Structural transitions in tau k18 on micelle binding suggest a hierarchy in the efficacy of individual microtubule-binding repeats in filament nucleation

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Abstract: The protein tau is found in an aggregated filamentous state in the intraneuronal paired helical filament deposits characteristic of Alzheimer's disease and other related dementias and mutations in tau protein and mRNA cause frontotemporal dementia. Tau isoforms include a microtubule-binding domain containing either three or four imperfect tandem microtubule binding repeats that also form the core of tau filaments and contain hexapeptide motifs that are critical for tau aggregation. The tau microtubule-binding domain can also engage in direct interactions with detergents, fatty acids, or membranes, which can greatly facilitate tau aggregation and may also mediate some tau functions. Here, we show that the alternatively spliced second microtubule-binding repeat exhibits significantly different structural characteristics compared with the other three repeats in the context of the intact repeat domain. Most notably, the PHF6* hexapeptide motif located at the N-terminus of repeat 2 has a lower propensity to form strand-like structure than the corresponding PHF6 motif in repeat 3, and unlike PHF6 converts to partially helical structure in the micelle-bound state. Interestingly, the behavior of the Module-B motif, located at the beginning of repeat 4, resembles that of PHF6* rather than PHF6. Our observations, combined with previous results showing that PHF6* and Module-B are both less effective than PHF6 in nucleating tau aggregation, suggest a hierarchy in the efficacy of these motifs in nucleating tau aggregation that originates in differences in their intrinsic propensities for extended strand-like structure and the resistance of these propensities to changes in tau's environment.

Keywords: tau; Alzheimer's; amyloid; microtubule-binding repeat; microtubule-binding domain; protein aggregation; paired helical filaments; PHF6; PHF6*

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Introduction

Tau is a microtubule-associated protein (MAP) that functions in microtubule nucleation, assembly, and stabilization and is found principally in axons of the central nervous systems, where it exists in six different isoforms.^{1–4} Tau contains an N-terminal projection domain, two proline-rich regions, and a C-terminal domain that includes three (3R) or four (4R) imperfect 31- or 32-residue repeats (Fig. 1). The proline-rich regions and the repeat domain constitute the microtubule-binding domain (MBD) of tau but only the repeat domain presents the ability to

Figure 1. Schematic representation of the primary sequence of tau protein, indicating the alternatively spliced exons (2, 3, and 10), the two proline-rich domains (P1 and P2) the microtubule-binding repeats (R1-R4) and the pseudorepeat R'. The amino acid sequence of the tau K18 construct used in these studies is shown, with each repeat on a separate line for ease of comparison. The K19 construct is identical to K18 except that R2 is excised. The PHF6* (in R2) and PHF6 (in R3) hexapeptide motifs are shown in boldface and underlined.

both bind microtubules and to promote their assembly.⁵

Neurofibrillary tangle (NFT) deposits containing paired helical filaments (PHFs) and/or other forms of aggregated tau are found in a series of related neurodegenerative disorders and mutations in tau are responsible for frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17). To date, over 35 tau mutations have been reported, with most of the mutations occurring within or near the repeat region (see^{6,7} for reviews). Tau mutations lead either to alternative splicing of tau mRNA and consequently abnormal 3R/4R ratios (silent or intronic mutations), or alterations in tau fibrillogenicity, microtubule-binding affinity or phosphorylation (missense mutations).

Despite early controversy⁸ a consensus has emerged that the core of the PHFs adopts the cross- β conformation common to other amyloidogenic proteins.^{9,10} Tau fibrillization *in vitro* requires anionic cofactors such as heparin,^{11,12} RNAs,¹³ detergent micelles,^{14,15} fatty acids,^{16,17} polyglutamate,¹⁸ and cytomembranes.¹⁹ *In vivo*, tau in NFTs is found to be hyperphosphorylated, possibly because tau phosphorylation appears to increase the pool of cytoplasmic tau available for polymerization by detaching the protein from the microtubules.

Although the intrinsically disordered nature of tau^{2,20,21} has prevented the characterization of any unique tau structure, NMR methods have been successfully applied to a number of different tau constructs. The structural properties of each isolated MBD repeat have been studied by ¹H NMR,²²⁻²⁴ while we and others reported on the conformation of the intact repeat domain of 3R²⁵ or 4R²⁶ tau. In

parallel, partial,^{21,27} and then nearly complete²⁸ NMR backbone resonance assignment of full-length tau was achieved. Most recently, reduced dimensionality NMR experiments have been shown to greatly facilitate resonance assignment of full-length tau isoforms.^{29,30} Although interesting structural tendencies, both local and long-range, were identified in these studies, it is clear that tau is very highly disordered and dynamic when isolated in solution.

In light of tau's association with cytomembranes both in the normal^{31,32} and the diseased brain,^{19,33} and reports of direct interactions between the tau MBD and membranes *in vitro*,^{34,35} we also examined how interactions of the 3R tau K19 fragment with detergent and lipid micelles and membranes influence its structure.³⁶ We observed that each of the three repeats in tau K19 contains an 8-9 residue region that folds into an ordered helical structure. In addition, the PHF6 hexapeptide motif located in repeat 3 and identified as a critical nucleator of tau aggregation³⁷ retains a preference for extended structure, similar to its behavior in the free state.²⁵

Here, we report on how the structural properties of a 4R tau fragment, K18, change in going from a free state to a micelle-bound state. The behavior of repeats 1, 3, and 4 in K18 is essentially identical to that previously observed in the context of the K19 fragment. For repeat 2, which does not occur in K19, we observe the formation of an 8 residue helix in the region corresponding to the micelle-induced helices previously observed in the other three repeats. Surprisingly, however, the N-terminal region of repeat 2, containing the PHF6* hexapeptide motif identified as a secondary PHF nucleation site,³⁸ becomes helical on micelle binding. Our

Figure 2. Tau K18 gains helical structures on binding to SDS micelles and PAPC lipid vesicles. The far UV CD spectrum of the free state has a lineshape characteristic of disordered peptides with a minimum at 198 nm. When bound to SDS, the lineshape corresponds to a mixture of disordered and helical secondary structure with a shoulder at 222 nm and a deeper minimum at around 205 nm. In the presence of PAPC lipid vesicles, the spectrum shifts slightly from that of the micelle-bound state toward the free state spectrum, likely reflecting the presence of a small amount of unbound protein.

results suggest a structural underpinning for a hierarchy in the potency of the different tau repeats in nucleating PHF formation for tau both free in solution and bound to membranes or other negatively charged surfaces.

Results

Circular dichroism (CD) shows tau K18 forms helical structure on binding to micelles and vesicles

CD spectra show that similarly to tau K19,³⁶ tau K18 is disordered when free in solution, as evidenced by a deep minimum at 198 nm, but acquires helical structure in presence of SDS micelles or PAPC vesicles, as indicated by minima at 222 nm and near 208 nm (Fig. 2). The helical content of K18 C322A appears somewhat lower in presence of PAPC SUVs than in presence of micelles, yielding a less pronounced signal at 222 nm and a shift of the second minimum toward lower wavelength, likely resulting from an equilibrium between vesicle bound K18 and K18 free in solution. The fractional helix content of the micelle-bound polypeptide can be estimated from the value of θ_{222} ^{39,40} to be about ~17%.

NMR proton-nitrogen correlation spectra confirm structural change on micelle binding by tau K18

Evidence for structural changes induced in K18 on micelle binding are also indicated by an increased resonance dispersion in two-dimensional NMR

proton-nitrogen correlation (HSQC) spectra (Fig. 3). The amide proton dispersion is increased from ~0.8 ppm in the free state [Fig. 3(a)] to ~1.4 ppm in the bound state [Fig. 3(b)], similarly to previous observations with K19.³⁶ This result is consistent with the modest gain in helical structure that is indicated by the CD spectra. We note that the CD data suggest that micelle-binding results in structural changes similar to those that K18 undergoes on binding to lipid bilayers, and that previous detailed NMR studies of K19 showed that detergent micelles result in structural changes essentially identical to those induced by lysophospholipids with physiologically relevant headgroups.³⁶ Nevertheless there are clearly differences between detergent micelles and biological membranes, including their small size and highly curved surfaces, which could influence their mode of interaction with proteins.

NMR chemical shifts, RDCs, and relaxation parameters reveal both differences and similarities between R2 and other repeats in free tau K18

Chemical shifts. We obtained de novo NMR backbone resonance assignments for nearly all of the backbone carbon and C β NMR resonances of tau K18 in both the free and micelle-bound states (deposited in BioMagResBank under accession number 19253). Figure 4 shows the deviations of the C α , and CO chemical shift from the values expected for a random coil⁴¹ in both the free and the micelle-bound states. These deviations, often termed secondary chemical shifts, are empirically related to the polypeptide backbone torsion angles ϕ and ψ .^{42,43} For the sake of comparison with our earlier studies of K19,^{25,36} we used the same random coil chemical shifts as in that work. An analysis employing more recently reported sequence corrected random coil shifts is shown and discussed in detail in the Supporting Information and Supporting Information Figure S1, and the results support the analysis as originally performed, although the secondary shifts are somewhat smaller in amplitude.

For repeats 1, 3, and 4, the chemical shift deviations in the free state (Fig. 4, panels a and b) are nearly identical to those observed for K19, both confirming that the presence or absence of the R2 repeat has little or no influence on the structural preferences of the other repeats, and further validating the accuracy and reproducibility of the chemical shift measurements. Repeat 2 shows a region of weak positive C α shift deviations for residues L284-V297, corresponding to equivalent regions in the other three repeats. Surprisingly, the C α shift deviations of the N-terminal nine residues of repeat 2, which contain the PHF6* hexapeptide (V275-K280) are slightly positive. This contrasts with the

Figure 4. Residual secondary structure in the free state of tau K18 is altered on micelle-binding. NMR secondary chemical shifts are shown for tau K18 C α (a) and CO (b) nuclei in the free state and C α (c) and CO (d) nuclei in the micelle-bound state. In (a) small but contiguous positive C α deviations for residues 284-297 in the free state of R2 are indicated by a horizontal black bar. The first 9 residues of R2 (275-283), exhibiting smaller but mostly positive deviations, are indicated by a horizontal red bar. In (b) the first 8 residues of R2, exhibiting negative CO deviations, are also indicated by a horizontal red bar. In (c) and (d), residues 284-291 of R2 become highly helical, exhibiting large positive C α and CO deviations, indicated by horizontal black bars. Residues 275-283 also exhibit increased (positive) C α deviations. Microtubule binding repeats are delineated by dashed orange lines. Corresponding data from our previous studies of K19 are shown in black lines. In (c) the free state data of panel (a) is also shown as a green line for purposes of comparison.

negative values for the PHF6 region in repeat 3. Generally, the behavior of the N-terminal region of repeat 2 is more similar to that of repeat 4, which contains the previously identified Module-B motif⁴⁴ than to the PHF6 region of repeat 3.

Residual dipolar couplings. We obtained RDCs for K18 aligned using alkyl-polyethylene glycol bicelles (Fig. 5). As previously noted,⁴⁵ the region in repeat 3 that includes the PHF6 hexapeptide (V306-D314) presents the highest amplitude negative NH

Figure 5. Extended structure propensity is strongest in PHF6. NMR residual dipolar couplings (RDCs) for bicelle-aligned K18 show strong negative RDCs at the N-terminal region of each repeat, including a proline-rich region at the beginning of R1, the PHF6* containing region of R2, the PHF6 region of R3 and the Module-B region of R4. The highest amplitude RDCs are in the PHF6 region. Subsequent to each region of negative RDCs is a region of smaller amplitude mixed positive and negative signals. Microtubule binding repeats are delineated by dashed orange lines and the C α secondary shifts from Figure 4, scaled arbitrarily for ease of comparison, are shown for reference.

Figure 6. Regions that become helical on micelle-binding also exhibit decreased fast timescale backbone motions. NMR relaxation parameters R_1 , R_2 and the heteronuclear NOE for the free (a) and micelle-bound (b) states of K18 show that regions that become highly helical on micelle-binding, such as residues 284–291, also experience an increase in the R_2 and heteronuclear NOE values. Microtubule binding repeats are delineated by dashed orange lines and the C_α secondary shifts from Figure 4, scaled arbitrarily for ease of comparison, are shown for reference.

RDCs, indicating the strongest β -strand propensity in tau K18. The N-terminal regions of repeats 1, 2, and 4 also exhibit notable negative RDCs, including a proline-rich region in repeat 1 (Q244–D252), the PHF6* region of repeat 2 (G273–D283), and the Module-B region (V337–K343) of repeat 4. Between these regions we observe RDCs of a markedly decreased amplitude, including a number of positive RDCs for residues that fall in regions where positive C_α secondary chemical suggest residual helical structure, although we do not see contiguous positive RDCs for the latter regions. The difference

between the RDCs we report here and those previously reported⁴⁵ may be due to the different alignment media used in the two studies (bicelles vs. phage), but as we observe more robust indications for helical structure in both RDC and secondary shift data, the somewhat different conditions used in the two studies may also contribute to the discrepancies.

Backbone dynamics. ^{15}N relaxation rates R_1 and R_2 , and steady-state heteronuclear NOE measurements, reflecting ps-to-ns timescale motions occurring in the free state of tau K18 [Fig. 6(a)] are

Figure 7. PHF6* becomes compact or helical in micelle-bound state, in contrast with PHF6. HN-HN NOEs in micelle-bound K18 are strong in regions that become helical on micelle-binding, as for example at residues 284–291 in R2 (horizontal black bar). Regions subsequent to the helices also exhibit strong NOEs, suggesting compact structures. PHF6 at the start of R3 shows relatively weak NOEs, consistent with a more extended conformational ensemble. In contrast, the region containing PHF6* at the beginning of R2 shows some of the strongest NOEs, suggesting, when combined with chemical shift deviations, that this region populates helical conformations in the micelle-bound state. Microtubule binding repeats are delineated by dashed orange lines and the $C\alpha$ secondary shifts from Figure 4, scaled arbitrarily for ease of comparison, are shown for reference. NOEs are shown as the average of the forward and reverse NOEs between residues i and $i+1$, with the standard deviation shown as an error bar.

largely consistent with a previous study,⁴⁶ except for the NOE data which were measured at different field strength and were surprisingly featureless when previously reported. As expected, the N- and C-terminal regions are more flexible with decreased values of R_1 , R_2 , and steady-state NOEs. The PHF6 region shows a peak in the R2 data that is also suggested in the heteronuclear NOE data and indicates that this region is the most motionally restricted part of the molecule on fast time scales. Data for the equivalent PHF6* and Module-B regions suggest somewhat less rigidity, more similar to that observed in the immediately subsequent regions where positive secondary $C\alpha$ shifts indicate transient helicity.

NMR chemical shifts, NOEs, and relaxation parameters show that PHF6* is less resistant to helix formation than PHF6 on K18 binding to micelles

Chemical shifts. Many, though not all, intrinsically disordered proteins fold into well-defined structures on binding to their natural partners.⁴⁷ We previously showed that the 3R tau K19 fragment gains significant helical structure on binding to lipid or detergent micelles or vesicles³⁶ located where transient helical tendencies are observed in the free state. Repeats 1, 3, and 4 of K18 behave in the same fashion when the protein is bound to detergent micelles (Fig.

4, panels c and d). As for these other repeats, repeat 2 exhibits an 8-residue segment with contiguous large (mostly > 2 PPM) positive $C\alpha$ deviations (L284–C291) with corresponding continuous positive CO deviations and mostly negative $C\beta$ deviations, indicating well-formed α -helical structure. Interestingly, the N-terminal segment of repeat-2, V275–K280, corresponding to PHF6*, presents intermediate amplitude positive $C\alpha$ shift deviations of 1–2 ppm, suggesting significant, if not fully formed, helical structure. Three out of six CO (and four out of six $C\beta$) shifts are also positive (negative) in this region, supporting the presence of some helical character. This is in contrast to the PHF6 sequence, which features several negative $C\alpha$ secondary shifts and strong negative CO and positive $C\beta$ deviations, indicating that it maintains its preference for extended structures. The N-terminal proline-rich region of the K18 construct (P247–D252), also maintains its preference for extended structures in the bound state, whereas the Module-B region of repeat 4 exhibits an intermediate behavior with positive $C\alpha$ secondary shifts of lower amplitude and mostly small negative CO and positive $C\beta$ deviations.

HN-HN NOEs. Amide proton NOEs were measured for K18 in the SDS-micelle-bound state (Fig. 7) as these NOE intensities are stronger in helical or compact structures and weaker in more extended

β -strand-like structures. As expected, we observe strong NOE intensities in the K19 helices previously identified based on chemical shifts in repeats 1, 3, and 4, as well as in the corresponding helix now observed in repeat 2 as well. Also as previously noted, the regions immediately following these helices also present strong NOE intensities (in some cases even stronger than within the helical regions) indicating that these regions likely form compact conformations. The data in the PHF6 region are incomplete but are consistent with a more extended conformation, with weaker NOE intensities. On the other hand, the region in repeat 2 containing PHF6* gives rise to very high NOE intensities, suggesting, when coupled with the chemical shift data described above, that this region favors helical conformations, and clearly does not favor extended conformations in the micelle-bound state.

Backbone dynamics. Measurements of backbone dynamics in the micelle-bound state of K18 [Fig. 6(b)] show that R_2 and NOE values increase considerably within each helical segment, including for residues 284-291 in repeat 2, when compared with the free state ($R_2 \sim 15 \text{ s}^{-1}$ and $\text{NOE} > 0.6$), consistent with a gain in structure as well as an increase in overall tumbling time of the molecule on micelle-binding. Similar behavior was observed for repeats 1, 3, and 4 in K19.²⁵ Interestingly, in repeat 2, the 10 N-terminal residues, preceding the helical region and containing PHF6*, exhibit R_2 s as high as those in the subsequent helix, and similarly high heteronuclear NOEs. This again contrasts with the PHF6 region and the corresponding Module-B peptide in the fourth repeat, which have distinctly lower R_2 and NOE values than the subsequent helical regions.

Discussion

Interest in the interactions of tau with membranes is based both on growing evidence that such interactions may feature in aspects of tau's normal function and on well established observations that such interactions are able to influence tau's abnormal aggregation. Tau localizes to membranes in certain contexts *in vivo*, and this interaction appears to play a role in modulating the growth of neuronal processes.³¹ Originally reported to be mediated by the N-terminal projection domain of the protein, more recent data suggest that the C-terminal region of tau is also implicated in membrane localization, with both possibly mediated by interactions with membrane-associated proteins such as Fyn or annexin A2.^{32,48,49} *In vivo* membrane interactions have also been shown to depend on the phosphorylation state of tau.⁵⁰⁻⁵² *In vitro*, tau has been shown to bind directly to phospholipids through its repeat domain.^{34,35} Although the functional role of direct tau-membrane interactions remains unclear, it is

well documented that tau interaction with fatty acids^{16,17} and lipid membranes^{14,53-55} can trigger its aggregation *in vitro*, and possibly also *in vivo*.^{19,33} Interestingly, it was recently demonstrated that anchoring the tau repeat domain at the plasma membrane seeds PHF formation by full length tau.⁵⁶

Earlier work by our group demonstrated that on binding of the K19 fragment of tau to lipid or detergent micelles, three short amphipathic helices are formed in regions exhibiting transient helical structure in the free state of K19. Here, we compare the structural changes of the K18 tau construct and in particular of the second microtubule-binding repeat, R2, on binding to detergent micelles. The presence of R2 has little or no influence on the conformational preferences of the other three repeats (R1, R3, and R4) in the free or bound state of the polypeptide, as the data in those repeats are nearly identical to those observed for K19,^{25,36} where R2 is absent. R2 itself behaves like the other three repeats in some respects, in that it contains a region (L284-V306) where secondary chemical shifts indicate a preference for transient helical structure in the free state, a segment of which forms a short highly-populated 8-residue helix (284-291) in the micelle-bound state. Surprisingly, however, the behavior of the N-terminal region of R2, which contains the PHF6* sequence, is quite different from that observed for the corresponding PHF6-containing region in R3. PHF6 shows a consistent propensity for extended strand structure in the free state, based on three different chemical shifts as well as RDCs, and this tendency is largely maintained in the micelle-bound state.³⁶ In contrast, for the PHF6*-containing region, secondary chemical shifts indicate mixed secondary structure preferences, implying a significantly weaker tendency toward β structure in the free state, whereas in the micelle-bound state this region appears to actually populate significantly helical conformations, based on both chemical shifts and NOEs. PHF6 exhibits restricted fast time scale backbone dynamics in the free state, which may play a role in facilitating inter-molecular beta-sheet formation. This is also true for PHF6*, as well as the Module-B region, but to a lesser extent than for PHF6.

Notably, subsequent to the original description of these two hexapeptide motifs, a study comparing their respective efficacy in nucleating tau PHF formation clearly indicated that PHF6 is a much more potent nucleator than PHF6*,⁵⁷ consistent with the stronger β -strand propensity we observe for PHF6 in the free state, and the ability of PHF6, but not PHF6* to maintain this propensity in the micelle-bound state. Interestingly, the PHF6 hexapeptide has been crystallized in an amyloid fibril conformation and its structure solved at high resolution by X-ray crystallography,⁵⁸ whereas no such structure of the PHF6* hexapeptide has been obtained or

reported, again consistent with the different secondary structure propensities we observe. The Module-B region of tau, contained in the N-terminal region of repeat 4, has also been identified as an important modulator of tau filament morphology, but unlike mutations in the PHF6 region, mutations in Module-B do not significantly decrease tau filament formation.⁴⁴ Our data and those of others indicate that this region also exhibits some propensity for extended structures, but as for PHF6*, we observe that this propensity is weaker in the free state, and is largely eliminated on micelle binding. Thus, it appears that while the N-terminal regions of each of repeats 2, 3, and 4 may play a role in tau filament formation, there is a hierarchy of efficacy amongst them that corresponds to their secondary structure propensities, and the robustness of these propensities in different environments. Although the preference for extended structures in each of these regions has been reported by several groups,^{25,26,59} this hierarchy amongst them has not been previously noted. Finally, we note the N-terminal region of repeat 1 also exhibits a preference for extended structures (Fig. 4), but this is very likely due to the presence of several proline residues in this region, the presence of which make it highly unlikely that this region would participate in nucleating beta-sheet formation, as has been noted before.²⁶

The helical regions we observed in the bound state of K18 correspond to some extent with TFE-induced structures previously identified in the isolated individual repeats,^{22–24} supporting the idea that the repeats behave largely independently of one another. In particular, well-formed helices were identified in each isolated repeat that included positions 10–18 within the repeat, corresponding to the helices that we observe to form on micelle binding within each repeat. However, in TFE, these helices extended further in the C-terminal direction (until position 24 in repeats 1 and 2 and until position 20 in repeats 3 and 4), likely reflecting the strong helix-inducing capability of TFE, which can apparently bridge the glycine-induced break observed at the end of each helix of the micelle-bound state. Interestingly, for repeats 2 and 4, the helical structure also extended N-terminally to repeat position 10, beginning at position 3 for R2 (corresponding to Ile 277) and 5 for R4 (corresponding to Ser 311), consistent with the strong (R2) or moderate (R4) helical propensity that we observe for the PHF6* and Module-B regions. The N-terminal extension of helical structure in R2 and R4 is not observed in R1 and R3 in either the micelle-bound state or in TFE, and this may be ascribed at least in part to the presence of helix-disrupting proline residues at repeat positions 8 and 7, respectively, which are absent in R2 and R4. Although the N-terminal region of R1 contains several other prolines and is not involved in

nucleating tau aggregation, the PHF6 motif of R3 is separated from the subsequent helical region by a proline, which appears to play an important role in preserving the preference of this motif for extended structure in different environments, including the TFE and micelle-bound states. Notably, substituting the R3 proline at repeat position 7 with a lysine leads to a significant increase of helicity in TFE, and to a dramatic decrease in TFE-induced R3 aggregation.⁶⁰ Replacing the corresponding lysine in R2 with a proline did not lead to enhanced aggregation of R2, supporting our conclusion that the PHF6* region has a lower intrinsic efficacy of nucleating aggregation, even when isolated from the subsequent helical region.

The implied hierarchy in nucleation efficacy of different tau microbule-binding domain motifs likely influences a variety of tau aggregation pathways, including that of the free protein, of the protein in organic solvents such as TFE, and perhaps most interestingly of the micelle-bound, and by implication, of the membrane-bound protein. At present, the context for the initiation of tau aggregation *in vivo* is not known, but the ubiquity of lipid membranes in nearly all cell types, combined with the documented ability of tau to interact with such membranes, and of membranes in turn to enhance tau aggregation, make the membrane surface a potentially important initiation site. Interestingly, in biopsies of AD brains, early non-fibrillar truncated tau aggregates are associated to membranous organelles³³ and early reports indicate that PHFs may originate from cytomembranes.¹⁹ The ability of PHF6, but not PHF6* or the Module-B motif, to remain extended in the micelle-bound state suggests that PHF6 is likely critical for membrane-induced aggregation. At the same time, the robust helical structure induced in R3, as well as in the other repeats, on detergent or lipid binding, may also play a role in membrane-driven aggregation. Several studies^{61–63} have suggested that tau aggregation triggered by anionic surfaces may involve a helical intermediate. More generally, models in which intermolecular interactions driven by amphipathic helices lead to an increased local concentration of highly amyloidogenic regions have been proposed for the aggregation of other amyloid proteins.^{64,65}

Methods

Recombinant proteins were expressed in *E. coli* cells transformed with a plasmid containing the construct for the K18 or the K19 fragments of tau under the control of the T7 promoter. On the basis of numbering for the longest tau isoform, the K18 sequence spans the residues 244–372 and corresponds to the repeat domain of the 4R-tau isoforms, whereas the K19 construct corresponds to the repeat domain of the 3R-tau isoforms and spans the residues 244–274

and 306–372.⁶⁶ To eliminate any intramolecular disulfide bond formation, Cys 322 in K18 was substituted with alanine using a Stratagene mutagenesis kit. All work was performed with the C322A variant of K18. We previously showed that the C322A mutation has no structural consequences for K19.²⁵ Purification of the recombinant proteins was performed as described.²⁵

NMR samples contained 400–800 μ M of protein, 0 or 100 mM ^2H -SDS, 100 mM NaCl, 10 mM Na_2HPO_4 , 4 mM DTT, in 10/90% $^2\text{H}_2\text{O}/\text{H}_2\text{O}$ at pH 7.4. NMR spectra were acquired at 10°C for the free protein and 30°C for the protein bound to SDS micelles using Bruker DRX700 and DRX800 spectrometers (New York Structural Biology Center). Experiments run to assign resonances included HNCA, HNCACB, CBCA(CO)NH, HNCO, HN(CA)CO, and HN(CA)NNH. HSQC-NOESY-HSQC was run with mixing times of 250 ms (note the use of deuterated micelles helps to limit spin diffusion). Modern versions of all experiments were used to incorporate sensitivity enhancement and gradient coherence selection. Spectral widths were 7000–8000 Hz in the direct dimension, 1500–1700 Hz in the ^{15}N -amide dimension, 9900–10600 Hz in the C_α/C_β dimension, and 1200–1800 Hz in the carbonyl dimension.

^{15}N relaxation data was measured at a tau K18 concentration of 500 μ M. Pseudo 3D experiments were used to determine R_1 and R_2 relaxation rates. For R_1 , the relaxation delays were 20, 50, 200, 100, 800, 600, 500, 400, 700, 300, 900, 1000, 1200, 200, 200, 700, and 700 ms. For R_2 , the delays were 16.3, 65, 82, 147, 114, 49, 33, 131, 98, 16.3, 16.3, 82, and 82 ms. Several delay times were run in triplicate in the R_1 and R_2 measurements to evaluate the experimental error. Four pairs of steady-state NOE spectra were recorded in an interleaved fashion. In each pair of spectra, one spectrum was recorded with saturation during the relaxation delay and the other spectrum was recorded without. The experimental error was evaluated from the deviation from the average of the four data sets.

^1H - ^{15}N residual dipolar couplings (RDCs) were measured by running IPAP-HNCO experiments on a Bruker DRX900 spectrometer (New York Structural Biology Center). Partial peptide alignment was achieved in solution containing 7.4% (w/v) bicelles prepared by mixing pentaethylene glycol monooctyl ether and 1-octanol in the same buffer and at the same pH used for other measurements.

The NMR data was processed with NMRPipe⁶⁷ and analyzed with NMRView.⁶⁸ Data were extensively zero filled in the carbon indirect dimension to ensure sufficient digital resolution. Secondary chemical shifts were calculated first using the random coil values from Wishart *et al.* with the necessary corrections for the residues preceding a proline⁴¹ for consistency with previous work on tau K19.

Secondary shifts were also calculated using more recent sequence-corrected random coil values,⁶⁹ as described in the Supporting Information.

Circular dichroism (CD) spectra were acquired using an AVIV 410 spectrometer. The temperature was set to 10°C for the free protein and 30°C for the protein bound to SDS micelles or PAPC small unilamellar vesicles (SUVs), in agreement with our NMR conditions. The protein concentration was $\sim 100 \mu\text{M}$ for all samples. The cell path length was 0.2 mm. PAPC SUVs were prepared as described.⁷⁰

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