The Toxic Conformation of the 42-residue Amyloid β Peptide and Its Relevance to Oxidative Stress in Alzheimer's Disease

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Abstract: Senile plaques in the brain of patients with Alzheimer's disease mainly consist of aggregates of amyloid β peptides (Aβ42, Aβ40). Aβ42 is more neurotoxic than Aβ40. This review describes recent findings from a structural analysis of Aβ42 aggregates and discusses their relevance to neurotoxicity through the formation of radicals.

Key Words: Alzheimer's disease, amyloid β, cerebral amyloid angiopathy, ESR, neurotoxicity, proline, oxidative stress, solid-state NMR.

1. INTRODUCTION

Alzheimer's disease (AD) is characterized by extracellular neuritic amyloid aggregates/fibrils (senile plaques) and intracellular neurofibrillary tangles [1]. The senile plaques are composed mainly of the 40-mer and 42-mer amyloid β peptides (Aβ40 and Aβ42) [2,3]. Numerous studies on the Aβ peptides have been done in the last two decades because the senile plaques are especially characteristic of AD. Since Aβ42 shows more potent neurotoxicity with more intense aggregative ability than Aβ40, it would play a critical role in the pathogenesis of AD [4,5]. There is substantial evidence to support the amyloid cascade hypothesis [6]: Aβ42 aggregates to form amyloid that induces cell death. Recent investigations [7-9] indicated that oxidative stress contributes to the neurodegeneration associated with AD (Fig. **1**). Radicalization of Aβ42 at Tyr-10 and Met-35 accompanied by the formation of H_2O_2 could play a critical role in the neurotoxicity. Although recent research suggested that oligomeric species of Aβ42, for example, oligomers [10-12], protofibrils [13], Aβ-derived diffusible ligands [14], and amylospheroid [15], might be toxic species that induce AD, the molecular mechanism of Aβ42 neurotoxicity has not been fully elucidated. Difficulty in the synthesis and purification of Aβ42 is one of the factors hampering research into the chemistry and biology of Aβ42 [16-18].

An efficient methodology to synthesize long peptides of over 50 amino acid residues without fragment condensation has been developed by us [19,20]. This method uses HATU [21] as an activator for Fmoc chemistry on a polyethylene glycol-polystyrene support in a continuous flow-type peptide synthesizer (Pioneer™ of Applied Biosystems). We adopted this method to the synthesis of Aβ42 in combination with purification by reversed-phase HPLC under alkaline conditions to obtain a highly pure Aβ42 [22,23]. This enabled us to investigate the aggregative ability and neurotoxicity of a large number of Aβ42 derivatives and to analyze the tertiary structure of their aggregates. This review describes our recent findings from the structural analysis of Aβ42 aggregates and proposes the toxic conformation of Aβ42, whose relevance to the neurotoxicity of Aβ42 is also discussed in context with the oxidative stress through the formation of radicals.

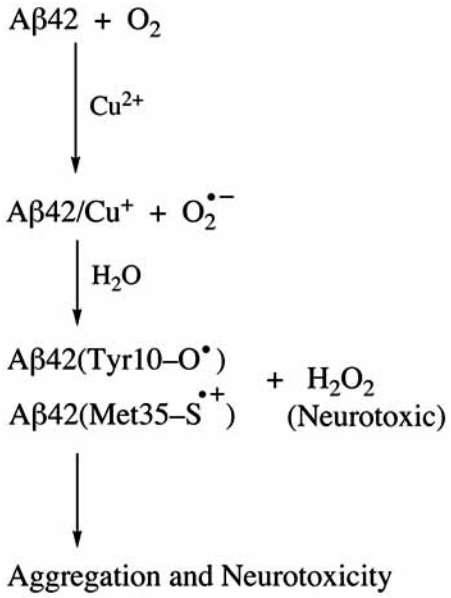

Fig. (1). Aβ42 aggregates to induce neurotoxic effects through a multistep cascade involving oxidative stress [6-9]. The phenoxy radical of Tyr-10 and the *S*-oxidized radical cation of Met-35 both play critical roles in the aggregation and neurotoxicity of Aβ42. Details are discussed in section **3**.

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2. IDENTIFICATION OF THE TOXIC CONFORMATION OF Aβ42 BY SYSTEMATIC PROLINE REPLACEMENT

Since Aβ peptides aggregate to induce neurotoxicity, elucidation of the tertiary structure of the Aβ aggregates is indispensable to understand their mechanism of action. Previous pioneering works [24] suggested that aggregates of Aβ42 and Aβ40 mainly consist of intermolecular β-sheets. However, the technical barriers to using X-ray crystallography or solution NMR hampered any precise determination of three-dimensional structure. Systematic replacement with proline in peptides is an efficient way to predict secondary structure, particularly β-sheets and turns. Prolines do not easily form a β-sheet because of a lack of NH functionality and the bend structure, while they are present in various turns as a Pro-*X* corner where *X* is a variable amino acid residue [25]. This methodology was first applied to the fragment peptides of Aβ (Aβ15-23, Aβ12-26) and I31P-Aβ42 [26,27]. However, our preliminary results obtained using whole Aβ42 were not consistent with the results obtained using the fragment peptides; E22P-Aβ42 aggregated extensively with potent neurotoxicity compared with wild-type Aβ42 [28], while E22P-Aβ12-26 did not aggregate at all [26]. This prompted us to systematically replace each proline of Aβ42, and measure the aggregative ability and neurotoxicity in PC12 cells of these proline mutants [29]. Although only the aggregation kinetics is shown in Fig. (**2**), the neurotoxicity correlated very well with the aggregative ability.

Among the mutants with replacement in the central region (positions 15-32), only E22P-Aβ42 aggregated more rapidly with stronger neurotoxicity in PC12 cells than wild-type Aβ42, suggesting the presence of a turn at positions 22 and 23 in the Aβ42 aggregate. The position of this turn may be related to familial AD with cerebral amyloid angiopathy, the Dutch (E22Q), Italian (E22K), and Arctic (E22G) mutations [30-32] since the sequences at positions 22 and 23 (QD, KD, and GD) are more frequently found in the two-residue β-turn compared with the sequence in the wild-type (ED) [25]. The aggregative ability and neurotoxicity of these mutants were significantly greater than those of wild-type Aβ42 [33], indicating that the turn at positions 22 and 23 is critical for the aggregation and neurotoxicity of Aβ42. A similar conclusion regarding Aβ40 was made by Williams and colleagues using systematic proline replacement [34].

All of the proline mutants altered at positions 3-13 aggregated extensively with significant neurotoxicity [29], suggesting that the N-terminal 13 residues could not adopt a β-sheet structure. This was in good accordance with previous reports on the structural analyses of Aβ aggregates [35-37]. In contrast, C-terminal proline mutants altered at positions 35-37 and 40-42 aggregated little and had extremely low neurotoxicity, while those altered at positions 34 and 38 aggregated extensively and exhibited substantial neurotoxicity [29]. These results suggest the presence of the additional two turns along with two β-sheet regions. Based on these findings and a steric requirement for a parallel intermolecular β-sheet at positions 12 through 39 in the Aβ40 aggregate [38,39], we proposed the model of the Aβ42 aggregate as shown in Fig. **3** (A) [29]. Wetzel's model of the Aβ40 aggregate is also shown in Fig. **3** (B) [34]. Although the N-terminal and central regions including the turn at positions 22 and 23 are very similar to each other, the C-terminal structure is quite different; the C-terminal three residues of Aβ42 adopt the conformation of a β-sheet while those of Aβ40 do not. This C-terminal β-sheet would explain the greater aggregative ability and neurotoxicity of Aβ42 than Aβ40. Kim and Hecht [40] also pointed out a correlation between aggregation and the propensity of the residues at positions 41 and 42 to form a β-sheet. However, proline mutagenesis cannot reveal whether the C-terminal β-sheet is intermolecular or intramolecular. Quite recently, Takano and colleagues [41] have succeeded in the crystallization of a

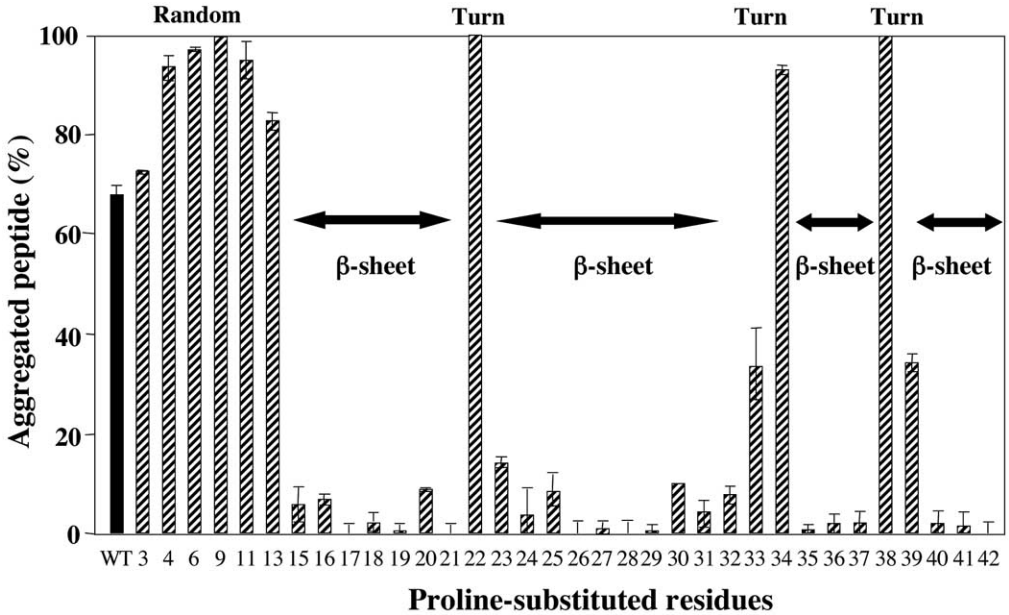

Fig. (2). Aggregation kinetics of a series of proline-substituted Aβ42 mutants after an 8-h incubation at 25 μM, 37 °C [29]. There was a good correlation between the aggregative ability and the neurotoxicity in PC12 cells [29]. WT: wild-type Aβ42.

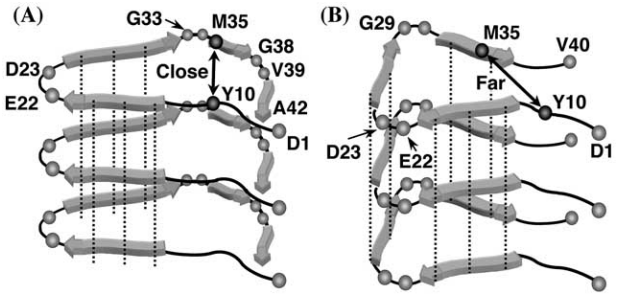

Fig. (3). Models of Aβ42 (A) [29] and Aβ40 (B) [34] aggregates based on systematic proline replacement. Dotted lines indicate intermolecular parallel β-sheets. The distance between the side chains of Tyr-10 and Met-35, both of which are critical to the radical-generating activity of Aβ42 [8,9], is shorter in the model of Aβ42 than in that of Aβ40.

fusion protein comprising Aβ28-42's N-terminus and the C-terminal region of ribonuclease HII from a hyperthermophile, *Thermococcus kodakaraensis*, to reveal the presence of an anti-parallel intramolecular β-sheet with a turn at positions 36 and 37. A turn at positions 37 and 38 has also been revealed by the *in silico* study of Aβ42 folding [42]. These results strongly suggest that the C-terminal structure in our aggregate model could also adopt an intramolecular anti-parallel β-sheet.

3. VERIFICATION OF THE TURN AT POSITIONS 22 AND 23 OF THE AGGREGATE OF Aβ42 WITH THE ITALIAN MUTATION USING SOLID-STATE NMR SPECTROSCOPY

Solid-state NMR spectroscopy is a powerful tool for analyzing the three-dimensional structure of a non-crystalline powder like Aβ aggregates. Antzutkin and colleagues [38] first analyzed the tertiary structure of whole Aβ40 aggregate using solid-state NMR, and obtained unambiguous evidence that the β-methyl carbons of Ala-21 and Ala-30 exist within 5 Å, respectively, indicating that Aβ40 forms parallel β-sheets to aggregate. The Aβ42 aggregate was also shown to have parallel β-sheets using site-directed spin labeling and solid-state NMR [37,43]. Recent biophysical research using electron microscopy, atomic force microscopy, Fourier transform infrared spectroscopy, and circular dichroism, suggested that Aβ fibrils, 70-120 Å in diameter, are composed of several protofilaments whose diameter is 30-60 Å [24,44]. Since the length of Aβ40 and Aβ42 in an extended β-strand is estimated to be 139 Å and 146 Å, respectively, Aβ40 and Aβ42 need to have at least one turn or bend structure. The existence of a turn in Aβ40 aggregates was supported by a set of experimental constraints based on the solid-state NMR [36,45,46]. The proposed structure is that Aβ40 forms an intermolecular parallel β-sheet at positions 10-22 and 30-40, respectively, with a bend at positions 23-29. Quite recently, Tycko and colleagues reported the quaternary structure of the Aβ40 aggregate based on experimental constraints using solid-state NMR [46,47]. However, this structure is slightly different from the aggregate models obtained by systematic proline replacement where the turn at positions 22 and 23 is critical for the aggregation and neurotoxicity of Aβ peptides [29,34]. We hypothesized the existence of the toxic conformation (Fig. **3** (A) or (B)) other than the physiological conformation with a bend at positions 23-29 like Fig. **4** (C). The toxic conformation is defined as that leading to marked aggregative ability and neurotoxicity. Since some Aβ42 mutants altered at position 22 (E22K-, E22Q-Aβ42), related to hereditary cerebral hemorrhage with amyloidosis, aggregated far more rapidly than wild-type Aβ42, the proportion with the toxic conformation might be significantly higher.

In order to verify the existence of the toxic conformer, we analyzed the tertiary structure at positions 21-24 of the E22K-Aβ42 aggregate by solid-state NMR [48] using dipolar-assisted rotational resonance (DARR) which enables a broadband ^{13}C-^{13}C correlation 2D experiment [49,50]. E22K-Aβ42 where all carbon atoms at positions 21-24 were labeled with ^{13}C was used in this study. Two sets of chemical shifts for Asp-23 were observed in a ratio of about 2.6:1.0 (major and minor conformers). The 2D DARR spectra at a mixing time of 500 ms, which can detect dipole-dipole interaction of about 5 Å, suggested that the side chains of Lys-22 and Asp-23 in the minor conformer could be located on the same side (Fig. **4** (A)). That means the presence of a turn structure at positions 22 and 23 in the E22K-Aβ42 aggregate. If this region were in a parallel β-sheet, adjoining side chains would face in opposite directions to each other and the distance between these chains would be long (8-10 Å) (Fig. **4** (B)). Although the structure at positions 21-24 of the major conformer could not be fully identified [48], it might be similar to that of the Aβ40 aggregate reported by Petkova and colleagues [46,47] (Fig. **4** (C)).

In Tycko's model [46,47], the bend structure at positions 23-29 could be stabilized by a salt bridge between the carboxyl group of Asp-23 and the amino group of Lys-28 (Fig. **4** (C)). Recent studies [47,51] suggested that this ionic inter-

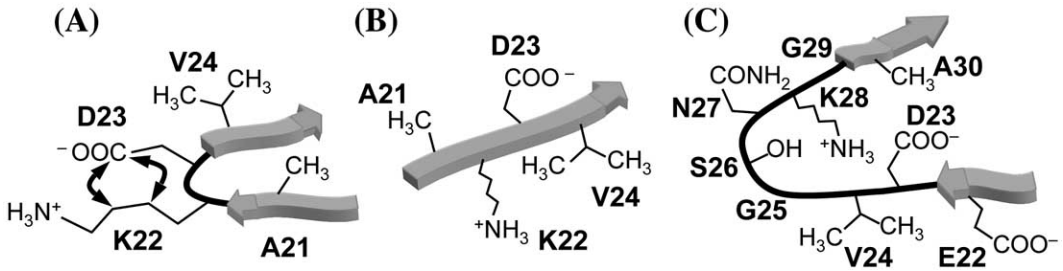

Fig. (4). (A) Possible structure of the minor conformer at positions 21-24 of the E22K-Aβ42 aggregate [48]. The ratio of major to minor conformers was estimated to be about 2.6:1.0. The double-headed arrows show the dipole interactions. (B) Putative structure at positions 21-24 in the case of the β-sheet. (C) The conformation at positions 22-30 in the Aβ40 aggregate reported by Tycko's group [46].

action might be intermolecular. Interestingly, this interaction was only detected in the aggregate of Aβ40 prepared under the gentle agitation, but not under the quiescent condition [47]. In contrast, in the minor conformer in the aggregate of E22K-Aβ42, the carboxyl group of Asp-23 could interact ionically with the amino group of Lys-22 (Fig. **4** (A)). This ionic interaction would promote the formation of a turn at positions 22 and 23. Since such ionic interaction is not possible in the wild-type Aβ42 with both anionic side chains (Glu-22 and Asp-23), the ionic interaction between Lys-22 and Asp-23 in the minor conformer of E22K-Aβ42 might be a reason why E22K-Aβ42 is more pathogenic than wild-type Aβ42. This speculation is partially supported by *in silico* studies and solution NMR analyses of Aβ21-30 with several mutations at position 22 [52,53]. Although based on results of solid-state NMR spectroscopy, Sato and colleagues [54] recently proposed a quite similar structure in the region of positions 17-40 of wild-type Aβ42 and Aβ40, the Aβ42 aggregate may contain a small amount of another conformer, the toxic conformer with the turn at positions 22 and 23, because it is far more neurotoxic than wild-type Aβ40. Detailed structural analyses of the aggregate of wild-type Aβ42 using solid-state NMR is being conducted by our group.

4. MECHANISM OF THE NEUROTOXICITY OF Aβ42 THROUGH THE FORMATION OF RADICALS

It is controversial whether or not the redox chemistry plays a critical role in the neurotoxicity of Aβ peptides. However, there is much circumstantial evidence that oxidative stress contributes to the neurodegeneration associated with AD [7-9]. The possible targets are considered to be unsaturated lipids and proteins in the membrane of the neuronal cells [55]. The impairment of enzymatic antioxidants like superoxide dismutase and glutathione reductase also increases oxidative stress in patients with AD [56]. However, it remains unclear at the molecular level how Aβ peptides radicalize to induce neurotoxicity.

Transition metals, Cu(II), Zn(II), and Fe(III), are abundant in Aβ plaques [57]. Cu(II) is most effective at strengthening the neurotoxicity of Aβ peptides in cell culture, and the strength of the neurotoxicity correlates with the ability to reduce Cu(II) to Cu(I) and to form H_2O_2 [58]. Cu(II) coordinates with three histidine residues at positions 6, 13, and 14 of Aβ42 (Fig. **5**), and the phenol hydroxyl group of Tyr-10 as well as some N-terminal nitrogen atoms might be involved in the formation of this copper complex [59,60]. Barnham and colleagues [61] proposed a molecular mechanism for the generation of H_2O_2 by Aβ peptides in the presence of Cu(II) and ascorbic acid as a reducing agent. Using density functional theory calculations, they showed that Tyr-10 is a pivotal residue to drive the catalytic production of H_2O_2 by Aβ peptides in the presence of Cu(II). This was supported by the mutation of Tyr-10 to alanine that diminished the H_2O_2 production. Moreover, they suggested that the phenoxy radical of Tyr-10 produced by the reaction with reactive oxygen species could cause neurotoxicity and result in the formation of dityrosine that could accelerate the aggregation of Aβ peptides. This hypothesis explains well why the rodent homologue of Aβ42 (R5G,Y10F,H13R-Aβ42) bound to metal ions more weakly and produced lower levels of H_2O_2 than the wild-type Aβ42 [62]. Since some N-terminal residues as well as His-6, Tyr-10, His-13, and His-14 might be involved in the binding [58], H6R (UK) and D7N (Tottori) mutations at the N-terminal in patients with familial AD might change the binding mode of metal ions with Aβ peptides to enhance the production of H_2O_2.

It has been suggested that Met-35 of Aβ peptides plays a critical role in AD [63]. Yanker and colleagues [64] found that Aβ25-35 containing a methionine residue was as neurotoxic as Aβ40. The substitution of Met-35 with norleucine diminished the oxidative stress and neurotoxicity caused by Aβ42 [65], and Aβ1-28 without a methionine residue caused very little oxidative stress or neurotoxicity [64,66]. In addition, Bitan and colleagues [67] suggested that oxidation (radicalization) of the sulfur atom of Met-35 of Aβ peptides would occur prior to their aggregation. The importance of the methionine residue was also shown by using *Caenorhabditis elegans* expressing human Aβ42; substitution of Met-35 with cysteine resulted in no protein oxidation [65]. Moreover, Crouch and colleagues [68] have recently suggested that inhibition of cytochrome *c* oxidase by Aβ42 could involve the formation of a redox active methionine radical.

It is concluded that the radicalization of both Tyr-10 and Met-35 is likely to be important to the neurotoxicity of Aβ peptides. We also confirmed that both residues are important in the generation of radicals and neurotoxicity as shown in Fig. **6** (B) [69]. The radical generating ability was estimated by electron spin resonance (ESR) spectrometry using phenyl-*tert*-butylnitrone (PBN) as a spin-trapping reagent. Y10F-Aβ42, M35*n*V-Aβ42, and Y10F,M35*n*V-Aβ42 produced significantly weaker ESR signals with less neurotoxicity in

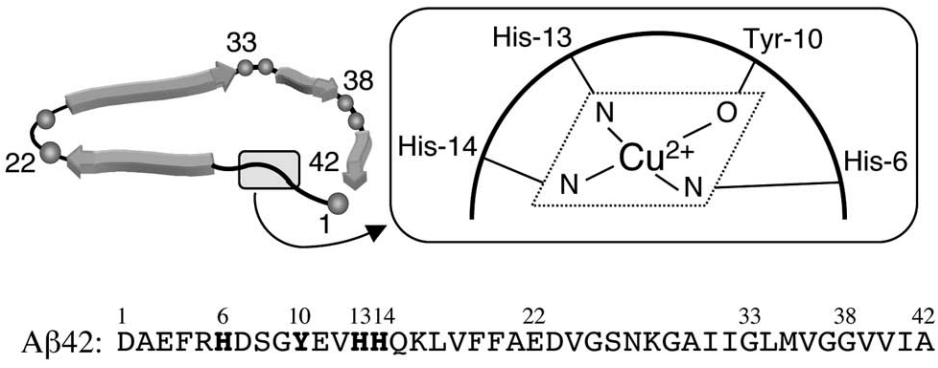

Fig. (5). Aβ peptides coordinate Cu(II) to generate H_2O_2 through reduction to Cu(I) [8]. See also Fig. (**1**).

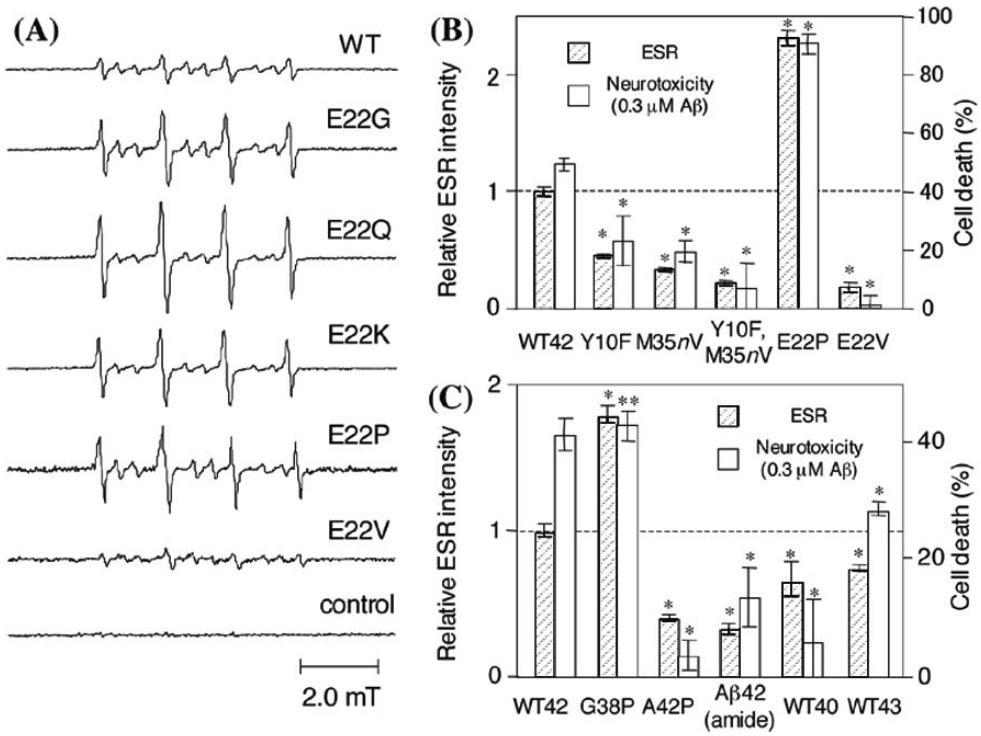

Fig. (6). (A) ESR spectra of Aβ42 mutants (165 μM) altered at position 22 after 48 h at 37 °C obtained using PBN as a trapping reagent [69]. ESR spectrometer: Bruker EMX8/2.7, microwave power: 20 mW, microwave frequency: 9.8 GHz, modulation amplitude: 1.0 G, modulation frequency: 100 kHz, conversion time: 40.96 ms, scans: 100. (B), (C) Relative ESR intensity after 48 h of incubation and neurotoxicity in PC12 cells at 0.3 μM of Aβ mutants [69]. *p<0.01 versus wild-type Aβ42, **p<0.05 versus wild-type Aβ42.

PC12 cells than did wild-type Aβ42. However, it is not clear how these residues cause oxidative damage to neuronal cells. As mentioned in section **1**, our recent research on Aβ peptides mutated at position 22 (E22G, E22Q, and E22K) that would preferentially form a β-turn at positions 22 and 23, and on the systematic replacement with proline of Aβ42, clearly showed that the turn at positions 22 and 23 plays a crucial role in the neurotoxicity of Aβ42 [29,33]. To investigate the effects of this turn on the generation of free radicals, a series of Aβ mutants altered at position 22 were examined for their ability to produce free radicals (Fig. **6** (A)) [69]. The predominant shape of the spectrum was four-line as Butterfield's group reported [65]. All mutants prone to form a β-turn at position 22 generated large numbers of free radicals, while the free radical production of E22V-Aβ42 (valine is known as a turn breaker) was lower. Since there was a good correlation between the free radical production and neurotoxicity *in vitro*, these results suggest that the turn at positions 22 and 23 would be important for radical-induced neurotoxicity in Aβ42. This turn structure was clearly detected in the aggregate of E22K-Aβ42, one of the familial AD mutants with potent aggregative ability and neurotoxicity [48]. In our aggregate model of Aβ42 (Fig. **3** (A)), the phenol hydroxyl group of Tyr-10 could be in the vicinity to the sulfur atom of Met-35, suggesting that the phenoxy radical derived from Tyr-10 by oxidation in the presence of metal ions could easily interact with the sulfur atom of Met-35 to yield the *S*-oxidized radical cation. The distance between the phenol oxygen atom of Tyr-10 and the sulfur atom of Met-35 can be roughly estimated based on recent research on the structure of Aβ42 in hexafluoro-2-propanol/H_2O and Aβ40 in a water-micelle environment [70,71]. The distance in 30 conformers of Aβ42 was in the range of 12-39 Å, while that in 10 confor-mers of Aβ40 was in the range of 23-38 Å, supporting the more effective interaction between Tyr-10 and Met-35 in Aβ42.

To verify the interplay between Tyr-10 and Met-35 in Aβ42, we measured the distance between these residues by ESR spectrometry using the methanethiosulfonate nitroxide spin label (MTSSL) (manuscript in preparation). Three kinds of Aβ42 derivatives with MTSSL at position 10, position 35, and both positions 10 and 35, were prepared for the measurement of the continuous wave ESR spectrometry. A clear dipole coupling was detected in 10,35-MTSSL-Aβ42 while no significant signal was observed in 10-MTSSL-Aβ42 or 35-MTSSL-Aβ42 as controls, suggesting that the spin labels at positions 10 and 35 could be located within 15 Å. In contrast, 10,35-MTSSL-Aβ40 did not give any signals ascribable to the dipole interaction. A distance measurement in 10,35-MTSSL-Aβ40 was made using Fourier transform ESR spectrometry. The distance between these two residues was estimated to be 30 Å that is twice as much as that in 10,35-MTSSL-Aβ42. These data strongly suggest that the side chains of Tyr-10 and Met-35 would interact with each other, more favorably in Aβ42 than Aβ40. This might be one of the reasons why Aβ42 is more neurotoxic than Aβ40.

The above data suggest that the *S*-oxidized methione radical at position 35 rather than the phenoxy radical at position 10 would be the ultimate toxic radical species in the Aβ-induced neurotoxicity. Kadlcik and colleagues [72] reported that the *S*-oxidized radical cation of Met-35 could cause neu-

rotoxic effects, while the phenoxy radical of Tyr-10 could not, supporting our hypothesis. However, the *S*-oxidized radical cation is too short-lived to induce neurotoxic effects in neuronal cells where diffusion is the rate-determining step of neurotoxicity because of the highly viscous environment [73]. Varadarajan and colleagues [73] proposed that the C-terminal carboxylate anion of Met-35 in Aβ25-35 could stabilize the *S*-oxidized radical cation by forming a six-membered ring *via* the intramolecular *S*-*O*-bond as reported in the *N*-acetylmethionine amide [74] (Fig. (**7**)). In the case of full-length Aβ40, Kanski and colleagues [75] suggested that the glycine centered radicals at positions 29 and 33 could be produced by the attack of the *S*-oxidized radical cation of Met-35. Theoretical calculations also predicted a role for the glycyl radicals at positions 29 and/or 33 as long-lived species that can induce damage to cellular membranes [76]. Our aggregate model of Aβ42 [29] is also consistent with this specific association of Gly-29 and Gly-33 with Met-35 because of the presence of the turn at positions 33 and 34. However, another mechanism of stabilization of the *S*-oxidized radical cation seems to exist in Aβ42.

As shown in Fig. **6** (C), Aβ40 without the C-terminal two residues produced lower levels of radicals with less neurotoxicity than Aβ42, suggesting that the C-terminal region enhances the radical-induced neurotoxicity of Aβ42. In our aggregate model of Aβ42 (Fig. **3** (A)), there are two β-sheet regions at positions 35-37 and 40-42. G38P-Aβ42, which was more aggregative than wild-type Aβ42 [29], showed greater radical-generating activity and neurotoxicity than the wild type. On the other hand, the radical-generating activity and neurotoxicity of A42P-Aβ42, which was far less able to aggregate than the wild type, were very weak (Fig. **6** (C)). These results strongly suggest that the C-terminal turn at positions 38 and 39 along with the β-sheet at positions 35-37 and 40-42 would be closely related to the neurotoxicity through the formation of radicals. On the assumption that the C-terminal β-sheets were intramolecular as discussed in section **1**, the β-sheet would promote the association of the *S*-oxidized radial cation with the C-terminal carboxylate anion to stabilize the methionine radical by the formation of an *S*-*O*-bond. The resultant hydrophobic C-terminal core would accelerate the aggregation to produce Aβ42 oligomers and fibrils. In support of this idea, Y10F-Aβ42, M35*n*V-Aβ42, and Y10F,M35*n*V-Aβ42 with less radical-generating activity than Aβ42 showed a slower aggregation kinetics than wild-type Aβ42, suggesting that the formation and stabilization of the *S*-oxidized radical cation of Met-35 would play a significant role in the aggregation as well as neurotoxicity of Aβ42 [69]. To test this assumption, Aβ42(amide), whose C-terminal carboxylic acid was converted into an amide, was examined for radical-generating ability and neurotoxicity. As shown in Fig. **6** (C), Aβ42(amide) was less neurotoxic with less radical-generating activity than wild-type Aβ42, suggesting that the interaction of the C-terminal carboxylate anion with the *S*-oxidized radical cation through the intramolecular anti-parallel β-sheet is vital to the neurotoxicity of Aβ42. Since the C-terminal four residues are not involved in the intramolecular β-sheet formation (Fig. **3** (B)) [34], such interaction at the C-terminus is not possible for Aβ40 which is far less neurotoxic than Aβ42. Moreover, Aβ43 produced fewer radicals and was less neurotoxic than Aβ42 (Fig. **6** (C)), suggesting that the C-terminal length of Aβ42 might be best for stabilization of the methionine radical. Bitan and colleagues [77] reported that Aβ42(amide) could not form a substantial amount of oligomers with lower molecular weight, supporting the above-mentioned hypothesis.

Based on these results, we proposed a mechanism of neurotoxicity of Aβ42 through the formation of radicals (Fig. **8**). The phenoxy radical at position 10 of Aβ42 would be produced by a redox reaction involving metal ions. The turn at positions 22 and 23 would bring the phenoxy radical in close to the sulfur atom of Met-35 to induce the formation of the *S*-oxidized radical cation, which could be stabilized by the C-terminal carboxylate anion through the intramolecular β-sheet. The resultant C-terminal hydrophobic core would accelerate the aggregation of Aβ42 to produce a variety of oligomers where the radical species are confined. Cleavage of the *S*-*O* bond would yield a carboxyl radical which would be the species that damages the neuronal cells. Since this radical species could be confined to the aggregate as a stable form, this damage (oxidative stress) would be long-lasting due to equilibration between the monomer and the oligomer.

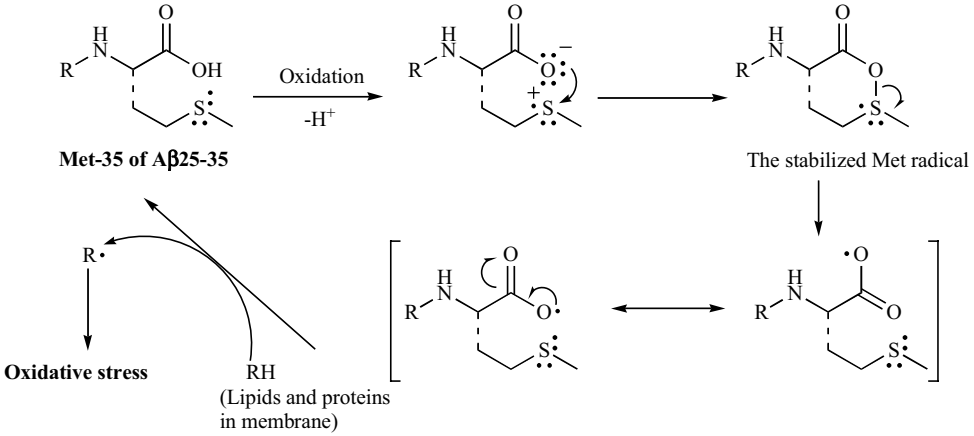

Fig. (7). Possible mechanism of stabilization of the *S*-oxidized radical cation of Aβ25-35 [73]. Such neighboring group participation is not possible in Aβ25-36 and Aβ25-35(amide) which exhibited very weak radical-producing activity and neurotoxicity.

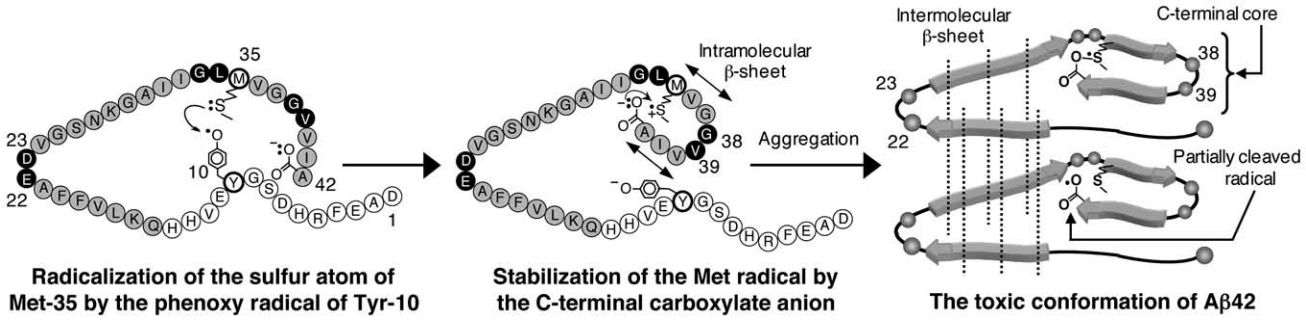

Fig. (8). Mechanism of neurotoxicity of Aβ42 through the formation of radicals [69]. Two turns at positions 22, 23 and 38, 39 are characteristic of the toxic conformation of Aβ42. The former would enhance the radicalization of the sulfur atom of Met-35, while the latter would stabilize the *S*-oxidized radical cation of Met-35 through the intramolecular β-sheet. The resultant C-terminal core would accelerate aggregation of Aβ42 to confine the radical species to the aggregate. The carboxyl radical liberated from the C-terminal core would cause oxidative stress among neuronal cells for a long period of time.

5. CONCLUDING REMARKS

The mechanism proposed here (Fig. **8**) shows the interplay between Tyr-10 and Met-35 that is critical to the neurotoxicity of Aβ42 through the formation of radicals, and explains clearly the large difference in aggregative ability and neurotoxicity between Aβ42 and Aβ40. However, some reports do not support the notion that the methionine residue at position 35 is critical to the neurotoxicity of Aβ peptides. Pike and colleagues [78] reported that the substitution of the methionine residue of Aβ25-35 with serine or cysteine led to peptides of similar neurotoxicity to Aβ25-35 itself. However, our proposed mechanism (Fig. **8**) is applicable to the neurotoxicity of whole Aβ42, not to that of fragment peptides without a tyrosine residue and the C-terminal hydrophobic core. Recently, Ciccotosto and colleagues [79] found that the neurotoxic effect of M35V-Aβ42 on primary mouse neuronal cortical cells was more potent than that of Aβ42. Since M35V-Aβ42 binds Cu(II) to produce similar amounts of H_2O_2 as wild-type Aβ42 *in vitro*, and since its neurotoxicity was attenuated by catalase, a radical species other than the *S*-oxidized radical cation of Met-35 might be produced by interaction of the phenoxy radical at position 10 with the valine residue at position 35 to produce a more stable secondary radical species. On the other hand, Crouch and colleagues [68] reported that M35V-Aβ42 abrogated the inhibitory activity of Aβ42 toward cytochrome *c* oxidase, suggesting that the methionine radical at position 35 could play an important role in the inhibition of cytochrome *c* oxidase. Several mutants of Aβ42 containing acidic or basic residues at positions 41 and/or 42 were less prone to aggregate than Aβ40, suggesting that properties of the side chains at positions 41 and 42, rather than the additional two C-terminal residues, cause higher aggregative ability of Aβ42 [40]. However, this is not contradictory to our mechanism since these alterations produce a plus or a minus charge in the C-terminal core formed by the intramolecular β-sheet to cause its repulsion.

Whether the mechanism shown in Fig. (**8**) contributes to the pathology of AD *in vivo* remains to be investigated. However, this model could contribute to the design of antibodies or RNA aptamers with fewer side effects, and to the development of inhibitors that are specific to the toxic conformation of Aβ42 (Fig. **8**). Such efforts are under way.

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