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Insights into the aggregation mechanism of Aβ(25–40)

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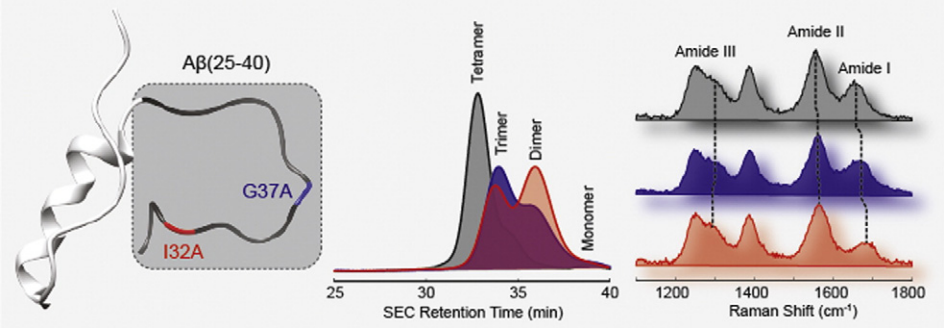
HIGHLIGHTS

- Substitution of alanine at position 32 or 37 shifts the oligomeric distribution from tetramers/trimers to trimers/dimers.
- Increased backbone hydration reduces Aβ(25–40)'s propensity to fibrillize.
- Structural changes in Aβ(25–40) induced by myricetin are eliminated when the hydrophobicity at residue 32 is reduced.
- Fibrillization depletes the store of the largest oligomeric species, with little impact on the amountof smaller oligomers.

GRAPHICAL ABSTRACT



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ABSTRACT

The hydrophobic fragment of the Alzheimer's related β-amyloid (Aβ) peptide, Aβ(25–40), aggregates and forms insoluble amyloid fibrils at a rate similar to the full-length peptide. In order to gain insight into the fibrillization of Aβ(25–40) and the ability of the flavonoid myricetin to inhibit its aggregation, the isoleucine at position 32 (I32A) and the glycine at position 37 (G37A) in the full-length peptide were replaced with alanine. Thioflavin T assays indicate that substitution of isoleucine for alanine significantly reduces the rate and extent of fibrillization compared to the Aβ(25–40) and G37A peptides. Although all three peptides are fully disordered initially, circular dichroism studies suggest the structure of the I32A and G37A peptides are different from the parent peptide Aβ(25–40). Introduction of myricetin to the peptide samples results in modest structural changes for the Aβ(25–40) and G37A peptides but not the I32A peptide. Aβ(25–40) oligomers were predominantly tetramers, whereas I32A and G37A oligomers were a mixture of trimers and dimers. After 48 h of incubation at 37 °C, the amount of tetramers and trimers in solution dropped for the Aβ(25–40) and G37A peptides but remained similar for the I32A peptide. Incubation of Aβ(25–40) with myricetin increased the relative proportion of trimers to tetramers. Ultraviolet resonance Raman studies suggests that the I32A peptide may be more hydrated than the Aβ(25–40) and G37A peptides. Taken together, these data indicate the structural changes observed for the Aβ(25–40) and G37A peptides upon introduction of myricetin are localized around residue 32 and could arise from hydrophobic interactions between the peptide and the flavonoid or interference with the self-association of the peptide in this region. Substitution of isoleucine at position 32 with alanine had little effect on the peptide's secondary structure but dramatically decreased the propensity of the peptide fibrillize.

Abbreviations: AD, Alzheimer's disease; Aβ, β-amyloid; ThT, thioflavin T.

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1. Introduction

Alzheimer's disease (AD), named after the German physician Alois Alzheimer, is now the most common form of dementia in the US [1]. AD involves neuronal damage and protein deposition in the extracellular (plaques) and intraneuronal (neuofibrillary tangles) regions of the brain. Extracellular plaques are composed predominantly of the β-amyloid (Aβ) peptide whereas hyperphosphorylated tau protein comprises neurofibrillary tangles [2]. Clinical symptoms include apraxia (loss of coordination), agnosia (impaired recognition), and aphasia (loss of language skills, difficulty speaking, and writing) [3]. In 2011, an estimated 5.4 million Americans developed AD; by 2040 that number could reach 81 million [4].

AD was first identified more than 100 years ago, but the cause or causes is still under debate [5]. However, insoluble plaques are considered to be one of the pathological hallmarks of AD. The aggregation mechanism of Aβ is complex and could involve multiple pathways [6,7]. The diffusion-limited aggregation (DLA) model was proposed in 1992 to explain Aβ aggregation. According to the DLA model, Aβ monomer seeds spontaneously convert to octamers, which stack to form fibrils. Fibrils are then proposed to elongate by diffusion limited end-to-end association, eventually forming fibrils [8]. The DLA model was modified in 2001 to include formation of a multimeric nucleus, from which filaments elongate and aggregate to form fibrils that grow though end-to-end association [9]. While the presence of plaques has been considered a hallmark of AD, high concentrations of soluble Aβ tend to correlate more with the presence of clinical AD symptoms [10].

Potential treatments for AD include Aβ production inhibitors, small anti-oligomerization molecules, anti-inflammatory drugs, and neuroprotective drugs [5]. However, interest in the therapeutic use of naturally occurring polyphenolic compounds for neurodegenerative diseases is on the rise [11–13]. Polyphenols and flavonoids are naturally abundant in a large variety of edible plants [14] and have beneficial effects including anti-oxidant, anti-bacterial, and anti-inflammatory activity [15,16]. Additionally, a number of polyphenolic compounds, including some flavonoids, have been shown to have anti-amyloidogenic activity [17–19]. Polyphenols have been shown to interact/bind to proteins through hetero-aromatic interactions, hydrophobic interactions, and hydrogen bonding with the backbone or positively charged amino acid side chains [20,21], which could be related to their anti-amyloidogenic activity. Antibody therapies represent another potential therapeutic avenue. Studies in transgenic mice models have shown that peripherally administered antibodies against Aβ can enter the central nervous system (CNS) and facilitate clearance of Aβ plaques [22] and soluble Aβ [23,24].

Previous studies have shown that the aggregation propensity of full length Aβ(1–40) is similar to its hydrophobic fragment Aβ(25–40) and the flavonoid myricetin fully inhibits fibrillization of both peptides [25]. While there is evidence of aromatic interactions between full length Aβ and myricetin [21], the mechanism of interaction between myricetin and the hydrophobic fragment Aβ(25–40) is not known. Since there are no aromatic residues in the Aβ(25–40) region, two site mutations were made to examine the effects of diminished hydrophobic interactions and backbone hydrogen bonding on myricetin's anti-amyloidogenic activity. It has been suggested that sequential glycines allow the polyphenol to hydrogen bond with the peptide backbone [26]. To prevent the phenol groups from hydrogen bonding with the peptide backbone, glycine 37 was changed to alanine (G37A). Similarly, sequential isoleucines are present at positions 31 and 32. Isoleucine 32 was changed to alanine to slightly reduce the hydrophobicity of the peptide, and interfere with hydrophobic interactions (I32A) [27]. Mutation of these residues should conserve the amyloidogenic properties of Aβ(25–40) [28], while potentially reducing the binding affinity of myricetin. While the effects of these mutations should be moderate, dramatic differences were observed in their oligomer distributions and rates of aggregation.

2. Materials and methods

2.1. Materials

Aβ(25–40) and the two site mutants, G37A and I32A, were synthesized by the Structural Biology Core Facility, University of Missouri-Columbia. Each peptide was purified using an analytical Beckman System Gold HPLC system (Beckman Coulter, Brea, CA) fitted with a C18 column (Grace Vydac Protein & Peptide, Southborough, MA). Ultrapure water (18 MΩ) was obtained using a Barnstead Diamond system (Barnstead International, Dubuque, IA). The pH of the water was adjusted to 2.5 using concentrated HCl (Fisher Scientific, Pittsburgh, PA) to optimize purification. Water and acetonitrile (Fisher Scientific, Pittsburgh, PA) were applied as an eluent system. Sodium phosphate monobasic, sodium phosphate dibasic and sodium chloride (Fisher Scientific, Pittsburgh, PA) were used to prepare buffers. Thioflavin T (ThT), purchased from Sigma-Aldrich (St. Louis, MO), was dissolved in ethanol and diluted with 10 mM phosphate buffer to 150 μM.

2.2. Sample preparation

Aβ(25–40) (M.W. 1470) and two single site mutants G37A (M.W.1484) and I32A (M.W. 1428), were directly dissolved in 10 mM pH 7.4 sodium phosphate buffer and sonicated in an ice bath for 2 min to break up aggregates. Subsequently the solution was centrifuged for 30 min at 14,000 rcf and 4 °C to remove insoluble aggregates and the supernatant was collected for future use. The concentration of each solution was estimated using a molar extinction coefficient of 17,385 L mol^{-1} cm^{-1}, determined from the side chain and peptide bond extinction coefficients at 215 nm [29,30]. All solutions were adjusted to a final concentration of 50 μM. A 20 mM stock myricetin (MP Biomedicals, Solon, OH) solution was made by dissolving myricetin in ethanol. A small aliquot of the stock solution was then added to the peptide solution for a final myricetin concentration of 25 μM. The concentration of myricetin was chosen to maximize the potential for interaction with the peptide while minimizing the potential of myricetin to aggregate, which occurs at higher concentrations.

2.3. Thioflavin T (ThT) fluorescence assay

Sample solutions of each peptide were prepared as stated above and were incubated at 37 °C for 11 days. During the incubation period, 70 μL of each sample was collected on days 0, 1, 2, 3, 5, 7 and 11. A small amount of the 150 μM ThT stock solution was added to each sample for a final ThT concentration of 5 μM. Fluorescence spectra were collected using a Cary Eclipse fluorescence spectrometer (Varian, Palo Alto, CA), using a 3 mm path length quartz cell (Hellma USA, Plainview, NY). Spectra of each sample were measured using an excitation wavelength of 450 nm with a slit width of 5 nm. The emission spectrum was collected between 470 and 700 nm with a slit width of 5 nm. Data points from 478 nm to 482 nm were averaged for a mean intensity at 480 nm, which is reported in Fig. 1.

2.4. Size exclusion chromatography (SEC)

A BioLogic DuoFlow FPLC chromatography system (Bio-Rad, Hercules, CA) fitted with a Superdex 75 10/300 GL column (GE Healthcare, Uppsala, Sweden) was employed for the SEC. The system was pre-equilibrated with 50 mM phosphate buffer with 150 mM NaCl; the same buffer was used as the eluent. Peptide samples were made as described earlier and an injection volume of 250 μL was used. The SEC was kept at 4 °C. A flow rate of 0.5 mL/min was employed and the absorption of the eluent at 215 nm was monitored using a Quad-Tec detector (Bio-Rad, Hercules, CA). A molecular weight ladder consisting of BSA, ovalbumin, ribonuclease A and vitamin B_{12} was employed to estimate the M.W. of