

Tau Aggregation

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Abstract—Here we revisit tau protein aggregation at primary, secondary, tertiary and quaternary structures. In addition, the presence of non-aggregated tau protein, which has been recently discovered, is also commented on.

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Key words: tau, microtubule-binding repeats, tau conformations, w-tau isoform.

INTRODUCTION

In 1906, Alois Alzheimer described a new disease characterized by the presence of two aberrant brain structures, namely senile plaques and neurofibrillary tangles (NFTs), along with neuronal death (Alzheimer et al., 1995). Later on, with the development of electron microscopy (EM), it was found that NFTs are composed of protein filaments, described as paired helical filaments (PHFs) (Kidd, 1963). Almost simultaneously the knowledge of cytoskeleton and brain microtubules underwent a breakthrough (Brinkley, 1982). Senile plaques and NFTs were characterized, showing the presence not only of tubulin—the main component—but also microtubule-associated proteins, the smallest one being tau (Weingarten et al., 1975). At the same time, it was discussed whether any cytoskeletal protein could be present in PHFs (Brion et al., 1985; Anderton et al., 1987) and, later on, the presence of tau was indicated by Iqbal's group (Grundke-Iqbal et al., 1986). This observation was rapidly reproduced by other laboratories (Kosik et al., 1986; Nukina and Ihara, 1986; Wood et al., 1986). However, it was not clear whether tau protein could be just a compo-

nent or the main protein in the PHF. Later on, in the UK, Wischik and Goedert started to purify PHFs, in search of its main component (Goedert et al., 1988; Wischik et al., 1988). At that time, we purified a huge amount of porcine tau at our laboratory (very similar to human tau) to determine whether purified tau protein alone, without other proteins, was able to assemble into PHF-like structures (Montejo de Garcini et al., 1986). Results obtained from these studies concluded that tau was the main component of PHFs. Other pioneering work showed that the complete tau molecule was present in PHFs (Kosik et al., 1988), that the core of the polymer was a tau region (see below) involved in microtubule binding (Wischik et al., 1988) and that tau isolated from PHFs display the same biochemical properties as normal tau (Greenberg et al., 1992; Lee et al., 2001). Confirmation of tau as the main component of PHFs led to an increase in research into this protein. A brief history of tau research can be found in (Iqbal et al., 2016) and is more extensively covered in the book of reference by Perry et al. (2006). In recent years, new evidence indicates that tau protein can spread in a prion-like manner between connected cells or regions, supporting the hypothesis that tau pathology begins in a small area of the brain and then progressively spreads to other areas (Mudher et al., 2017). Moreover, how international research now addresses the role of tau in Alzheimer's disease (AD) is described in (Wortmann, 2012).

The role of chemical tau aggregation inhibitors is covered in the comprehensive review by (Wischik et al.,

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Abbreviations: AD, Alzheimer's disease; CBD, corticobasal degeneration; CD, circular dichroism; CNS, central nervous system; EM, electron microscopy; NFTs, neurofibrillary tangles; PHFs, paired helical filaments; PNS, peripheral nervous system.

2014). Regarding the function of tau as a microtubule-binding protein, a recent review provides an updated perspective of the interaction of this protein with microtubules (Baas and Qiang, 2019). In this review, we will comment on the ability of tau protein to aggregate into small or large polymers (forming different structures) (Avila et al., 2016; Goedert and Spillantini, 2019).

TAU PROTEIN

Human tau protein is expressed by a single gene located at chromosome 17 (Neve et al., 1986). The transcription of this gene in the central nervous system (CNS) leads to the appearance of a nuclear RNA that, upon alternative splicing and after translation, yields six distinct alternative tau isoforms (Liu and Gong, 2008).

Tau gene contains 16 exons (Himmler, 1989; Andreadis, 2005). Alternative splicing of exons 2, 3, and 10 results in the appearance of six tau isoforms in the CNS while in the peripheral nervous system (PNS), there is a high-molecular-weight tau isoform expressing the exon 4A, which yields a protein known as big tau. As a consequence, there are six isoforms in the CNS (Himmler, 1989; Goedert et al., 1992; Georgieff et al., 1993; Andreadis, 2005; Liu and Gong, 2008). Those containing exon 10 are known as tau 4R isoforms (R meaning the presence of a microtubule-binding repeat), whereas those isoforms lacking exon 10 are referred to as tau 3R isoforms (Avila et al., 2016). In the brain disorders known as tauopathies, including AD, some tau aggregates contain both 4R and 3R isoforms. Other disorders, like corticobasal degeneration (CBD), have mainly tau 4R aggregates, whereas in Pick's Disease (PiD), 3R aggregates predominate (Lee et al., 2001; Goedert et al., 2017). EM and cryo-EM have revealed differences between the structures of tau 4R and 3R aggregates (Falcon et al., 2018) depending on the type of tau pathology (see below).

INVOLVEMENT OF THE PRIMARY STRUCTURE OF TAU PROTEIN IN AGGREGATION

The primary structure of the longest human CNS tau molecule contains 441 residues. It contains up to 85 putative sites for phosphorylation (Ser, Thr or Tyr residues) and 102 hydrophobic residues (Ala, Val, Iso, Leu, Met, Phe). Tau self-aggregation can occur through ionic and/or hydrophobic interactions. Thus, the introduction of negative charges by phosphorylation could affect the former interactions (see for example (Avila et al., 2004; Hanger et al., 2009), thereby influencing tau aggregation. Hydrophobic residues are evidently

involved in hydrophobic interactions (see Fig. 1). The principal region of tau involved in its self-aggregation contains microtubule-binding repeats. The main characteristic of this tau region is that it has more basic residues like Lys or Arg. Posttranslational modifications of Lys residues by acetylation also regulates tau aggregation (Cohen et al., 2011).

Regarding the microtubule-binding region of tau, which spans residues 244 to 369 (Zeng et al., 2021), some specific residues like Cys 322 can form interlinks between tau molecules (Schweers et al., 1995), or residues like N279 (asparagine) can mutate to K (lysine) in some frontotemporal dementia (FTD) patients. Also, there is the loss of Lys 280 in other FTD mutations. Indeed, in these patients, other residues in the microtubule-binding region can mutate (see (Liu and Gong, 2008)). Moreover, Pro 232 lies close to the microtubule-binding region. Phosphorylation at the neighbour Thr 231 can facilitate the functions of Pin 1, a peptidyl-prolyl isomerase (changing cis–trans conformation), that binds to P-Thr 231/Pro 232 and regulates the extent of tau phosphorylation and consequently aggregation (Liu and Gong, 2008; Liou et al., 2011; Kimura et al., 2013).

THE THIRD MICROTUBULE-BINDING REPEAT OF TAU PROTEIN AND TAU AGGREGATION

The third microtubule-binding repeat (R3, residues 306–335) plays a key role in tau aggregation. Residues 306 to 311 present in this repeat (VQIVYK) can self-aggregate (von Bergen et al., 2000), and V306 or Q307 may be involved in this process. Residues K317, K321, H329, H330 and K331 may also participate in self-aggregation of R3 (Dong et al., 2021).

INVOLVEMENT OF THE SECONDARY STRUCTURE OF TAU PROTEIN IN AGGREGATION

Many decades ago, while performing a circular dichroism (CD) analysis of tau monomer, Cleveland and coworkers (Cleveland et al., 1977) found a low content of α -helix and β -sheet structures. However, there are sequences in tau polymers that can form β -sheet, α -helix or polyproline II helix structures (Mukrasch et al., 2009). β -sheet structures, for example at the initial six amino acids of R3, are involved in tau polymerization (Schweers et al., 1994; Mukrasch et al., 2005). Furthermore, there are regions in tau polymers that have α -helix structures (Sadqi et al., 2002; Kunjithapatham et al., 2005). The propensity for forming α -helix structures at residues 114–123 or 428–437 was described in (Mukrasch et al., 2005). Also, upon binding to lipid interfaces, tau may acquire a transitory α -helix structure (Barre and Eliezer, 2006). Finally, polyproline II helices have been reported to form at residues 170–181, 216–223 and 232–239 (Mukrasch et al., 2005). In any case, β -sheet formation at repeats R2 and R3 of tau play a major role in the aggregation of this protein (von Bergen et al., 2005; Fitzpatrick et al., 2017).

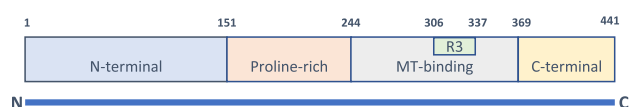


Fig. 1. The tau molecule can be divided into four distinct regions. The microtubule (MT)-binding region is the one involved in direct tau–tau interactions in self-assembly.

AGGREGATION *IN VITRO* OF SOME SMALL TAU PROTEIN FRAGMENTS

Using long incubation times, researchers tested tau aggregation *in vitro* in the presence of the denaturing agent urea (Montejo de Garcini et al., 1986), which may open tau molecules, facilitating interaction through their microtubule-binding regions. These regions of tau are enriched in basic residues and the addition of polyanions facilitates the self-aggregation of the protein (Goedert et al., 1996; Kampers et al., 1996; Perez et al., 1996; Arrasate et al., 1997; Wilson and Binder, 1997; Friedhoff et al., 1998). Furthermore, the presence of hydrophobic residues also facilitates tau aggregation (Li et al., 2008).

Focusing on R3 of tau, residues 306–335, the sequence containing the first six hydrophobic residues can self-assemble probably in an antisense direction (von Bergen et al., 2000; Li et al., 2008), a feature that was proposed to take place in PHFs (Iqbal et al., 2016) (see Fig. 2). In addition, the peptide containing residues 317–335 can also undergo polymerization, a process facilitated by the presence of polyanions (Perez et al., 2001) and which can also take place in antisense directions (Fig. 2). Ionic interactions may be responsible of the self-assembly of this peptide.

THE INVOLVEMENT OF THE TERTIARY STRUCTURE OF TAU PROTEIN IN AGGREGATION

Tau protein monomer has been recognized as an intrinsically disordered protein lacking a structured tertiary structure (Levine et al., 2015). Indeed, some EM analyses show that tau monomer has an extended non-globular shape (Hirokawa et al., 1988). However, the reaction of tau with some “conformational” antibodies, like Alz-50, pointed to an interaction between the N-terminal and C-terminal ends of tau and the existence of a defined tertiary structure (Carmel et al., 1996). Later studies suggested a structure, known as a “paper-clip” (Jeganathan

et al., 2006). These studies proposed that tau aggregation is facilitated by the opening of this structure. In this regard, the use of denaturing agents such as urea (Montejo de Garcini et al., 1986) favors this opening, thereby facilitating assembly of tau monomers.

Furthermore, an unusual conformation based on the stabilization of a putative small α -helix in a proline-rich region, caused by the phosphorylation at Thr 231, has been proposed (Sibille et al., 2012). Little is known about the role of this conformation in the assembly of tau polymers.

QUATERNARY STRUCTURE IN AGGREGATED TAU

Tau aggregates, ranging from dimers to complex structures, have been described. Thus, a relation between the small aggregates and the large structures has been proposed. For example, two intermediate aggregates, namely oligomers and granular oligomers (Maeda et al., 2007; Takashima et al., 2013), can be intermediate steps for the assembly of fibrillar polymers *in vitro*. Furthermore, *in vitro* experiments showed that monomeric tau can be added to the ends of purified PHFs, allowing the elongation of the polymerized filaments (Santa-Maria et al., 2008). The level of toxicity of these distinct tau aggregates is not the focus of this work since it has been previously reviewed (Avila, 2010; de Calignon et al., 2010).

Electro microscopy (EM) analysis revealed two types of polymerized structures in the brains of AD patients: PHFs and straight filaments (SFs) (Crowther, 1991). Similar structures were found when tau protein was assembled *in vitro* in the presence of heparin (Goedert et al., 1996; Perez et al., 1996). Recently, the use of cryo-EM analysis of larger tau structures has facilitated resolutions for atomic modeling (Zhang et al., 2019). Differences between heparin-induced polymers from tau 4R and 3R isoforms were found (Zhang et al., 2019).

Four distinct conformations were identified for tau 4R isoforms, while a different and unique conformation was described for tau 3R (Zhang et al., 2019). Cryo-EM analysis of 4R tau filaments has indicated the presence of the following types of filaments: snake, twister and jagged (Zhang et al., 2019) (Fig. 3). Also, a hose conformation that could stop twisting was reported. As mentioned above, a single conformation was observed for heparin-induced tau 3R filaments (Fig. 3) (Zhang et al., 2019).

Furthermore, filaments present in various tauopathies, including AD, which involves tau 4R and 3R isoforms; PiD (Pick disease), which involves the tau 3R isoform, and CBD (Cortico Basal Degeneration), with the tau 4R

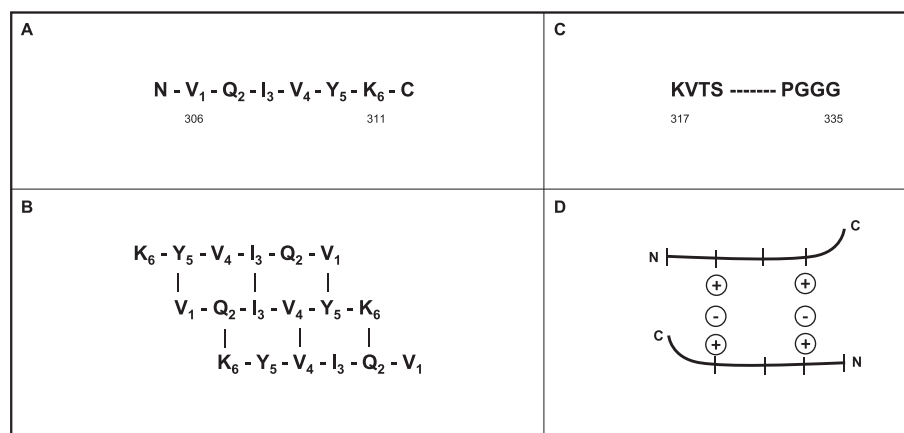


Fig. 2. Self-assembly model of the peptide comprising residues 306–311 based on (Li et al., 2008). (A) Sequence of the tau peptide. (B) Antiparallel interaction, as indicated in reference (Li et al., 2008). (C) Sequence of the ø peptide (Perez et al., 2001), comprising residues 317–335. (D) Suggested antiparallel interaction, facilitated by the presence of polyanions (Goedert et al., 1996; Perez et al., 1996).

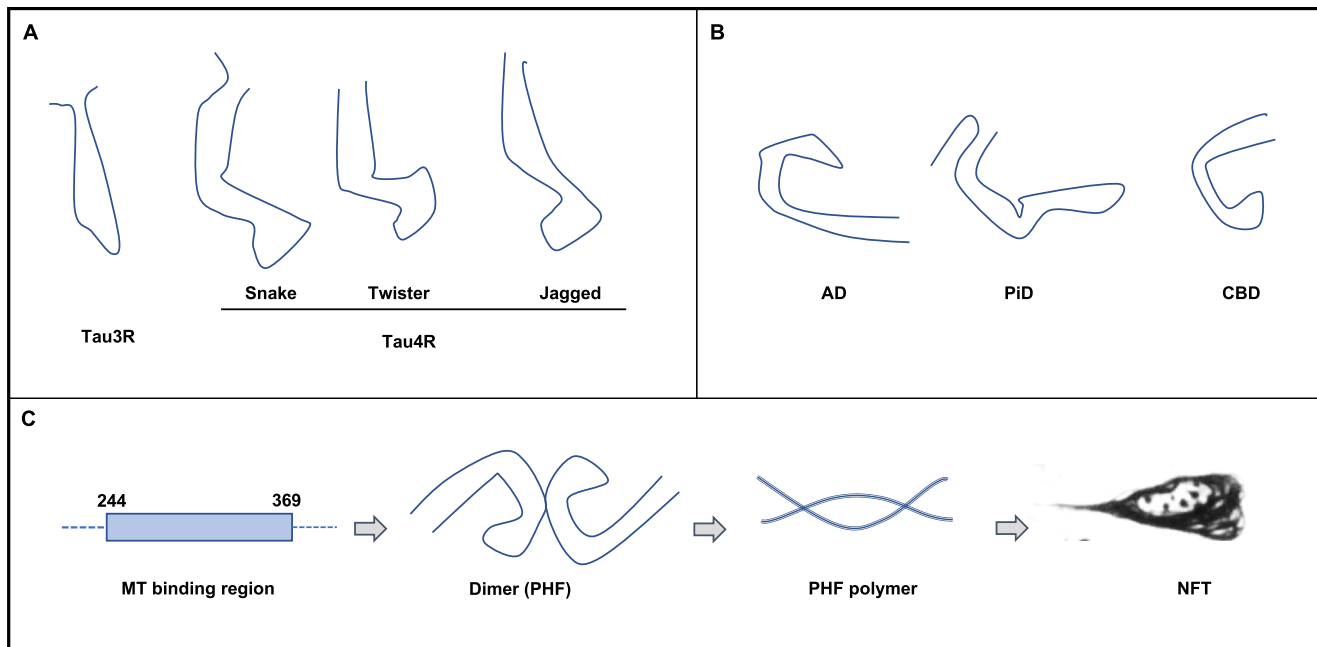


Fig. 3. Conformations at the microtubule (MT)-binding region of tau involved in aggregation. **(A)** Heparin polymers assembled from tau 3R (a single conformation) or from tau 4R (several conformations). **(B)** Conformations of tau polymers found in AD, PiD and CBD (Zhang et al., 2019; Zeng et al., 2021). **(C)** Models of tau assembly in AD, from the MT-binding region of tau to the formation of neurofilament tangles (NFTs).

isoform, were analyzed by cryo-EM (Zeng et al., 2021) and hierarchical classification of tauopathies on the basis of their filament folds has been proposed (Shi et al., 2021). It was found that AD polymers contain residues from 273 to 304/380, PiD from 254 to 378, and CBD from 274 to 380 (Zhang et al., 2019; Zeng et al., 2021). With respect to their relevance in the development of any specific disease, small tau oligomers are probably better targets.

However, the number of these structures related to tauopathies in the patients' brains is probably low. In this regard, further research is required to determine their relevance in the development of disease. In this context, the presence of small tau oligomers could be more relevant than that of large structures, the latter gaining significance in the confirmation of the diagnosis of the disease. In this regard, small tau oligomers may be more suitable targets to treat these tauopathies.

A NEW HUMAN TAU ISOFORM WITH A DECREASED ABILITY TO AGGREGATE

A new tau isoform, w-tau, caused by intron retention, has recently been described in the human brain (Garcia-Escudero et al., 2021). This human-specific tau isoform lacks exon 13 and contains, after exon 12, a translated sequence of intron 12 comprising 18 residues (Garcia-Escudero et al., 2021) (Fig. 4A). The amount of w-tau isoform (w indicates the presence of Trp residues) is decreased in the brains of AD patients compared to non-demented subjects. The main characteristic of this isoform is its reduced ability for self-aggregation. Indeed, the presence of w-tau may decrease the aggregation of other human tau isoforms. W-tau, therefore, emerges as

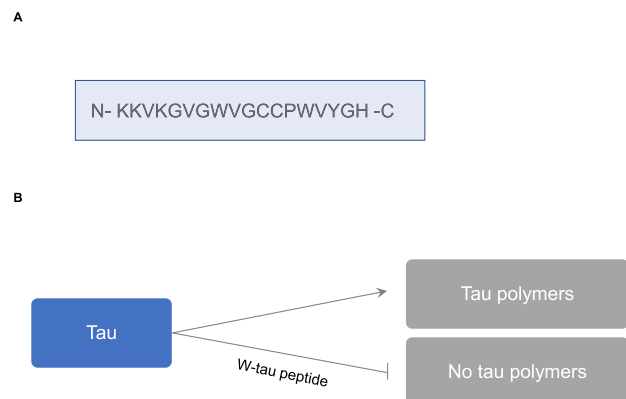


Fig. 4. Structure of extra human-specific sequence of w-tau peptide. **(A)** From reference (Garcia-Escudero et al., 2021) the sequence corresponding to the translated sequence after intron 12 retention, is indicated. This w-tau peptide has anti-aggregation properties (Garcia-Escudero et al., 2021) due to the presence of basic residues or tryptophan (w) motifs (Griner et al., 2019). **(B)** Hypothetical inhibitors of tau aggregation by w-tau peptide.

a potential therapeutic approach to tackle the toxicity due to tau aggregation, although more research is required to describe new characteristics of this new tau isoform (Fig. 4B).

In addition to the presence of Trp residues, in w-tau, the sequence of 18 residues, caused by intron retention is characterized by the presence of basic residues. As previously commented, polyanions facilitate tau assembly, while polycations have the opposite effect on tau polymerization. Future research should address whether w-tau peptide acts as a polycation on tau polymerization.

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