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Authors

Bitan, Gal
Tarus, Bogdan
Vollers, Sabrina S
[et al.](#)

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A Molecular Switch in Amyloid Assembly: Met³⁵ and Amyloid β -Protein Oligomerization

Gal Bitan,[†] Bogdan Tarus,[‡] Sabrina S. Vollers,[†] Hilal A. Lashuel,[†]
Margaret M. Condrón,[†] John E. Straub,[‡] and David B. Teplow^{*†}

Contribution from the Center for Neurologic Diseases, Brigham and Women's Hospital, and
Department of Neurology, Harvard Medical School, Boston, Massachusetts 02115, and
Department of Chemistry, Boston University, Boston, Massachusetts 02215

Received February 28, 2003; E-mail: teplow@cnd.bwh.harvard.edu.

Abstract: Aberrant protein oligomerization is an important pathogenetic process in vivo. In Alzheimer's disease (AD), the amyloid β -protein ($A\beta$) forms neurotoxic oligomers. The predominant in vivo $A\beta$ alloforms, $A\beta_{40}$ and $A\beta_{42}$, have distinct oligomerization pathways. $A\beta_{42}$ monomers oligomerize into pentamer/hexamer units (paranuclei) which self-associate to form larger oligomers. $A\beta_{40}$ does not form these paranuclei, a fact which may explain the particularly strong linkage of $A\beta_{42}$ with AD. Here, we sought to determine the structural elements controlling paranucleus formation as a first step toward the development of strategies for treating AD. Because oxidation of Met³⁵ is associated with altered $A\beta$ assembly, we examined the role of Met³⁵ in controlling $A\beta$ oligomerization. Oxidation of Met³⁵ in $A\beta_{42}$ blocked paranucleus formation and produced oligomers indistinguishable in size and morphology from those produced by $A\beta_{40}$. Systematic structural alterations of the C _{γ} ³⁵-substituent group revealed that its electronic nature, rather than its size (van der Waals volume), was the factor controlling oligomerization pathway choice. Preventing assembly of toxic $A\beta_{42}$ paranuclei through selective oxidation of Met³⁵ thus represents a potential therapeutic approach for AD.

Introduction

Amyloid β -protein ($A\beta$) is the main proteinaceous component of the amyloid plaques found in the brains of Alzheimer's disease (AD) patients.^{1,2} Neuritic plaques, pathognomonic features of AD, contain abundant fibrils formed from $A\beta$. These fibrils have been found to be neurotoxic in vivo and in vitro.^{3–5} Recent studies of structure–activity relationships among fibril assembly intermediates have revealed that many intermediates are neurotoxic, including dimers and trimers,⁶ $A\beta$ -derived diffusible ligands (ADDLs),⁷ and protofibrils.^{8,9} These oligomers may, in fact, be the proximal effectors of neuropathogenesis in

AD.^{10–12} This new insight into the assembly and biological activity of fibril precursors suggests that therapeutic strategies may need to target oligomeric assemblies. To facilitate the development of these strategies, an understanding of the mechanisms controlling the formation of oligomeric $A\beta$ assemblies is necessary.

The predominant forms of $A\beta$ found in brains of AD patients are 40 and 42 amino acids long (designated $A\beta_{40}$ and $A\beta_{42}$, respectively).^{13,14} Despite the small primary structure difference between $A\beta_{40}$ and $A\beta_{42}$, i.e., the dipeptide Ile⁴¹-Ala⁴², the clinical impact and biophysical behavior of the two $A\beta$ alloforms are distinct. $A\beta_{40}$ and $A\beta_{42}$ exist in the plasma and cerebrospinal fluid at a concentration ratio of $\sim 10:1$, respectively, yet $A\beta_{42}$ is deposited first during the development of AD^{15,16} and is more neurotoxic than $A\beta_{40}$.^{17,18} Alloform-specific patterns of amyloid deposition are observed. $A\beta_{42}$ is the predominant

[†] Center for Neurologic Diseases, Brigham and Women's Hospital, and Department of Neurology, Harvard Medical School.

[‡] Department of Chemistry, Boston University.

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component in parenchymal plaques, whereas A β 40 is the predominant component in vascular deposits.^{19,20} An increase in the A β 42/A β 40 ratio is associated with early onset familial AD.^{21,22} Oligomeric A β 42 has been shown to be toxic to neuronal cells at nanomolar levels in vitro,^{7,23–29} whereas A β 40 oligomers are significantly less toxic.^{18,30} In addition, A β 42 has been shown to form fibrils substantially faster than A β 40.^{31,32}

Recently, we demonstrated that the early assembly of A β 42 involves formation of pentamer/hexamer units termed paranuclei.³³ Following their formation, paranuclei self-associate into larger oligomers, which appear to give rise to protofibrils.³³ Paranucleus formation is the earliest observable A β 42 assembly event. Early A β 40 assembly produces an equilibrium mixture of monomer, dimer, trimer, and tetramer.³⁴ Assemblies in this mixture are largely amorphous, in contrast to the quasiglobular paranuclei observed in A β 42 preparations.³³ These biophysical differences between A β 40 and A β 42 are likely to underlie the distinct biological behaviors of the two peptides. Therapeutic agents capable of directing A β 42 down oligomerization pathways akin to that of A β 40, i.e., delaying or inhibiting paranucleus formation and self-association, thus could be of clinical value.

It has been postulated that Met³⁵ contributes to the neurotoxic activity of A β . In particular, oxidation of Met³⁵ to the corresponding sulfoxide (Met(O)³⁵) has been suggested to be involved in A β -induced oxidative damage.^{35,36} However, a confusing observation is that the toxicity of A β increases with assembly of the monomeric peptide into oligomers and fibrils, but its oxidative power is highest for monomers and decreases with assembly.³⁷ In addition, solution NMR studies of reduced and oxidized A β 40 and A β 42 have shown little conformational difference among the four peptides,³⁸ suggesting that the effect of Met³⁵ on the biological activity of A β is exerted in the

assembled state, rather than in the monomeric state. [Met(O)³⁵]A β has been reported to display both accelerated^{39,40} and delayed⁴¹ fibrillogenesis rates relative to wild type (WT) A β . The biological implications of these data are unclear. Recent reports have shown that formation of small oligomers by A β 40⁴² and of protofibrils by A β 40⁴³ and A β 42⁴⁴ is delayed upon Met³⁵ oxidation. If oligomeric assemblies, rather than fibrils, are the key effectors of neurotoxicity in AD, then differences in assembly kinetics may not underlie the differences in biological activity between the reduced (native) and oxidized A β forms. Rather, the distinct neurotoxic activity of different A β alloforms may reflect qualitative differences such as formation or stabilization of particular oligomeric assemblies. Here, we examined the effect of Met³⁵ oxidation on the oligomer size distribution of A β 40 and A β 42 using the technique of Photoinduced Cross-linking of Unmodified Proteins (PICUP).^{45,46} PICUP provides the means to identify and quantitate small, metastable oligomers. These analyses are difficult using other biochemical or biophysical methods.³⁴ The current study reveals that oxidation of Met³⁵ transforms the oligomer size distribution of A β 42 into one that is indistinguishable from that of A β 40, suggesting a key role for Met³⁵ in the formation of paranuclei and in the control of oligomerization pathway choice.

Results

Characterization of the Oligomer Size Distributions of Reduced and Oxidized A β 40 and A β 42. To determine the effects of oxidation of Met³⁵ to Met-sulfoxide [Met(O)³⁵] on the oligomer size distributions of A β 40 and A β 42, low molecular weight (LMW⁴⁷) fractions of the reduced and oxidized peptides were isolated using size exclusion chromatography (SEC) and immediately cross-linked. SDS-PAGE analysis of cross-linked wild-type (WT) and oxidized A β 40 revealed little difference between the oligomer size distributions (Figure 1A, lanes 1 and 3). Both distributions showed comparable abundances of monomer through tetramer, followed by a sharp decrease of the abundances of pentamer through heptamer, as described previously.³⁴ The distribution of WT A β 42 comprised three groups of oligomers (Figure 1A, lane 2): monomer through trimer, displaying decreasing intensity with increasing oligomer order; a Gaussian-like distribution between tetramer and octamer, with a maximum at pentamer and hexamer; and oligomers of $M_r \approx 30$ –60 kDa showing intensity maxima consistent with dodecamer and octadecamer. In contrast, the oligomer size distribution of [Met(O)³⁵]A β 42 differed dramatically from that of WT A β 42. It was, in fact, indistinguishable

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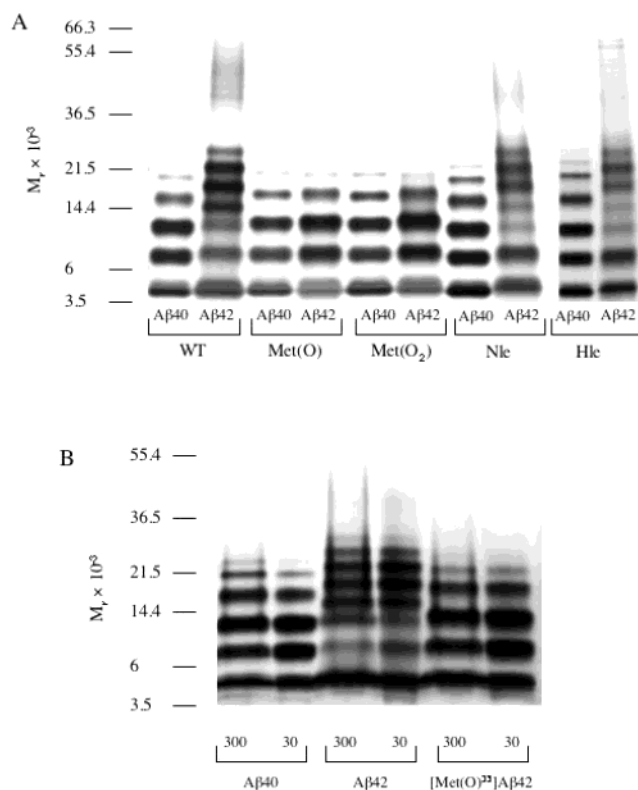


Figure 1. Effects of residue 35 side-chain structure and peptide concentration on A β oligomerization. A) PICUP was performed on SEC-isolated LMW A β 40 and A β 42 analogues containing the modifications Met³⁵ \rightarrow Met(O), Met³⁵ \rightarrow Met(O₂), Met³⁵ \rightarrow Nle, and Met³⁵ \rightarrow Hle. WT A β 40 and A β 42 were used as controls. Following cross-linking, the products were analyzed by SDS-PAGE and silver staining. Positions of molecular weight markers are shown on the left. The gel is representative of each of 3 independent experiments. B) PICUP was applied to freshly dissolved A β 40, A β 42, and [Met(O)³⁵]A β 42 at nominal concentrations of 30 and 300 μ M. Each peptide first was dissolved in water at a nominal concentration of 4 mg/mL. The pH then was adjusted to 10 by addition of 1N NaOH, after which 20 mM sodium phosphate, pH 7.4, was added to yield a final nominal peptide concentration of 300 μ M. The solutions were sonicated for 1 min. Aliquots from these solutions were diluted 10-fold in 10 mM sodium phosphate, pH 7.4, to produce the 30 μ M solutions. Following cross-linking, the products were analyzed by SDS-PAGE and silver staining. The volumes loaded were adjusted so that equal amounts of protein were loaded in each lane. Positions of molecular weight markers are shown on the left. The gel is representative of each of 3 independent experiments.

from the oligomer distributions of reduced and oxidized A β 40 (Figure 1A, lane 4). This result suggested a crucial role for Met³⁵ in the oligomerization of A β 42, particularly in the formation of A β 42 paranuclei. Moreover, the data demonstrated that the particular interactions leading to the oligomer size distribution observed for WT A β 42, which of necessity involve Ile⁴¹ and Ala^{42,33,48} were abolished by oxidation of Met³⁵ to the corresponding sulfoxide.

In principle, the differences in the oligomerization patterns among A β 42 and oxidized A β 42 or A β 40 may reflect solubility differences rather than distinct oligomerization behavior. If so, [Met(O)³⁵]A β 42 and A β 40, at higher concentrations, might form paranuclei. To address this question, we compared the oligomer size distributions of A β 40, A β 42, and [Met(O)³⁵]A β 42 at nominal concentrations of 30 and 300 μ M (Figure 1B). For all three alloforms, the oligomer size distributions obtained at 30

Table 1. Calculated Dipole Moments and van der Waals Volumes for C γ -Substituent

| Residue | X | Dipole Moment (Debye) ^a | van der Waals volume (\AA^3) ^a |
|----------------------|------------------------------------|------------------------------------|--|
| Met | H ₃ C—S— | 1.60 | 86.86 |
| Met(O) | | 5.28 | 96.35 |
| Met(O ₂) | | 5.20 | 102.70 |
| Nle | H ₃ C—CH ₂ — | 0.53 | 87.27 |
| Hle | | 0.58 | 106.65 |

^a Values calculated for the chemical group X attached to the γ -methylene of residue 35.

and 300 μ M were essentially identical, demonstrating that the effect of oxidation on the oligomerization pattern was concentration-independent over this range of concentration, at a minimum.

Elucidation of Structural Elements at C γ ³⁵ which Control A β 42 Paranucleus Formation. To determine the structural elements of residue 35 which affect paranucleus formation, three additional modifications were examined, oxidation of Met³⁵ to Met-sulfone [Met(O₂)³⁵] and substitutions by norleucine (Nle) and homoleucine (Hle). To compare the relative influence of electrostatic and steric forces, the dipole moments and van der Waals volumes of side chain portions beyond the γ -methylene were computed for each of the residues used (Table 1). Oxidation of Met to Met(O₂) is similar to oxidation to Met(O) in that it increases both the size and the polarity of the side chain (Table 1). Substitution of Met by Nle maintains the size and decreases the polarity of the side chain, whereas substitution by Hle increases the size and decreases the polarity (Table 1).

SEC-isolated LMW A β 42 containing Met(O₂), Nle, or Hle in position 35 were cross-linked and analyzed by SDS-PAGE. The A β 40 counterparts of these peptides were used as controls. The effect of oxidation of Met to Met(O₂) was identical to that of oxidation to Met(O) (cf., Figure 1A, lane 4 with lane 6). In fact, [Met(O₂)³⁵]A β 42 yielded a distribution indistinguishable from those of [Met(O)³⁵]A β 42 and all of the A β 40 analogues examined (Figure 1A). By contrast, the distributions of [Nle³⁵]A β 42 and [Hle³⁵]A β 42 were qualitatively similar to that of WT A β 42 (cf., Figure 1A, lanes 2, 8, and 10). They contained the same three groups of oligomers and the predominant cross-linking products were pentamer/hexamer. The relative abundance of the third oligomer group (\sim 30–60 kDa) was decreased in the distributions of the Nle and Hle analogues, relative to WT A β 42. The sulfur atom in residue 35 thus was not required for the formation of paranuclei, but the Met side chain facilitated the self-association of paranuclei more than the all-hydrocarbon side chains. The data suggest that the unique oligomer size

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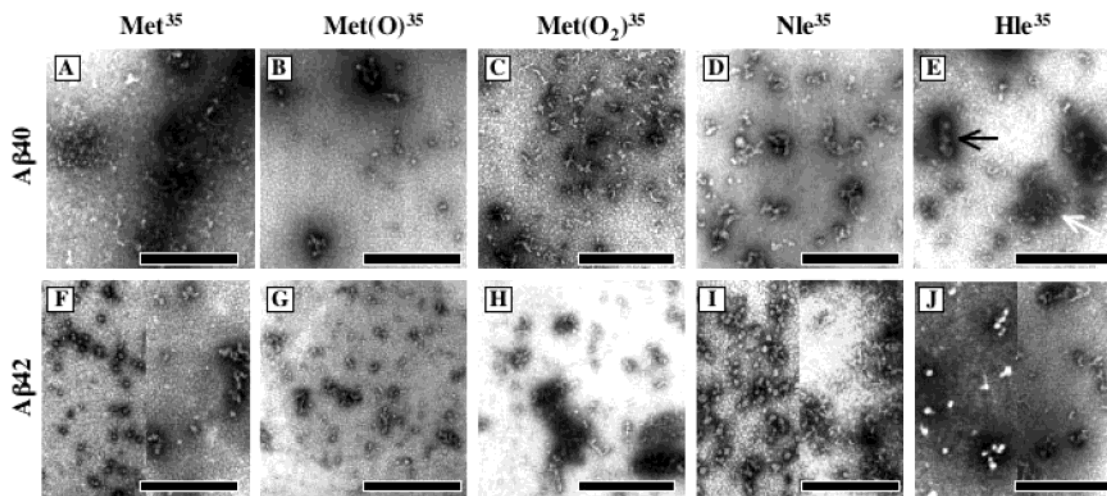


Figure 2. Morphologic analysis of LMW $A\beta$. $A\beta_{40}$ (Panels A–E) and $A\beta_{42}$ (panels F–J) containing Met^{35} (A,F), Met(O)^{35} (B,G), $\text{Met(O}_2\text{)}^{35}$ (C,H), Nle^{35} (D,I), or Hle^{35} (E,J) were spotted on glow-discharged, carbon-coated grids immediately after isolation by SEC. The samples then were fixed with glutaraldehyde, stained with uranyl acetate, and examined by EM. Scale bars are 100 nm. The micrographs shown are representative of multiple fields recorded for each of several grids prepared in at least three independent experiments.

distribution of $A\beta_{42}$ requires the hydrophobic, rather than the steric characteristics of Met^{35} . Thus, although the van der Waals volume of the Hle side chain is larger than those of Met(O) or $\text{Met(O}_2\text{)}$ (Table 1), the oligomer distribution of $[\text{Hle}^{35}]A\beta_{42}$ was qualitatively similar to that of the WT peptide. The change in polarity, rather than the change in size, of the Met side chain thus appears responsible for the major alteration of the $A\beta_{42}$ oligomer size distribution following oxidation. The oxidation of Met^{35} may destabilize paranuclei due to the increased energetic cost of desolvating the oxidized side chain (see Discussion).

Morphology of $A\beta$ Oligomers. WT LMW $A\beta_{40}$ and $A\beta_{42}$ display distinct morphologies immediately following their preparation.³³ LMW $A\beta_{40}$ is relatively amorphous whereas LMW $A\beta_{42}$ produces quasi-globular structures $\sim 2\text{--}10$ nm in diameter. We determined here the effect of structural modifications at position 35 on the morphology of LMW $A\beta$ (Figure 2). To do so, LMW fractions of each of the peptides used in the PICUP experiments were isolated and examined by EM. The morphology of oxidized $A\beta_{42}$ resembled that of $A\beta_{40}$.³³ The globular structures observed for WT $A\beta_{42}$ were not detected for $[\text{Met(O)}^{35}]A\beta_{42}$ or $[\text{Met(O}_2\text{)}^{35}]A\beta_{42}$. The change in the morphology of oxidized $A\beta_{42}$ relative to WT $A\beta_{42}$ correlates with the distinct oligomer size distributions observed for the two peptides.

All $A\beta_{40}$ analogues produced irregular, prolate aggregates. Two main classes were observed, one with mean diameter $\sim 1\text{--}2$ nm (Figure 2E white arrow) and the other with mean diameter $\sim 5\text{--}10$ nm (Figure 2E black arrow). WT and oxidized $A\beta_{40}$ produced mainly the thinner structure whereas $[\text{Nle}^{35}]A\beta_{40}$ and $[\text{Hle}^{35}]A\beta_{40}$ produced more of the thicker structures. Similar structures also were observed for the $A\beta_{42}$ analogues. However, WT $A\beta_{42}$, $[\text{Nle}^{35}]A\beta_{42}$, and $[\text{Hle}^{35}]A\beta_{42}$ also showed quasi-spherical structures of diameter $\sim 5\text{--}8$ nm, either alone or associated into small groups. In agreement with the PICUP data, these quasi-spherical structures were not observed for $[\text{Met(O)}^{35}]A\beta_{42}$ and $[\text{Met(O}_2\text{)}^{35}]A\beta_{42}$, or for any of the $A\beta_{40}$ analogues, supporting the conclusion that these morphologies correspond to paranuclei.³³

Conformation of LMW $A\beta_{40}$ and $A\beta_{42}$ Analogues. We have shown previously that, immediately following isolation, LMW $A\beta_{40}$ and $A\beta_{42}$ both are predominantly unstructured.³³ To assess the conformational effects of side-chain modifications in position 35, the conformations of all the analogues used in this study were examined by CD immediately following their isolation by SEC (The CD spectra are available as Supporting Information). The predominant secondary structure element for all alloforms was “random coil.” Small amounts of β -sheet, β -turn, and α -helix also were observed. The relative amounts of each structural element were similar for each of the analogues. These data show, as observed for WT $A\beta$, that the initial oligomerization of the analogues containing modifications in position 35 does not involve substantial conformational rearrangement.

Discussion

An important and contentious area in studies of the chemical biology of Alzheimer’s disease is the role of redox chemistry and the involvement of $A\beta$ in it. It has been suggested that the redox state of Met^{35} is critical in determining the biological activity of $A\beta$, yet the mechanism by which this effect is exerted is not well understood. One hypothesis proposes that oxidation of Met^{35} , possibly through interaction with transition metal ions, is directly related to generation of cytotoxic reactive oxygen species.^{35,36} Alternatively, oxidative stress may be cellular in origin and result from the interaction of $A\beta$ with neurons, microglia, and/or astrocytes.^{49,50} If so, the assembly state of $A\beta$ could be vital in controlling these cellular interactions. Consistent with this notion, Met^{35} oxidation has been reported to affect $A\beta$ assembly. However, a consensus on precisely how Met^{35} does so does not exist. For example, it has been suggested that $[\text{Met(O)}^{35}]A\beta$ forms fibrils faster,^{39,40} at a similar rate,^{37,51} or slower^{38,41} than does WT $A\beta$. Recent studies have reported

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that oxidation of Met³⁵ retarded the formation of A β 40 trimer and tetramer,⁴² and of A β 40⁴³ and A β 42⁴⁴ protofibrils. In addition, there is no consensus with regard to the question of whether oxidation of Met³⁵ attenuates A β toxicity.^{37,52} We show here that oxidation of Met³⁵ dramatically changes the way in which A β 42 oligomerizes. Paranuclei, which recently have been shown to be characteristic of A β 42 oligomerization,³³ do not form when Met³⁵ is oxidized. In fact, [Met(O)³⁵]A β 42 yields an oligomer size distribution characteristic of A β 40. Replacement of the sulfur atom by a methylene group, as in [Nle³⁵]A β 42, yields a characteristic A β 42 oligomer size distribution. Substitution of Met³⁵ by Nle was reported to reduce A β fibril-induced neuronal death.^{53,54} Studies are now required to determine whether a similar effect is produced by oligomeric [Nle³⁵]A β 42.

Our studies of A β oligomerization using [Nle³⁵]A β 42 demonstrate that the sulfur atom is not essential for production of A β 42 paranuclei. This conclusion is supported by the observation that [Hle³⁵]A β 42 also produces a typical A β 42-like oligomer distribution. Hle is as nonpolar as Nle but is significantly larger than either Nle or Met (Table 1). Therefore, the polarity, rather than the size, of the residue 35 side-chain appears to be the key factor controlling oligomerization pathway choice. One mechanistic explanation for this phenomenon is that the increased energetic cost of desolvating the oxidized (polar) side chain of Met(O) or Met(O₂) disfavors paranucleus formation. During paranucleus formation, Met likely participates in hydrophobic interactions leading to its exclusion from the solvent-accessible volume—an energetically favorable process for the relatively nonpolar Met side-chain. This hypothesis is supported by comparative examination of the energetics of solvation of dimethyl sulfide (DMS) and dimethyl sulfoxide (DMSO), compounds corresponding to the side chain groups beyond the β -methylene in Met and Met(O), respectively (Table 1). Experimental and computational data demonstrate that the hydration of DMSO is a more favorable process than is the hydration of DMS. The partial molar enthalpy of DMSO at infinite dilution in water is -4.33 kcal/mol.⁵⁵ The solvation free energy for DMS is 0.15 kcal/mol⁵⁶ and the solvation enthalpy is -2.37 kcal/mol.⁵⁶ The increased solubility of DMSO in water can be explained by its tendency to act as a hydrogen bond acceptor.⁵⁵ In addition, DMSO has a large dipole moment (3.96 D)⁵⁷ compared to the dipole moment of DMS (1.5 D).⁵⁷ The dipole moment of dimethyl sulfone (DMSO₂) is even larger (4.25 D) than that of DMSO. Oxidation of Met to the sulfoxide or sulfone forms thus produces a substantial dipole moment and makes the oxidized Met³⁵ a strong hydrogen bond acceptor. This makes the solvation free energy of the residue substantially larger than that of the relatively nonpolar Met residue, a change which would disfavor movement of the side-chain into the apolar environment of the assembled A β oligomer. In support of this hypothesis, when A β 42 and [Met(O)³⁵]A β 42 are mixed in different ratios and cross-linked, the observed oligomer size

Table 2. Calculated Side-chain Aqueous Solvation Energy^a

| Residue | G_{cav} | $G_{\text{elec}}(\epsilon = 4)$ | $G_{\text{elec}}(\epsilon = 80)$ | ΔG_{elec} |
|----------------------|------------------|---------------------------------|----------------------------------|--------------------------|
| Met | 2.85 | -0.37 | -0.54 | 0.17 |
| Met(O) | 3.05 | -3.98 | -5.85 | 1.87 |
| Met(O ₂) | 3.18 | -3.86 | -5.68 | 1.82 |
| Nle | 2.85 | -0.04 | -0.06 | 0.02 |
| Hle | 3.30 | -0.05 | -0.07 | 0.02 |

^a The cavitation free energy G_{cav} , the peptide solvation free energy $G_{\text{elec}}(\epsilon = 4)$, the aqueous solvation free energy $G_{\text{elec}}(\epsilon = 80)$, and the “desolvation” energy ΔG_{elec} were calculated as described in the Discussion. Each is expressed in units of kcal/mol.

distributions appear to be simple composites of the distribution of each alloform alone (data not shown). This “titration” of the oligomer size distribution is consistent with a homotypic peptide association process in which reduced and oxidized A β molecules do not interact appreciably with each other. Also consistent with this idea is the recent observation that upon oxidation of Met³⁵, A β no longer interacts with phospholipid vesicles.⁵²

The relationships between the dipoles and van der Waals volumes corresponding to Met and Met(O₂) demonstrate this effect quantitatively. We assume that the difference in the dipole moment (~ 3.6 D) between these two side chains is produced by a point dipole placed at the center of the sulfur atom. The distance between the center of the sulfur atom and the solvent exposed surface of the oxygen bound to sulfur is taken to be 3.23 Å.⁵⁸ This distance is the minimal radius of the spherical cavity in which the point dipole is placed. The electrostatic contribution to the free energy of the dipole in water can be estimated by the classical theory of Onsager⁵⁹

$$G_{\text{elec}} = -\frac{1}{2}\phi_{\text{RF}}\mu = -\frac{\mu^2(\epsilon - 1)}{a^3(2\epsilon + 1)}$$

where ϕ_{RF} is the reaction field with which the solvent responds to the dipole, μ is the dipole moment, ϵ is the relative dielectric constant of the solvent (80 for water), and a is the radius of the sphere in which the dipole is placed. For the most polar residues, Met(O) and Met(O₂), the aqueous solvation energy (G_{elec}) is large, thus the sulfoxide and sulfone functions interact favorably with solvent water (Table 2). The influence of the difference in the van der Waals volume can be estimated by evaluating the work necessary to create a cavity with radius a using a solvent accessible surface area (SASA) proportionality relation^{60,61}

$$G_{\text{cav}} = \gamma A_{\text{SAS}}$$

where γ has the dimensions of a surface tension and A_{SAS} is the SASA. The results listed in Table 2 were obtained by choosing 30 cal/(mol Å²) for γ (for alkanes, the typical value is 20 – 30 cal/(mol Å²)⁶¹). Increasing the volume of Met by oxidation to Met(O) or Met(O₂) leads to a relatively small, unfavorable energetic cost.

In considering the energetic cost of removing an amino acid side-chain from an aqueous solvent ($\epsilon = 80$) and placing it in an internal, hydrophobic cavity ($\epsilon = 4$) in an oligomeric form of a peptide, the “desolvation” energy is the most important

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factor. This energetic cost is estimated to be

$$\Delta G_{\text{elec}} = G_{\text{elec}}(\epsilon = 4) - G_{\text{elec}}(\epsilon = 80)$$

A Met(O) or Met(O₂) side-chain partitions preferentially into the aqueous environment, thus its burial in a less polar oligomer core, as would occur during paranucleus formation, is unfavorable. In contradistinction to solvation effects, the relatively large excluded volume of the Hle does not significantly disrupt the formation of small or large oligomers, indicating that steric effects in the packing about residue 35 are of little significance. Our observations are consistent with those of Craik and co-workers,⁶² who studied Aβ structural changes due to oxidation of Met³⁵ in monomeric Aβ40 solvated in a water–micelle environment. In the unoxidized peptide (PDB code 1BA4), the side chain of Met³⁵ lies on the backbone and does not disrupt the local helical structure.⁶² In the oxidized peptide (PDB code 1BA6), the side chain of Met(O)³⁵ is repositioned to make better contact with the aqueous solvent. The exposure of the methionine sulfoxide residue to solvent disrupts the helical structure observed in the WT peptide.⁴¹ Disruption of Aβ helical structure by an Ile³¹ → Pro substitution (a region proximal to Met³⁵) has been proposed by Butterfield et al. as a mechanism for an observed suppression of oxidative stress and neurotoxicity.⁶³ Our CD data do not reveal a significant reduction in α-helix content following Met oxidation, suggesting that solvation effects, as opposed to secondary structure changes, are the key determinants of oligomerization state in our experiments.

Previously,³³ we demonstrated that paranucleus formation required an Aβ peptide extending to Ile⁴¹. The association of paranuclei into higher order assemblies required the addition of Ala⁴². This second assembly step was facilitated by Thr⁴³ or by replacement of the C-terminal carboxyl by a carboxamide.⁴⁸ The data presented here demonstrate that Met³⁵ is involved directly in formation of Aβ42 paranuclei. It is possible that paranucleus formation involves direct interaction between Met³⁵ and the Aβ C-terminus, particularly Ile⁴¹. Experimental evidence exists that the C-terminus of monomeric Aβ42 in aqueous solution does not form a stable structure.^{38,64} Thus, an interaction between Met³⁵ and Ile⁴¹ likely would be intermolecular in nature and occur during Aβ42 oligomerization. Alternatively, both Met³⁵ and Ile⁴¹ may be critical in controlling the oligomerization of Aβ42 but act independently. Further studies are necessary to evaluate these possibilities.

The observation that the oligomer size distribution and morphology of oxidized Aβ42 are similar to those of Aβ40 suggests that Met³⁵ oxidation would alter the biological behavior of the resulting Aβ42 oligomers. Oligomeric Aβ40 is less neurotoxic than oligomeric Aβ42.^{18,30} If these distinct neurotoxic activities correlate with the quaternary structure of Aβ40 and Aβ42, and oxidation of Met³⁵ reduces the neuronal injury mediated by Aβ42 oligomers, then oxidizing agents specifically targeting Met³⁵ within Aβ42 could be valuable therapeutic tools for AD.

Materials and Methods

Peptides and Reagents. Aβ(1–40), [Met(O)³⁵]Aβ(1–40), [Met(O₂)³⁵]Aβ(1–40), [Nle³⁵]Aβ(1–40), [Hle³⁵]Aβ(1–40), Aβ(1–42),

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[Met(O)³⁵]Aβ(1–42), [Met(O₂)³⁵]Aβ(1–42), [Nle³⁵]Aβ(1–42), and [Hle³⁵]Aβ(1–42) were synthesized by 9-fluorenylmethoxycarbonyl (Fmoc) chemistry, purified by reverse phase HPLC, and characterized by mass spectrometry and amino acid analysis (AAA), essentially as described.⁶⁵ Mass spectrometry was performed using a ThermoFinnigan LCQDeca electrospray ionization/ion trap mass spectrometer linked to a Surveyor HPLC system. Tris(2,2'-bipyridyl)dichlororuthenium(II) (Ru(Bpy)) and ammonium persulfate (APS) were purchased from Sigma (Milwaukee, WI). Polyacrylamide gels, buffers, stains, standards, and equipment for SDS-PAGE were from Invitrogen (Carlsbad, CA).

Isolation of Low Molecular Weight (LMW) Aβ Peptides. LMW Aβ isoforms were isolated by size exclusion chromatography (SEC), as described.³⁴ Briefly, 170 μL of a 2 mg/mL peptide solution prepared in DMSO was fractionated using a 10/30 Superdex 75 HR column eluted at 0.5 mL/min with 10 mM sodium phosphate, pH 7.4, and the central portion of the included peak was collected during ~30 s. A 10-μL aliquot of each isolate was taken for AAA to determine the peptide concentration.

Cross-Linking and SDS-PAGE Analysis. Freshly isolated LMW peptides were immediately subjected to PICUP, as described.³⁴ Briefly, 1 μL of 1 mM Ru(Bpy) and 1 μL of 20 mM APS in 10 mM sodium phosphate, pH 7.4, were added to 18 μL of freshly isolated LMW peptide. The mixture was irradiated with visible light, and the reaction was quenched immediately with 10 μL tricine sample buffer (Invitrogen) containing 5% β-mercaptoethanol (β-ME). The cross-linked oligomer mixtures were analyzed by SDS-PAGE and the intensities quantified by densitometry, as described.³⁴ The amounts taken for SDS-PAGE analyses were adjusted according to the peptide concentration so that equal amounts of protein were loaded in each lane.

Electron Microscopy. SEC-isolated Aβ peptides were used for these experiments, which were done essentially as described.⁴⁷ Briefly, 8 μL of sample were applied to glow-discharged, carbon-coated Formvar grids (Electron Microscopy Sciences, Washington, PA) and incubated for 19 min. The solution was gently absorbed using Whatman grade 1 qualitative filter paper. The grid was incubated with 5 μL of 2.5% glutaraldehyde for 4 min and wicked dry with filter paper. The peptide then was stained with 5 μL of 1% uranyl acetate (Pfaltz & Bauer, Inc., Waterbury, CT) for 3 min. This solution was wicked off, and the grid was air-dried. Samples were examined using a JEOL CX100 electron microscope.

Circular Dichroism Spectroscopy. Immediately following isolation of LMW Aβ, aliquots were placed into a 0.1-cm path length quartz cell (Hellma, Forest Hills, NY). The spectra were recorded on an Aviv Model 62A DS spectropolarimeter (Aviv Associates, Lakewood, NJ) over the wavelength range of 195–260 nm, at 22 °C, as described.⁶⁶

Computational Methods. The electric dipole moments and the excluded volumes for Met, Nle, and Hle were calculated using the version 22 parameter set of the CHARMM program.⁶⁷ For Met(O), the parameters proposed by Strander and Feller⁵⁸ for dimethyl sulfoxide were used. For the sulfur and the sulfur oxygens in Met(O₂), the HF/6-31g* calculated partial charges for dimethyl sulfone⁶⁸ were used.

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Supporting Information Available: CD spectra for all the $A\beta$ alloforms used in this work. This material is available free of charge via the Internet at <http://pubs.acs.org>

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