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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

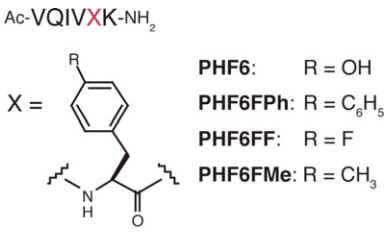

Figure 1. Structures of PHF6 peptide derivatives used in this study. Tyr at the position X corresponds to the Tyr-310 residue of native tau.

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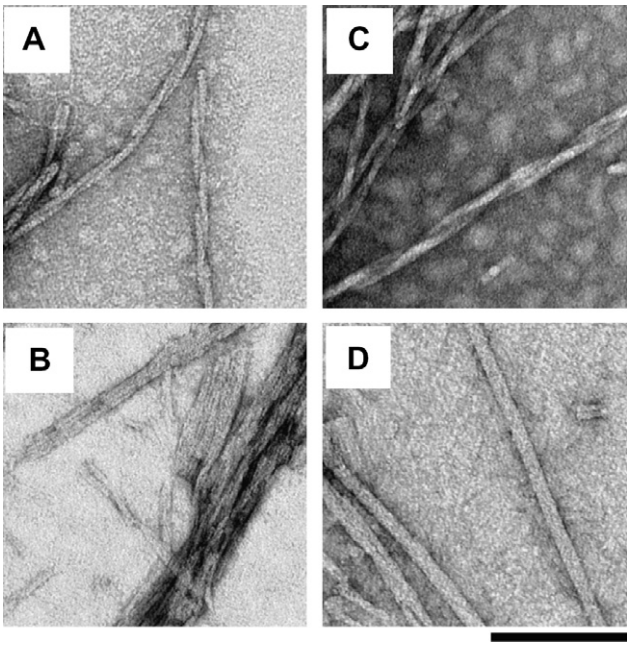

Figure 2. TEM images of amyloid-type fibers formed from (A) PHF6 and (B) 4-phenyl derivative PHF6FPh, (C) 4-fluoro derivative PHF6FF, (D) 4-methyl derivative PHF6FMe. Samples were negatively stained with 2% uranyl acetate. Scale bar: 100 nm.

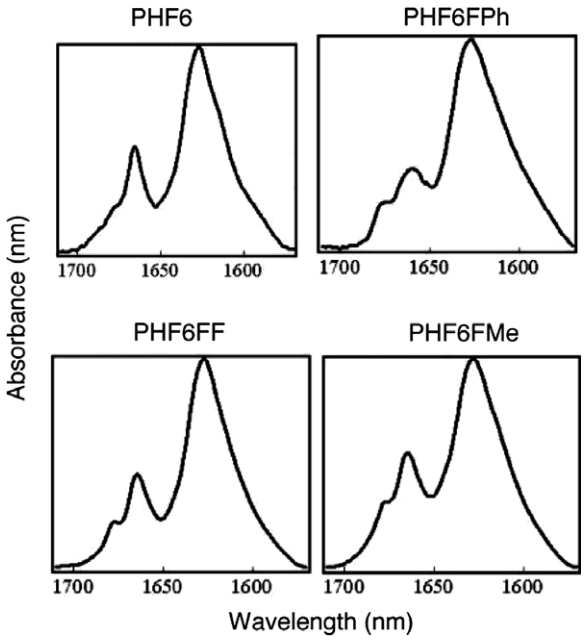

Figure 3. Bands characteristic of β-sheet secondary structure are shown at the amide I region of the IR spectra. The sample containing the peptide aggregates was prepared from 0.5 mM peptide solution of 20 mM MOPS, 0.3 M NaCl, pH 7.5, then centrifuged at 15,000 rpm for 10 min. After removal of the supernatant, 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

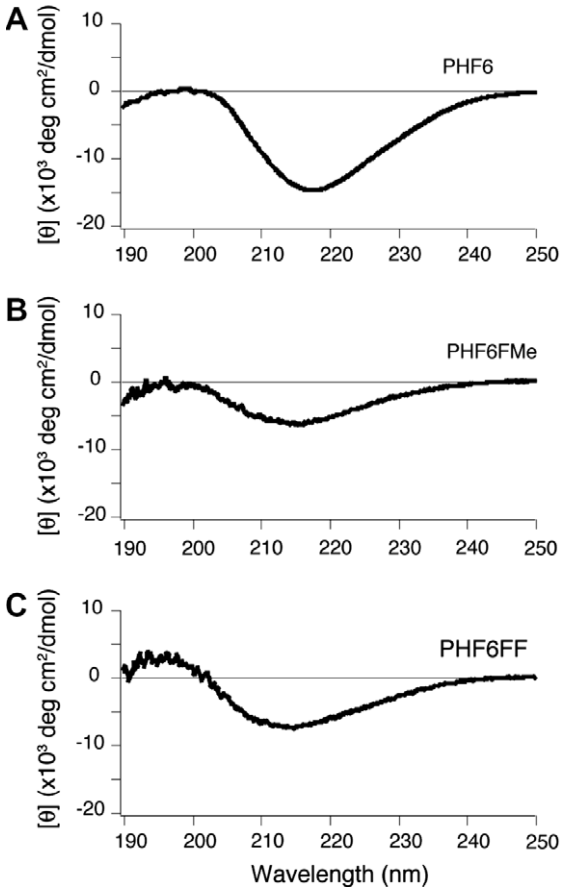

Figure 4. CD spectra of (A) PHF6, (B) PHF6FMe, and (C) PHF6FF in 20 mM MOPS buffer (pH 7.5).

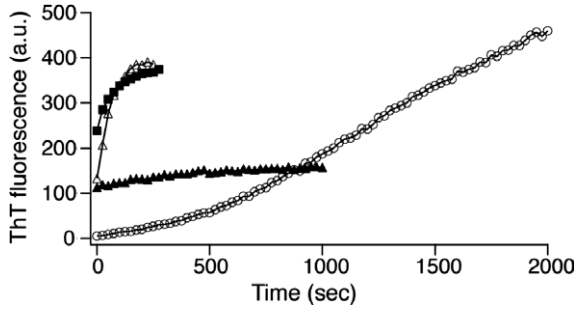

Figure 5. Time-course changes of the ThT fluorescence intensity of each peptide (0.2 mM) in 20 mM MOPS buffer (pH 7.5) in the presence of 0.1 M NaCl and 0.47 M HFIP (1,1,1,3,3,3-hexafluoro-2-propanol). PHF6 (open circles), PHF6FF (open triangles), PHF6FMe (filled squares), and PHF6FPh (filled triangles) dissolved in HFIP were polymerized by the addition of an excess amount of 20 mM MOPS buffer (pH 7.5) containing 10 μM ThT and 0.1 M NaCl.

formation kinetics for PHF6FMe and PHF6FF followed a single exponential approach to equilibrium. On the other hand, PHF6 aggregation kinetics showed an initial retardation of the fluorescence evolution followed by the faster change in the later phase, indicating that the fibril formation of PHF6 is a nucleation-dependent process, as is widely known for amyloid-type aggregations. PHF6FPh had the fastest aggregation kinetics, and PHF6FMe and PHF6FF had faster aggregation rates than PHF6. The hydrophobic group substituted at the 4-position generally accelerated the aggregation rate of PHF6 derivatives. Although the fluorescence intensities of ThT induced by PHF6F, PHF6FF, and PHF6FMe at equilibrium were higher than that of PHF6FPh, it was difficult to compare quantitatively the amount of fibril formation by different peptides from the fluorescence intensity of ThT.

The amount of aggregates formed by PHF6, PHF6FPh, PHF6FF, and PHF6FMe was compared in 20 mM MOPS containing 0.1 M NaCl and 1.0 M urea (pH 7.5). After centrifuging at 15,000 rpm for 10 min, concentration of each peptide in the supernatant was quantitated to estimate the fractions of peptide precipitated (Fig. 6A). Aggregates were formed in a buffered solution containing urea, which is a well-known agent that denatures the assembly of proteins and peptides. PHF6FPh showed remarkably low solubility even in the presence of urea. More than 90% of PHF6FPh existed in the precipitates. As indicated by the tightly segregated mass fibrils observed by TEM image (Fig. 2B) and rapid formation of amyloid type aggregates revealed by the ThT assay (Fig. 5), PHF6FPh possessed high amyloid aggregation propensity. PHF6FMe and PHF6FF precipitated more efficiently than PHF6. These results showed similar aggregation propensities observed in the kinetic behaviors for aggregates formation by the PHF6 derivative peptides (Fig. 5), namely, PHF6FPh was the most amyloidogenic peptide.

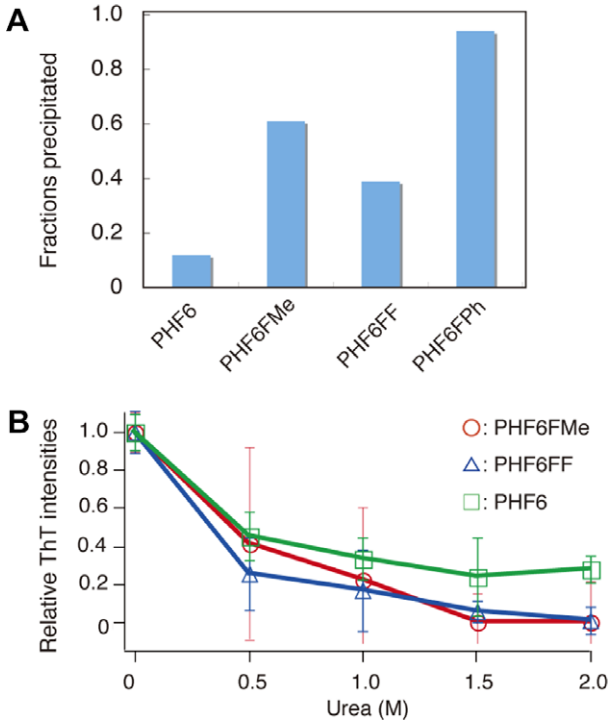

Figure 6. (A) Fractions of precipitated peptide indicated solubility of each peptide in the 20 mM MOPS buffer containing 0.1 M NaCl and 1.0 M urea (pH 7.5). (B) Relative ThT fluorescence intensity changes by increasing the urea concentrations (0–2 M) for the aggregates formed by PHF6FMe (circles in red), PHF6FF (triangles in blue) and PHF6 (squares in green) in 20 mM MOPS buffer (pH 7.5).

Stability of the aggregates of PHF6, PHF6FMe, and PHF6FF was quantitatively compared by monitoring the ThT fluorescence intensities in the presence of various concentrations of urea (Fig. 6B). The ThT fluorescence intensity with PHF6 was suppressed to 30% in the presence of 2 M urea, while the fluorescence intensities with PHF6FMe and PHF6FF were completely suppressed in the same condition. The results showed that the fibrils of PHF6 were more tolerant to the urea-induced denaturing condition than those of PHF6FMe or PHF6FF. It is likely that interactions between the ε-amino group of Lys residue and the phenolic hydroxyl group of Tyr residue stabilize the fibril of PHF6. The experiments above indicated that chemical nature of the aromatic residue in the fibril-forming core peptide of human tau strongly affected its amyloid-forming propensity. Order of the amyloidogenicity was: PHF6 < PHF6FMe = PHF6FF < PHF6FPh. The fibrils of PHF6 were more stable to the urea denaturation than those of PHF6Me or PHF6FF. Especially, we found that the phenyl group substituted at the 4-position of Tyr-310 position was a strong enhancer of the fibril assembly and promoted segregation of the amyloid fibers to form a tight mass of the aggregates from PHF6FPh (Fig. 2B).

All the findings above demonstrated that the aromatic and hydrophobic non-natural amino acids substituted at the Tyr residue of the fibril-forming core peptide of human tau play important roles in enhancing the amyloid-forming propensity of the peptide. PHF6FPh, PHF6FMe, and PHF6FF showed faster amyloid-forming kinetics than PHF6. It is likely that PHF6FPh accelerates association of peptides by interaction between the hydrophobic and aromatic side chains. Because the modification at the 4-position of phenyl group was accommodated in the fibrils of PHF6 derivatives, it would be possible to control the amyloid-forming propensity of short peptides by using non-natural amino acids.

Acknowledgments

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Supplementary data

Experimental details for the synthesis and spectroscopic measurements of peptides PHF6, PHF6FPh, PHF6FF, and PHF6FMe used in this study. This material is available free of charge via the Internet at http://www.elsevier.com. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.03.071.

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