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Amyloid-forming propensity of the hydrophobic non-natural amino acid on the fibril-forming core peptide of human tau

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Abstract—Amino acid residues with aromatic side chains, such as Tyr and Phe, are known to play essential roles in forming and stabilizing the amyloid fibrils of pathogenic polypeptides by affecting their amyloid forming propensity. We have studied the amyloid-type aggregation of peptides containing non-natural amino acid derived from a core part of human pathogenic protein, tau. The hydrophobic nature of the biphenyl group and its intermolecular aromatic interactions strongly alter their amyloid formation properties.

Proteins that form the amyloidal aggregates, such as microtubule-associated protein tau and islet amyloid polypeptide (IAPP), contain short peptide segments that play critical roles in the fibril formation. It has been shown that certain defined sequences nucleate amyloid formation of proteins. Aromatic amino acid residues tyrosine (Tyr) and phenylalanine (Phe) in the short peptide segments have been shown to stabilize the amyloid fibrils of pathogenic polypeptides,[1] as has been demonstrated for tau,[2] IAPP,[3] and beta-peptide.[4] A short peptide segment VQIVYK (PHF6) corresponding to the core part of tau fibril formation is one of such sequences capable of forming paired-helical filaments.[5] The N-terminal hydrophobic and the C-terminal charged residues of the PHF6 peptide are important to the fibril formation. PHF6 derivative peptides substituted at the Tyr position by natural amino acid residues with aromatic or large hydrophobic side chains showed high amyloidogenic propensities. Mutants of the PHF6 peptide formed twisted filaments, flat and rolled sheets, or spherical or annular particles.[6] In order to get further insights into the role of aromatic side chains on the amyloid fibrils by short peptides, PHF6 derivative peptides, in which the Tyr position of PHF6 was substituted with hydrophobic non-natural amino acids, were synthesized and were tested for their fibril-forming property. The hydrophobic non-natural amino acids influence the fibril-forming property of a peptide derived from core part of tau fibril formation.

PHF6 derivatives substituted at the Tyr-310 residue of PHF6 were synthesized to represent a variety of aromatic side chains. Non-natural amino acids, 4-phenylphenylalanine (PHF6FPh), 4-fluorophenylalanine (PHF6FF), and 4-methylphenylalanine (PHF6FMe), were incorporated at the Tyr-310 position of PHF6 (Fig. 1).

In a highly aggregating solution condition (0.5 mM peptide, 20 mM MOPS, 0.3 M NaCl, pH 7.5), PHF6

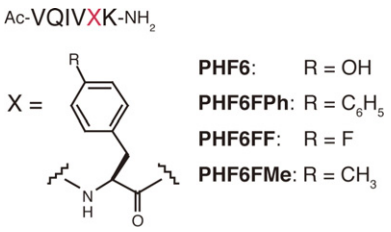

Figure 1. Structures of PHF6 peptide derivatives used in this study. Tyr at the position X corresponds to the Tyr-310 residue of native tau.

Keywords: Amyloid; Fibrils; Peptide; Non-natural amino acid; Aggregates; Tau; Pathogenic polypeptides.
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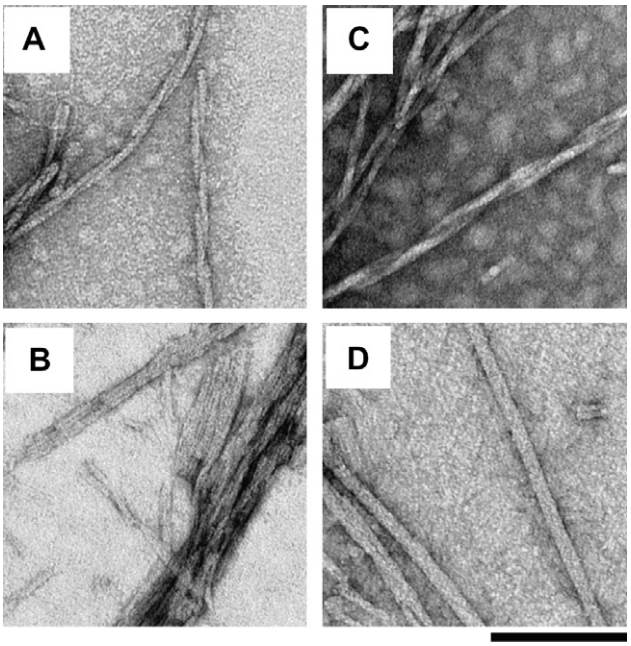

Figure 2. TEM images of amyloid-type fibers formed from (A) PHF6 and (B) 4-phenyl derivative PHF6FPh, (C) 4-fluoro derivative PHF6FF, (D) 4-methyl derivative PHF6FMe. Samples were negatively stained with 2% uranyl acetate. Scale bar: 100 nm.

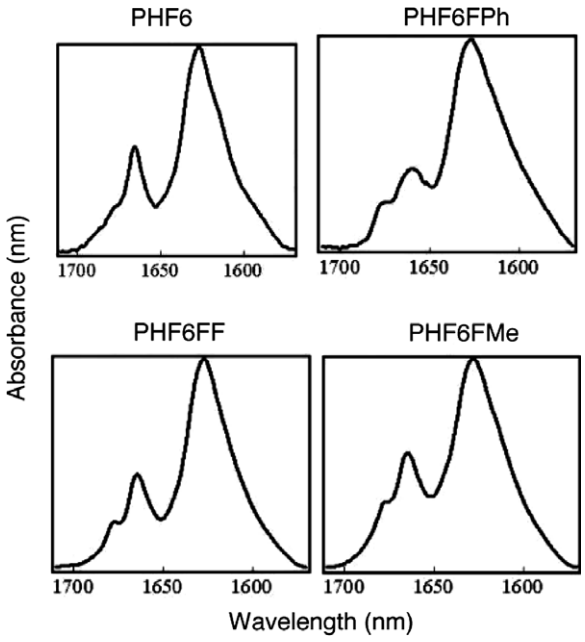

Figure 3. Bands characteristic of β-sheet secondary structure are shown at the amide I region of the IR spectra. The sample containing the peptide aggregates was prepared from 0.5 mM peptide solution of 20 mM MOPS, 0.3 M NaCl, pH 7.5, then centrifuged at 15,000 rpm for 10 min. After removal of the supernatant, the precipitated aggregates were washed with water two times. The water-rinsed aggregates were spread on a calcium fluoride plate and dried up. The measurement was performed with 2 cm^{-1} resolution and accumulation of 128 scans.

formed amyloid-type paired-helical fibers (Fig. 2A) as reported previously.[7] PHF6FPh formed tightly segregated mass of fibers (Fig. 2B). Fluorine substitution at the 4-position of phenyl ring (PHF6FF) caused formation of helical filaments with wider radius than that of PHF6 (Fig. 2C). Abundant straight filaments were observed when the 4-position of phenyl group was substituted with a methyl group in PHF6FMe (Fig. 2D). Thus even sharing the same aromatic phenyl group at the position Tyr-310, substitution at the phenyl ring with different groups caused formation of fibrils with different morphologies.

The amide I region of the IR spectra are shown for PHF6, PHF6FPh, PHF6FF, and PHF6FMe (Fig. 3). The aggregate of PHF6 exhibited strong maximum of absorbance around 1625 cm^{-1}, indicating a very high content of the β-sheet secondary structure as previously reported.[2] Each aggregate of PHF6FPh, PHF6FF, and PHF6FMe also exhibited the strong maximum of absorbance around 1630 cm^{-1}, indicating that the amyloid type fibers of PHF6FPh, PHF6FF, and PHF6FMe contained the β-sheet secondary structure as assessed by FT-IR.

Conformation of the PHF6 derivative peptides in solution was monitored by the CD spectra in 20 mM MOPS buffer (pH 7.5). Negative peaks at around 218 nm in the CD spectrum of PHF6 (Fig. 4A) indicated the tendency to form β-sheet secondary structures, as has been well documented for PHF6. The 4-methyl substituted derivative PHF6FMe also showed CD signals characteristic of the β-sheet secondary structure (Fig. 4B). PHF6FF showed the similar signal corresponding to the β-sheet formation (Fig. 4C). PHF6FPh showed quite high aggregation propensity. Because PHF6FPh was insoluble in the buffer, it was difficult to obtain reliable CD spectra of PHF6FPh in the solution. These data indicate that PHF6, PHF6FF, and PHF6FMe exist in the β-sheet secondary structure not only in the amyloid-type fibers, but also in the solution.

We next performed time-course analyses of the aggregates formation by the Tyr-310 mutants of PHF6 in 20 mM MOPS (pH 7.5) containing HFIP to assess the effect of substitution at the 4-position of phenyl group on the fibril formation. Peptide stock solutions were prepared by HFIP to avoid formation of oligomeric species of peptides in the initial stage of aggregation experiments (see Supporting information). Fibril formation of these peptides was monitored by thioflavin T (ThT) fluorescence[8] in the presence of 0.1 M NaCl. The time-course analyses of the aggregates formation revealed no induction time for PHF6FF, PHF6FMe, and PHF6FPh as shown in Figure 5. PHF6FPh revealed constant ThT fluorescence intensities immediately after the beginning of the aggregates formation experiment, indicating rapid formation of amyloid-type fibers by PHF6FPh. It is likely that the formation of fibrils by PHF6FPh reached a plateau right after the beginning of the aggregates-forming experiment. Because the maximum intensity of ThT fluorescence induced upon binding to peptide fibrils varies with the chemical nature of peptide, the observed smaller intensity of ThT fluorescence induced by PHF6FPh does not necessarily mean less efficient formation of PHF6FPh fibrils. Aggregates