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REVIEW

Structure of β-Amyloid Fibrils and Its Relevance to Their Neurotoxicity: Implications for the Pathogenesis of Alzheimer's Disease

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Alzheimer's disease and cerebral amyloid angiopathy are characterized by the deposition of β-amyloid fibrils consisting of 40- and 42-mer peptides (Aβ40 and Aβ42). Since the aggregation (fibrilization) of these peptides is closely related to the pathogenesis of these diseases, numerous structural analyses of Aβ40 and Aβ42 fibrils have been carried out. Aβ42 plays a more important role in the pathogenesis of these diseases since its aggregative ability and neurotoxicity are considerably greater than those of Aβ40. This review summarizes mainly our own recent findings from the structural analysis of Aβ42 fibrils and discusses its relevance to their neurotoxicity *in vitro*.

[**Key words:** Alzheimer's disease, Aβ40, Aβ42, amyloid, cerebral amyloid angiopathy, familial Alzheimer's disease, neurotoxicity, oxidative stress, solid-state nuclear magnetic resonance]

Alzheimer's disease (AD) is one of the most common neurodegenerative disorders associated with aging, and is characterized by fibrillar deposits of amyloid β (Aβ) peptides in the brain parenchyma and cortical blood vessels (1). Aggregates quite similar to amyloid fibrils are also observed in several other neurodegenerative diseases, for example, prion disease, Huntington's disease, and Parkinson's disease. AD is characterized by extracellular neuritic amyloid plaques (senile plaques) and intraneuronal neurofibrillary tangles. The senile plaques consist mainly of 40- and 42-mer peptides (Aβ40 and Aβ42) (2), whereas a major constituent of the neurofibrillary tangles is abnormally phosphorylated tau (3). Since the senile plaques are more characteristic of AD, much research on Aβ peptides has been carried out in the past 20 years.

Aβ peptides result from the proteolytic cleavage of β-amyloid precursor protein (APP) (4) by two proteases, β- and γ-secretase (5–7) (Fig. 1). Occasionally, 39- and 43-mer Aβ peptides occur. Under physiological conditions, the ratio of Aβ42 to Aβ40 is about 1:10. Aβ42 plays a critical role in the pathogenesis of AD since its aggregative ability and neurotoxicity are much greater than those of Aβ40 (5, 8). Aβ42 oligomers initially formed as a seed accelerate the aggregation of Aβ40 to form the amyloid plaques that eventually lead to the neurodegeneration (amyloid cascade hypothesis) (9). Although the direct involvement of Aβ peptides in AD is well documented and their aggregative ability is closely related to their neurotoxicity, the precise mechanism of the neurotoxic effects of Aβ peptides remains unclear. Moreover, it has recently been reported that the neurotoxicity of Aβ peptides might be ascribable to the oligomeric species, not the fibrils (10, 11).

The structural analysis of Aβ fibrils is one of the most promising ways of revealing the mechanism of AD. Recent biophysical investigations using electron microscopy, Fourier transform infrared spectroscopy (FTIR), and circular dichroism (CD) spectroscopy showed that Aβ fibrils adopt a β-sheet structure (12). However, a high-resolution structural analysis of Aβ fibrils has yet to be conducted since single crystal X-ray crystallography and solution NMR cannot be applied to insoluble Aβ fibrils. The systematic mutation of Aβ peptides and solid-state NMR spectroscopy are more reliable methods of establishing how particular amino acid residues are arranged within the β-sheet of Aβ fibrils, information that is indispensable to developing new agents with

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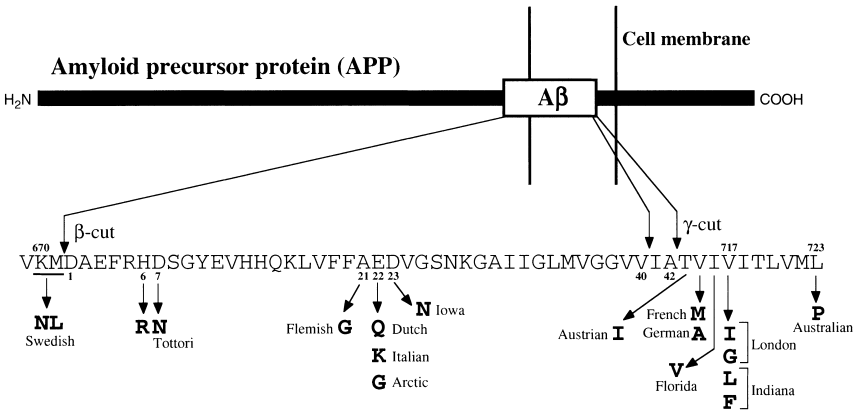

FIG. 1. Structure of Aβ42 and Aβ40, and APP mutations inside or outside the Aβ-coding region in familial Alzheimer's disease (FAD).

inhibitory activity toward the aggregation and subsequent neurotoxicity. This review focuses mainly on our own recent investigations of the structure of Aβ fibrils and discusses its relevance to their neurotoxicity *in vitro*.

I. SYNTHESIS OF Aβ PEPTIDES

To investigate the structure of Aβ fibrils, highly pure Aβ peptides are indispensable. However, Aβ42 with 14 hydrophobic and/or bulky amino acid residues at the C-terminus easily aggregates even in weakly acidic and neutral media (13). This aggregative propensity makes difficult its solid-phase synthesis, and Aβ42 is classified as a difficult sequence-containing peptide (14). In addition, conventional reversed-phase HPLC purification in aqueous acetonitrile containing trifluoroacetic acid is not effective for purifying crude Aβ42 because of its low solubility in water and broad elution on the C_{18} column under acidic conditions. Since commercially available Aβ42 sometimes contains impurities (15), previous biological and physicochemical data using Aβ42 are often problematic and controversial.

Several long peptides such as ribonuclease, HIV protease, and green fluorescence protein have been synthesized using fragment condensation of peptides of about 10 residues (16–18). However, fragment condensation requires a lot of labor and time. To circumvent this problem, we developed an efficient methodology to obtain long peptides of over 50 amino acid residues without fragment condensation, in collaboration with Drs. H. Fukuda and M. Shindo at Applied Biosystems (Foster City, CA, USA) (19–21). More than 50 kinds of Aβ42 peptides have been synthesized in a stepwise fashion with a Pioneer™ peptide synthesizer (Applied Biosystems) using *N*-[(dimethylamino)-1*H*-1,2,3-triazolo [4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) (22) as an activator for Fmoc chemistry (Fig. 2). The Pioneer™ is a continuous flow-type peptide synthesizer that allows for a high flow rate of the reagent solution to establish a high coupling yield. Each Fmoc amino acid activated by HATU in the presence of *N,N*-diisopropylethylamine in *N,N*-dimethylformamide was coupled with a preloaded polyethylene glycol-polystylene (PEG-PS) support containing Fmoc-Ala. The PEG-PS support is quite useful for synthesizing a long

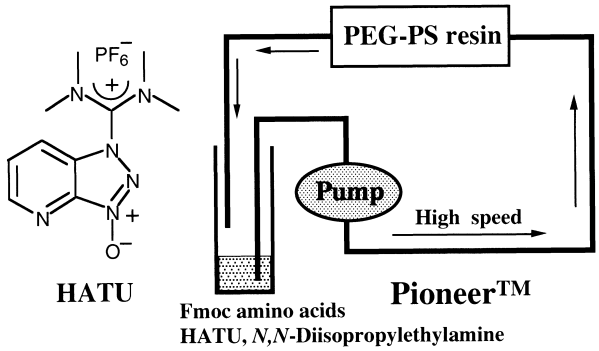

FIG. 2. Structure of HATU (22) and the continuous flow-type peptide synthesizer (Pioneer™).

hydrophobic sequence like Aβ42 because the PEG moiety reduces the hydrophobic interaction between the peptide chain and the polystyrene support. Although the PEG-PS resin is fragile, the continuous flow-type system does not damage it. This efficient synthesis followed by purification using reversed-phase HPLC under alkaline conditions gave each Aβ42 peptide in over 98% purity (23–27). Total yields of the Aβ peptides were generally 10–20%, indicating that the average coupling yield of each condensation step was over 96%.

Recently, a unique method of synthesizing Aβ42 using an *O-N* intramolecular acyl migration reaction (28), which is generally observed in Ser/Thr-containing peptides (29, 30), has been reported. 26-*O*-Acyl isoAβ42 did not aggregate rapidly and could be easily purified by the reversed-phase HPLC, possibly because the branched ester structure would suppress unfavorable intermolecular hydrophobic interactions. After purification, 26-*O*-acyl isoAβ42 was efficiently converted to intact Aβ42 under physiological conditions via *O-N* intramolecular acyl migration.

II. NEUROTOXICITY AND PHYSICOCHEMICAL PROPERTIES OF Aβ MUTANT PEPTIDES IN FAMILIAL ALZHEIMER'S DISEASE

Familial Alzheimer's disease (FAD) has been recognized since the 1930s (31). Hitherto, APP gene on chromosome 21 (4), the presenilin 1 gene on chromosome 14 (32), and