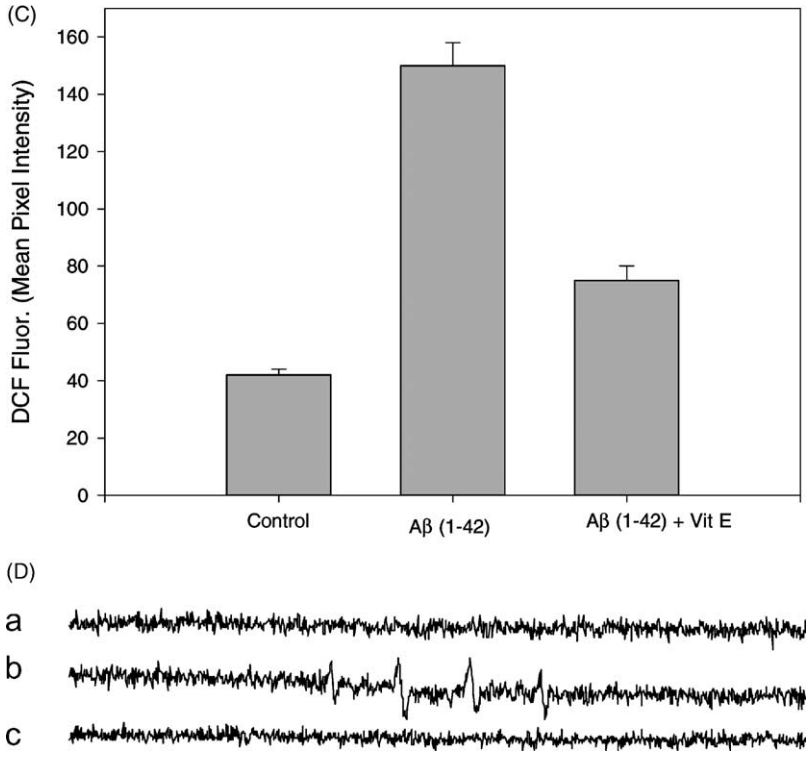


Fig. 3. (*Continued*).

if a free radical process is operative [86]. Replacement of the sulfur atom of methionine in Aβ(1–42) by a methylene moiety (–CH_2–) leads to a norleucine derivative [Aβ(1–42M35NLE)] (Fig. 2). Substitution of this one S-atom of this approximately 4000-Da Aβ(1–42) peptide by CH_2 is unlikely to cause significant alterations in peptide structure (same side chain length and hydrophobicity) although there is no data to support this speculation. Addition of Aβ(1–42M35NLE) to 9–11-day-old primary hippocampal cultures causes no protein oxidation, no neurotoxicity, and no free radical formation [84] (Fig. 3A). Further, in contrast to native Aβ(1–42), Aβ(1–42M35NLE) produces no electron paramagnetic resonance (EPR) spectrum in the presence of the highly purified spin trap PBN and metal ion chelators [84] (Fig. 3D). These results are consistent with the notion that methionine residue 35 is essential for the oxidative stress and neurotoxic properties of Aβ(1–42). In support of this idea, oxidation of the sulfur of methionine in Aβ(1–42), leading to Aβ(1–42M35Sox) (Fig. 2), is also not toxic to cells and produces no protein oxidation [80] (Fig. 3A). This result probably arises from the fact that the sulfur is already oxidized in the methionine residue of Aβ(1–42) and can not easily be oxidized further to the sulfone. However, although Aβ(1–42M35Sox) is not toxic to neurons, this peptide does inhibit mitochondrial function as assessed by the MTT reduction assay [80], a result confirming previous studies of Seilheimer et al. [67]. In one study, the substitution of norleucine for methionine in Aβ(1–42) still resulted in toxicity [60]. These researchers used different neuronal culture conditions than used in our laboratory, and, in contrast to usual studies of aged neurons (9–11 days in culture at which time transporters and other key cellular components are fully expressed), 2-day-old neuronal cultures were used to assess neurotoxicity.The totality of the above results suggest that the methionine residue of Aβ(1–42) is important for oxidative stress and neurotoxicity. However, since emerging evidence suggests that aggregation of Aβ peptides is essential for their toxicity, is the absence of oxidative stress and neurotoxicity in Aβ(1–42M35NLE) and Aβ(1–42M35Sox) due to the lack of fibril formation in these methionine-modified peptides? Fig. 4 shows that Aβ(1–42), Aβ(1–42M35NLE, and Aβ(1–42M35Sox form fibrils when viewed by electron microscopy, while the reverse sequence of Aβ(1–42) [Aβ(42–1)] is devoid of fibrils. This result, also seen if Vitamin E, which inhibits Aβ(1–42)-induced oxidative stress and neurotoxicity [86], is added to Aβ(1–42) (Fig. 4), [79], suggests that it is not the lack of aggregation of these methionine-modified peptides that prevents oxidative stress and neurotoxicity. Rather, we propose that is it is the absence or diminution of free radicals associated with the peptide that provides the explanation of the non-oxidative and non-toxic properties of these modified peptides.

The importance of methionine in Aβ(1–42)-associated oxidative stress also was demonstrated in vivo [84]. Transgenic *Caenorhabditis elegans* were genetically modified using an *unc*-54 promoter to produce human Aβ(1–42) that is deposited in the muscle wall of the worm [19]. Animals

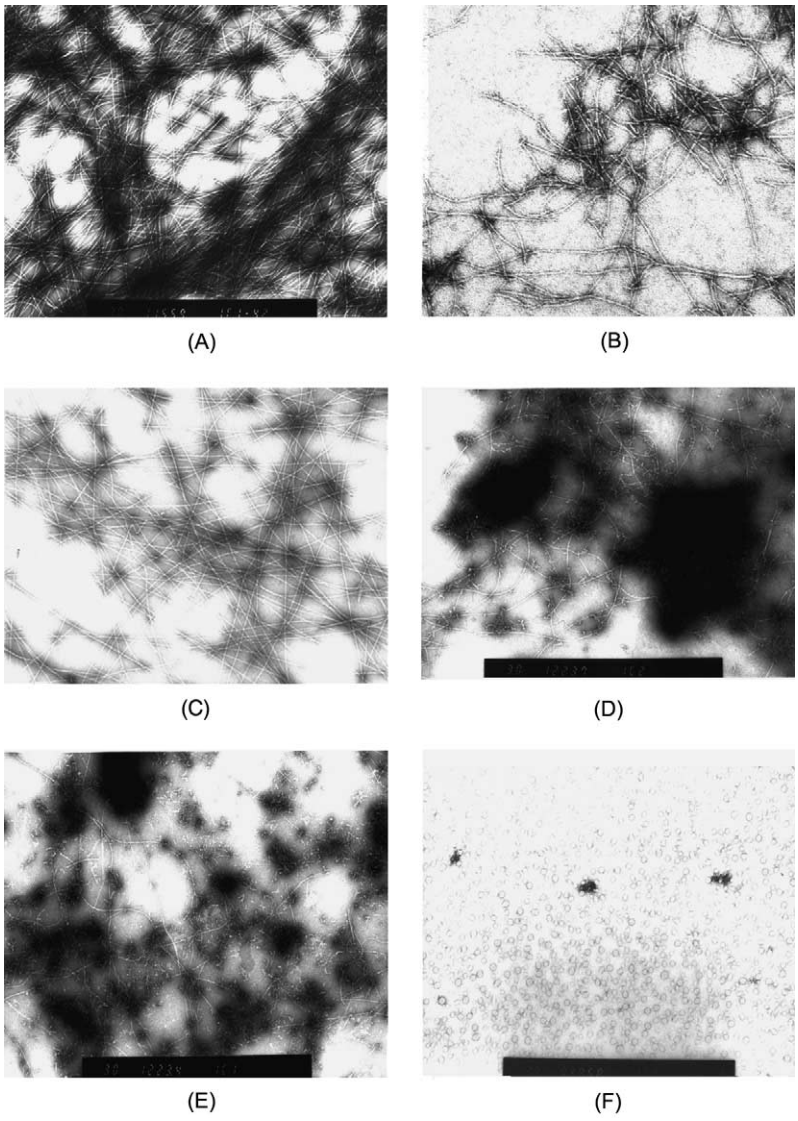


Fig. 4. Fibril formation assessed by electron microcopy. Peptides, 1 mg/ml, were dispersed in 500 μl deionized water and incubated for 48 h at 37 °C. Five microliter of each peptide mixture was placed on separate copper formvar carbon-coated grids. After 1–5 min at room temperature, excess water was drawn off, and samples were counterstained with 2% uranyl acetate. Air-dried samples were examined in a transmission EM at 75 kV. (A) Aβ(1–42), which induces oxidative stress in and is toxic to neurons. (B) Aβ(1–42M35NLE), which does not induce protein oxidation nor is it neurotoxic. (C) Aβ(1–42M35Sox), which does not induce protein oxidation nor is it neurotoxic. (D) Aβ(1–42) in the presence of Vitamin E (DMSO), which inhibits protein oxidation and neurotoxicity. (E) Aβ(1–42) in the presence of DMSO alone. (F) Aβ(42–1).

expressing human Aβ(1–42) phenotypically are paralyzed when placed on a food source in stark contrast to vector-only worms that are mobile. The Aβ(1–42) deposited is in the β-sheet conformation assessed by thioflavin T and the fluorescent Congo Red derivative, X-34 [41]. If our model for Aβ(1–42)-associated free radical oxidative stress [10,12,78] is correct, a prediction would be that worm proteins should have increased oxidation relative to the vector controls. Table 1 shows that this is the case [84]. A different transgenic *C. elegans* construct that express the same amount of peptide as that deposited in transgenic animals expressing native human Aβ(1–42) [19], but has methionine substituted by cysteine, no longer shows any increased protein

Table 1
In vivo protein oxidation in transgenic *C. elegans* in which human Aβ(1–42) is expressed

Construct	Protein oxidation[a]	*P* value
Human Aβ(1–42)	175 ± 6 (6)	<0.001
Human Aβ(1–42M35C)	96 ± 3 (4)	N.S.

[a] Percent of the *unc*-54 vector only controls. Mean ± S.E.M. (number of samples) are presented. Protein oxidation was determined by imaging Western blots of immunochemically-detected protein carbonyls relative to those of the vector control.

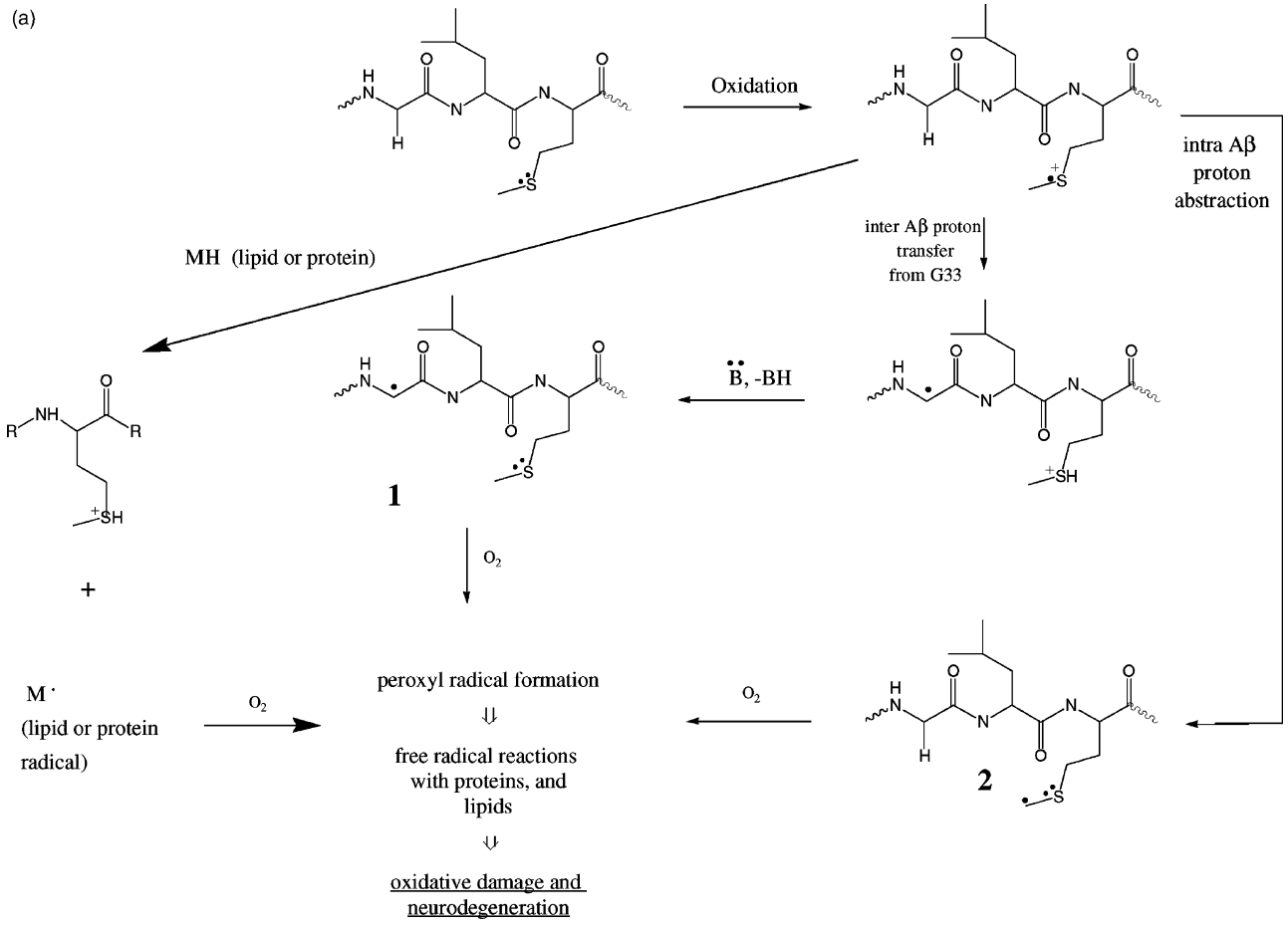
oxidation (Table 1). Methionine is a thio-ether, and, as such, can undergo free radical chemistry [56,57,63,65,66]. In contrast, cysteine is a thio-alcohol, which does not have the same chemistry as thio-ethers. Alternatively, the cysteine-containing Aβ(1–42) has a potential for disulfide formation, which could conceivably inhibit its reactivity. These in vivo results are consistent with the in vitro studies that suggest the importance of methionine in the oxidative stress and neurotoxic properties of Aβ(1–42). These transgenic *C. elegans* worms with methionine substituted by Cys are no longer paralyzed [19], consistent with the notion that it is the oxidative stress induced by the human Aβ(1–42) that produces the muscle paralysis in these worms. Currently, we are testing different amino acid substitutions in *C. elegans* with the aim of gaining additional insight into toxicity mechanisms of human Aβ(1–42).

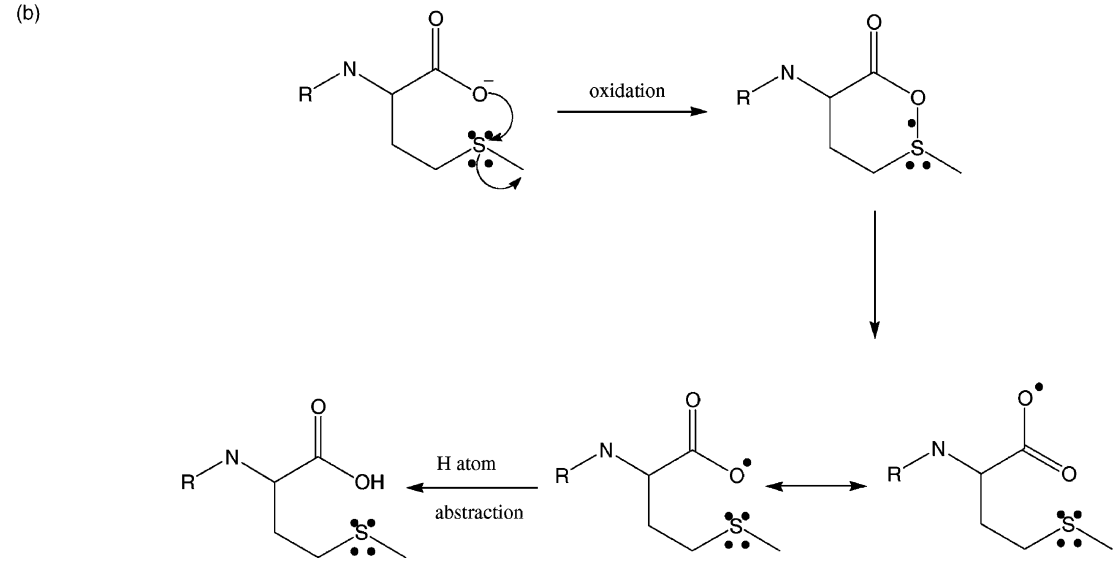
In addition to the criticality of methionine for the oxidative stress and neurotoxic properties of Aβ(1–42), similar findings were observed for Aβ(1–40) and Aβ(25–35) [77]. Many researchers have used this latter, 11-amino acid fragment of Aβ(1–42) as a low-cost alternative to full-length Aβ(1–42) peptide, since this smaller peptide mimics several of the oxidative and neurotoxic properties of full-length Aβ(1–42). However, these properties usually are manifested much sooner (hours) than those of Aβ(1–42) (1–2 days) and often are more pronounced. These differences may reflect different molecular mechanisms for oxidative stress and neurotoxicity between the peptides. The most obvious difference with respect to the methionine residue is that Met is C-terminal in the shorter peptide. To investigate the role of C-terminal Met in Aβ(25–35), several studies were conducted [77,80]. Truncation of Aβ(25–35) to produce Aβ(25–34), i.e. no Met, leads to a non-oxidative and non-neurotoxic peptide [77]. Similarly, elongation of the peptide to Aβ(25–36), i.e. Met is no longer C-terminal, also leads to a non-oxidative and non-neurotoxic peptide [80]. These results with this shorter peptide are consistent with the notion shown in Fig. 5 (bottom) that the carboxylate oxygen can attack the sulfur atom on Met, thereby making a six-membered ring, to form a sulfuramyl free radical. Either directly by H-atom abstraction or though other free radical propagating steps, this sulfuramyl free radical could lead to the observed protein and lipid peroxidation. Loss of H^+ from SH^+ (pK_a is −5) by any base present leads to the regeneration of methionine, and the whole process would be catalytic. Hence, this may account for the increased rapidity and increased oxidative stress and neurotoxicity with Aβ(25–35) relative to Aβ(1–42), which because the Met is not C-terminal can not participate in this mechanism of oxidative stress. Rather, Aβ(1–42) must have other means of leading to the sulfuramyl free radical on Met (see following sections).

The mechanism posited in Fig. 5 for Aβ(25–35) was strengthened by studies with Aβ(25–35amide) [80]. If the carboxylate oxygen of Aβ(25–35) were replaced by an amide (NH_2), then the initial attack on the sulfur would not be feasible. One would predict that this peptide would be non-oxidative and non-neurotoxic. This is exactly what is found [80].

For Aβ(1–42) the mechanism by which Met initiates a sulfuramyl free radical remains unclear. However, several possibilities are possible. In the elegant studies by Schöneich described before, it is clear that if the Met residue of Aβ exists in an α-helical environment, which has been demonstrated based on NMR studies of Aβ(1–40) [16] and in other biophysical studies with Aβ(1–42) [17], the carbonyl oxygen of the $i + 4$ residue (Ile-31) potentially can interact with the S-atom of Met, possibly changing its redox chemistry such that molecular oxygen can oxidize Met to the sulfuramyl free radical. If this were the case, then subsequent free radical propagation steps can ensue leading to lipid peroxidation and protein oxidation. A different mechanism has been proposed by Huang et al. [29,30]. These scientists report that Aβ(1–42) can reduce peptide-bound Cu(II) to Cu(I) and form H_2O_2 [29,30]. Cu(I), in turn, can react with the H_2O_2 to form hydroxyl free radicals. Bush states that if any amino acid is substituted for methionine, no toxicity ensues [9]. Additionally, if exogenous methionine is added to Aβ(1–28)—a fragment lacking toxic properties of the wild type-there is an increase rate of Cu(II) reduction supporting a critical role of methionine is such processes [17]. These researchers also demonstrated insertion of Aβ(1–42) into

Fig. 5. Top: Plausible mechanism for sulfuramyl free radical cation localized on Met residue 35 of Aβ(1–42) to induce lipid peroxidation and protein oxidation in neurons, ultimately leading to the death of the neuron. The S^+ radical is postulated to be formed either by (a) reaction of molecular oxygen with the S-atom of Met, whose electronic structure has been altered by interaction of the S-atom of Met with the carbonyl oxygen of Ile residue 31 in an α-helical conformation or (b) following peptide-bound redox metal ion associated chemistry (see text). Extraction of a H-atom from an unsaturated C-atom on a phospholipid acyl chain or from a susceptible protein would lead to a C-centered free radical, M. This, in turn, would immediately bind paramagnetic oxygen, forming a peroxyl free radical, thereby propagating the radical process. The latter could induce lipid and protein oxidation, ROS formation, reactive lipid peroxidation products, and, eventually, cell death. H-atom abstraction by the sulfuramyl radical also is postulated to occur either between adjacent chains involving Gly-33 or in the same chain, forming a CH_2 radical on the former methyl group of Met-35. In either case, oxygen would immediately bind forming a peroxyl free radical that would lead to neurotoxicity as described before. The SH^+ moiety on Met would immediately lose its H^+ since the pK_a is −5, reforming Met. Thus, the cycle could be catalytic. Vitamin E, a chain-breaking antioxidant, would intercept peroxyl free radicals, thereby stopping the propagation steps. See text for more details. Because Met on Aβ(25–35) is C-terminal, we propose that the Met-associated free radical process is different than that of Aβ(1–42). The carboxylate oxygen of C-terminal Met could attack the S-atom, making a six-membered ring containing a sulfuramyl free radical. Such an attack would not be possible with the C-terminal amide or in Aβ(25–34) that lacks Met, which may explain why these modifications render Aβ(25–35) to be non-oxidative and non-neurotoxic. Subsequent reactions lead back to Met, providing a catalytic route for oxidative stress and neurotoxicity. See text for more details.


(a)
Oxidation
intra Aβ proton abstraction
inter Aβ proton transfer from G33
MH (lipid or protein)
B̈, -BH
1
2
O2
+
M·
(lipid or protein radical)
O2
peroxyl radical formation
free radical reactions with proteins, and lipids
oxidative damage and neurodegeneration


(b)
oxidation
H atom abstraction

model lipid bilayer with the methionine located deep in the bilayer is as α-helix conformation. Such orientation could position a putative sulfuramyl radical to abstract H-atom from an allylic carbon on acyl chain of lipids and could suggest the potential interaction of Ile-31 and Met-35 as noted before. Our attempts to dissect these two possibilities (one, a redox metal ion-independent, structurally-based oxidation of Met, and the second, a process involving peptide-bound redox metal ions) are on-going. Progress has been made in one approach. If to the norleucine-substituted Aβ(1–42), Aβ(1–42M35NLE), which, as noted before, is non-oxidative and non-neurotoxic, Cu(II) is added, there still remains no oxidative stress and no neurotoxicity [78], consistent with the thesis of this review that methionine is the key player in these properties of Aβ(1–42). Supporting this view, if the putative Cu(II) binding sites of His 6,13,14 in Aβ(1–42) are substituted by Tyr, which binds Cu(II) at least a 100-fold less well than does His [51], then this Met-containing peptide is still oxidative and still inhibits mitochondrial function [78].

Recently, a paper reporting the results of theoretical calculations suggested that a sulfuramyl free radical on Met of one molecule of Aβ(1–42) could abstract a H-atom from the α-carbon of Gly-33 of an adjacent peptide in a β-sheet conformation [64]. Only Gly-33 was able to participate in this H-atom transfer according to the authors. To test this idea, we substituted Gly-33 in Aβ(1–42) by Val [Aβ(1–42G33V)], which would not be able to participate in H-atom transfer to Met-35. In contrast to native Aβ(1–42), Aβ(1–42M35V) caused far less protein oxidation and induced no neurotoxicity [31]. If this mechanism is applicable in AD brain, several points are worth noting. First, H-atom abstraction from the α-carbon of Gly-33 would form a C-centered free radical that would immediately bind molecular oxygen forming a peroxyl free radical (see Fig. 5). The latter can induce protein oxidation and lipid peroxidation [11], which can lead to cell death. Secondly, this mechanism does not require participation by redox metal ions, providing another mechanism by which oxidative stress can arise. Schöneich has shown that a sulfuramyl radical also can abstract a H-atom from the methyl group next to the sulfur atom in Met on the same peptide, forming a CH_2 free radical [64]. As before, such a free radical would immediately bind molecular oxygen forming a peroxyl free radical, and protein oxidation and lipid peroxidation could follow as before. More research to dissect these possibilities is needed and ongoing.

This review has summarized results of several studies that show the critical importance of methionine in the oxidative stress and neurotoxic properties of Aβ(1–42). Given the centrality of Aβ(1–42) to the pathogenesis of AD and the extensive oxidative stress under which the AD brain exists, these results may unite these aspects of this dementing disorder under the aegis a model for neurotoxicity in the AD brain based on methionine-initiated, Aβ(1–42)-associated free radical oxidative stress and neurotoxicity. If so, these results strongly support that notion that intervention in AD by brain accessible antioxidants may provide a promising therapeutic strategy for this disorder.

Addendum

Consistent with the notion that the secondary structure of Aβ(1–42) is important in the oxidative stress and neurotoxic properties of the peptide, we substituted the helix-breaking amino acid, proline, for isoleucine in residue 31. This breaks interaction of the carbonyl oxygen of Ile-31 with the S-atom of Met-35. As predicted, this substituted peptide is no longer oxidative nor neusotoxic [88]. Additionally, if the chemistry of methionine is maintained in Aβ(1–42), but the initial target of the posited sulfuramyl radical is no longer available, no neurotoxicity nor oxidative damage is predicted. This prediction was confirmed by substitution of the negatively charged amino acid aspartic acid for glycine-37. The negative charge is predicted to force the lipid bilayer-resident methionine out of the low dielectric medium of the lipid bilayer. When this modified peptide was incubated with hippocampal neurons, no oxidative stress nor neurotoxicity resulted [89], consistent with the notion that lipid peroxidation induced by Aβ(1–42) is an early event in the oxidative stress and neurotoxicity associated with this peptide [90] and perhaps in AD brain [91].

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