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Substituted tryptophans at amyloid-β(1–40) residues 19 and 20 experience different environments after fibril formation

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ABSTRACT

Amyloid-β protein (Aβ) is the principal component of the neuritic plaques found in Alzheimer's disease. The predominant Aβ morphology in the plaques is fibrillar which has prompted substantial *in vitro* work to better understand the molecular organization of Aβ fibrils. In the current study, tryptophan substitutions were made at Aβ(1–40) position 19 (F19W) or 20 (F20W) to ascertain environmental differences between the two residues in the fibril structure. Kinetic studies revealed similar rates of fibril formation between Aβ(1–40) F19W and F20W and both peptides formed typical amyloid fibril structures. Aβ(1–40) F19W fibrils displayed a significant tryptophan fluorescence blue-shift in λ_{max} (33 nm) compared to monomer while Aβ(1–40) F20W fibrils had a much smaller shift (9 nm). Fluorescence quenching experiments with water-soluble acrylamide and KI demonstrated that both W19 and W20 were much less accessible to quenching in fibrils compared to monomer. Lipid-soluble TEMPO quenched the fluorescence of Aβ(1–40) F19W fibrils more effectively than F20W fibrils in agreement with the fluorescence blue-shift results. These findings demonstrate distinct environments between Aβ(1–40) residues 19 and 20 fibrils and indicate that while W20 accessibility is compromised in Aβ fibrils it resides in a much less hydrophobic environment than W19.

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Introduction

The neuritic plaques found in the brain parenchyma of Alzheimer's disease (AD)[1] patients contain a dense core of amyloid-β protein (Aβ) fibrils. These fibrillar deposits originate from 40- or 42-residue Aβ monomers produced by proteolytic cleavage of the amyloid-β precursor protein [1]. *In vitro* studies show that Aβ can self-assemble via non-covalent nucleation-dependent polymerization [2] to form protofibrils and fibrils with significant β-sheet secondary structure [3]. Since the fibrils formed *in vitro* share many characteristics with those in the brain extensive studies have been conducted to better understand the structure and organization of Aβ fibrils. The Aβ(1–40) sequence can be subdivided into several regions that are important in understanding the self-assembly process. First, a relatively hydrophilic N-terminal region (residues 1–10) that solid state NMR [4], solution hydrogen–deuterium exchange NMR [5], and limited proteolysis [6] have shown is not involved in the fibril structure. Second, a short hydrophobic middle region encompassing residues 17–20 (LVFF) that is critical for fibril formation and stability based on proline-scanning mutagenesis [7]. Third, a hydrophobic C-terminal region comprising residues 29–40 that plays a major role in initiating Aβ self-assembly and determining the fibril structure.

An established model of Aβ(1–40) fibril structure by Tycko and colleagues elucidated using solid-state NMR proposed electrostatic and hydrophobic intramolecular interactions between regions encompassing residues 12–24 and 30–40 separated by a 180° bend [4]. Intermolecular hydrogen bonding between monomer units results in formation of two parallel in-register β-sheets perpendicular to the fibril axis. Based on this model, the core of the β-sheet structure that extends along the fibril axis is composed primarily of hydrophobic residues. Furthermore, the trans orientation of the side chains places phenylalanine 19 (F19) into a low dielectric center while phenylalanine 20 (F20) is oriented away from the fibril core. Additional work on this particular model confirmed that interactions between side chains L17/F19, and I32/L34/V36 create a hydrophobic cluster but revealed that these interactions were staggered by two monomer units [8]. The staggered orientation still puts these hydrophobic side chains in the interior of a molecular layer. Fibril ultrastructure also involves lateral association of β-sheet-rich protofilaments. Both Aβ(1–40) and Aβ(1–42) have been proposed to have 3–6 protofilaments per fibril with each

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[1] *Abbreviations used:* AD, Alzheimer's disease; Aβ, amyloid-β protein; HFIP, hexafluoroisopropanol; SEC, size exclusion chromatography; ThT, thioflavin T; TEM, transmission electron microscopy; TFA, trifluoracetic acid.

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protofilament containing 2 β-sheets [9,10]. This ultrastructural organization of Aβ fibrils likely creates several layers (or shells) with different environments.

Using the Tycko model of Aβ(1–40) fibril molecular structure [4] as a guide, recent work from our laboratory made individual tryptophan substitutions at phenylalanine residues 4 or 19 in the Aβ(1–40) sequence to understand the environmental changes surrounding these two residues during fibril formation [11]. Aβ(1–40) F19W underwent a marked blue-shift in fluorescence λ_{max} during fibril formation while Aβ(1–40) F4W did not, consistent with insertion of W19, but not W4, into the hydrophobic fibril inner core. The aforementioned structural model, particularly the cross sectional view of the fibril with minimal mass per length (Fig. 5a from [4]), has phenylalanines 19 and 20 oriented in opposite directions suggesting the two residues may experience significantly different environments after fibril formation. In order to further probe fibril molecular organization, the current study compared Aβ(1–40) F19W and Aβ(1–40) F20W in aggregation propensity, tryptophan fluorescence blue-shift, and accessibility to fluorescence quenchers.

Methods

Synthesis of Aβ peptides

Synthesis of Aβ(1–40) peptides was done using standard Fmoc chemistry and solid phase synthesis [12] on a 396 Omega automatic synthesizer (Advanced ChemTech, Louisville, KY). The starting scale was 25 μmol of Fmoc-Ala previously coupled on 2-ClTrt resin. Three equivalents of all protected amino acids were incorporated at every coupling and re-coupling was automatically performed at every cycle. The peptides were deprotected and cleaved from the resin in a single reaction by treatment with trifluoroacetic acid (TFA) and scavengers. Post-synthetic work up with diethyl ether gave a crude peptide product which was recovered and dried as a TFA salt. Analytical characterization was performed by LC–MS (Thermo Finnigan, San Jose, CA) using a C18 reverse phase column (Waters, Milford, MA) and multistep gradients of water and acetonitrile, each containing 0.1% TFA. The mass spectra confirmed the correct monoisotopic molecular weights of 4366 g/mol for Aβ(1–40) F19W and F20W.

Preparation and aggregation of Aβ peptides

Aβ(1–40) peptides were treated using a modification of a previously described protocol [13]. Briefly, the crude peptides from solid phase synthesis were dissolved in 100% TFA at a concentration of 1 mM by weight and bath-sonicated for 10 min. Solution aliquots were placed into multiple tubes, which were then dried in a vacuum centrifuge and reconstituted again with an equal volume of 100% hexafluoroisopropanol (HFIP) (Sigma–Aldrich, St. Louis). Following incubation for 1 h at 37 °C, the samples were again dried in a vacuum centrifuge and stored at −20 °C. When needed, the lyophilized peptides were reconstituted in 10 mM NH_4OH containing 6 M guanidinium hydrochloride (GuHCl). The solution was centrifuged at 18,000*g* for 10 min with a Beckman–Coulter Microfuge® 18 and monomeric peptides were isolated by size-exclusion chromatography (SEC).The centrifugation supernatant was eluted from a Superdex 75 HR 10/30 column (GE Healthcare) in 50 mM Tris–HCl pH 8.0 at 0.5 ml/min. Concentrations of monomeric Aβ obtained from SEC were determined by absorbance using an extinction coefficient of 6970 cm^{-1} M^{-1} at 280 nm for Aβ(1–40) F19W and F20W. For aggregation studies, solutions of SEC-purified Aβ peptides in 50 mM Tris–HCl pH 8.0 (30 μM) were prepared in siliconized tubes with 150 mM NaCl and 0.05% NaN_3 unless otherwise described. The tubes were agitated by gentle vortexing at 22 °C. After agitation each tube was briefly centrifuged on a table top model for 2 s and mixed thoroughly prior to analysis by fluorescence or microscopy. Aβ(1–40) F19W and F20W fibrils were isolated following aggregation by centrifugation at 18,000*g* for 10 min, removal of the supernatant, and resuspension in 50 mM Tris–HCl pH 8.0/150 mM NaCl/0.05% NaN_3.

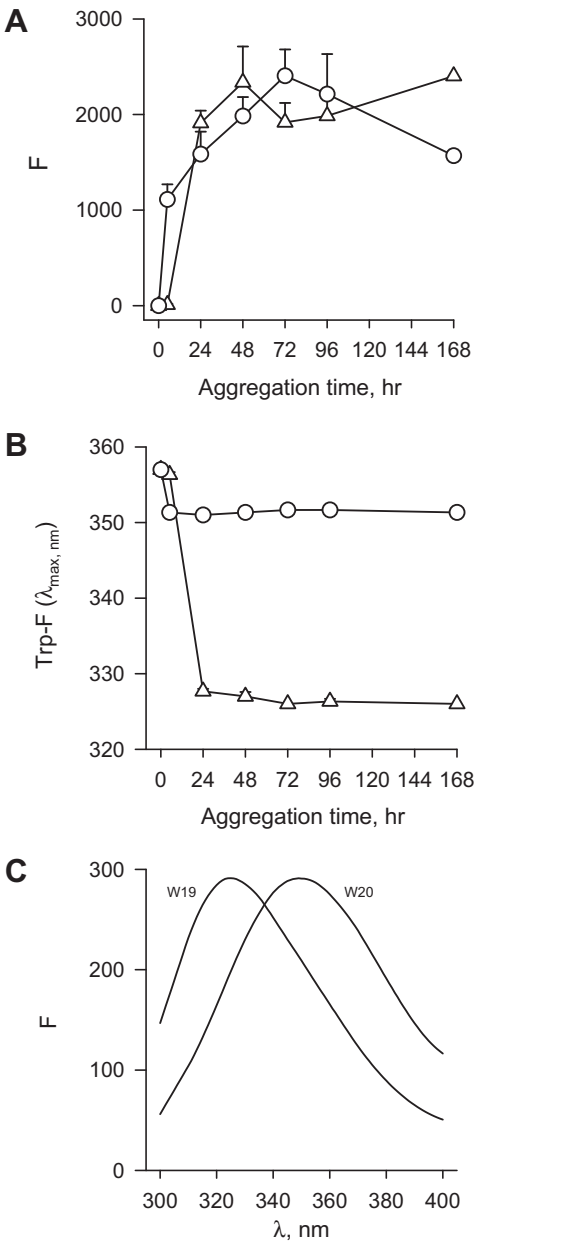

Fig. 1. Aggregation of tryptophan-substituted Aβ(1–40) peptides. Solutions of SEC-purified Aβ(1–40) F19W and Aβ(1–40) F20W (30 μM in 50 mM Tris–HCl pH 8.0/150 mM NaCl/0.05% NaN_3) were aliquoted into 3 tubes and gently agitated at 22 °C. (Panel A) ThT fluorescence was measured as described in the Methods for Aβ(1–40) F20W (circles) and Aβ(1–40) F19W (triangles). Data points in the graph for each Aβ solution are the average ± std error for $n = 3$ samples of each Aβ peptide. For the last time point the solutions for each peptide were combined and thus do not have a std error bar. At other points, the error bars are smaller than the symbols and are not visible. (Panel B) Tryptophan fluorescence λ_{max} values were obtained as described in the Methods at given time points from the Aβ solutions in Panel A. Std error bars for $n = 3$ samples each of Aβ(1–40) F20W (circles) and F19W (triangles) are presented but partially obscured by the symbols. (Panel C) Trp fluorescence scans of Aβ(1–40) F20W and F19W fibrils isolated as described in the Methods. The scans were smoothed in SigmaPlot and the overall fluorescence intensities were normalized to better indicate the λ_{max} values which were 325 and 349 nm for Aβ(1–40) F19W and F20W fibrils, respectively.

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