V. Abstract

Alzheimer's disease is accompanied by a number of structural and metabolic alterations in the brain. Two characteristic hallmarks are the protein aggregates in amyloid plaques (made up mostly of Aβ peptide) and in the neurofibrillary tangles (consisting largely of the microtubule-associated protein tau).

Tau protein is a natively unfolded protein in the solution, yet it is able to polymerize into the ordered paired helical filaments (PHF) which bundled into neurofibrillary tangles. Recently it was shown that a small fraction of tau, the hexapeptide motif PHF6 (^{306}VQIVYK^{311}) in the third repeat is capable of inducing tau aggregation via formation of a β-structure (von Bergen et al., 2000). In the present study, we performed scanning mutagenesis (proline mutants and tryptophan mutants) to investigate the *in vitro* structural requirements for PHF polymerization of tau protein containing four repeats. We show that there is a cross-talk between the two hexapeptide motifs PHF6* (^{275}VQIINK^{280}) in the second repeat and PHF6 (^{306}VQIVYK^{311}) in the third repeat during PHF aggregation. A single proline mutant in the hexapeptide motif PHF6* or PHF6 inhibits PHF formation completely, however, the inhibition by the I308P mutant in PHF6 can be overcome by turning PHF6* into a strong assembly promoter via one of the FTDP-17 mutants, ΔK280 (in the presence of heparin).

Using tryptophan scanning mutagenesis we observed the kinetics and structure of tau's polymerization into PHFs independently of exogenous reporter dyes. The fluorescence exhibits pronounced blue-shifts due to burial of the residue inside PHFs, depending on Trp position. The effect is greatest near the center of the repeat domain, showing that the packing is tightest near the β-structure inducing hexapeptide motifs. The tryptophan response allows measurement of PHF stability made by different tau isoforms and mutants. Unexpectedly the stability of PHFs is quite low (denaturation half points ~1.0 M GuHCl), implying that incipient aggregation should be reversible, and that the observed high stability of Alzheimer PHFs is due to other factors. The stability increases with the number of repeats and with tau mutants promoting β-structure,

arguing for a gain of toxic function in frontotemporal dementias. Fluorescence resonance energy transfer (FRET) was used to analyze the distances of Tyr310 to tryptophans in different positions. The degree of FRET in the soluble protein was position dependent, with highest signals within the second and third repeats but low or no signals further away. In PHFs most mutants showed FRET, indicating that tight packing results from assembly of tau into PHFs.