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Preparation and Characterization of Neurotoxic Tau Oligomers†

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ABSTRACT: Tau aggregation is a pathological hallmark of Alzheimer's disease, Parkinson's disease, and many other neurodegenerative disorders known as tauopathies. Tau aggregates take on many forms, and their formation is a multistage process with intermediate stages. Recently, tau oligomers have emerged as the pathogenic species in tauopathies and a possible mediator of amyloid-β toxicity in Alzheimer's disease. Here, we use a novel, physiologically relevant method (oligomer cross-seeding) to prepare homogeneous populations of tau oligomers and characterize these oligomers in vitro. We show that both Aβ and α-synuclein oligomers induce tau aggregation and the formation of β-sheet-rich neurotoxic tau oligomers.

Amyloid formation is a complex process that involves many morphologically and conformationally distinct species. The critical role of soluble amyloid oligomers in neurodegeneration has become generally accepted for multiple neurodegenerative diseases (*1*−*5*). Tau self-assembly, aggregation, and accumulation in neurofibrillary tangles (NFT) are hallmarks of Alzheimer's disease (AD), Parkinson's disease (PD), and many other neurodegenerative disorders (*6*, *7*). Recently, the significance and toxicity of NFT and other large metastable tau inclusions have been questioned (*2*, *8*−*10*). In animal models, cell death, microgliosis, and synaptic dysfunction occur in a manner independent of NFT formation (*11*−*13*). Moreover, neurodegeneration and behavioral impairments coincide with the accumulation of soluble aggregated tau species but are dissociated from the accumulation of NFT (*14*, *15*). Moreover, granular tau oligomers have been detected and isolated biochemically at very early stages of the disease, prior to the onset of symptoms or the formation of NFT (*16*, *17*).

This large body of evidence argues that tau oligomers are not fundamentally different from oligomers formed by other disease-associated proteins; they represent the acutely toxic structures of aggregated tau. Very little is known about tau oligomers, because reliable methods for preparing homogeneous populations of tau oligomers are lacking, which prevents researchers from studying them and testing chemical and other approaches to combatting their formation and toxicity.

Unlike amyloid-β (Aβ) peptide, which is highly prone to aggregation and spontaneously forms amyloid in vitro, tau is an unfolded, soluble protein. In vitro aggregation of tau into filaments can be achieved using high concentrations and via the addition of promoters (*18*−*20*). Mechanistic studies of full-length tau protein aggregation and filament formation in vitro have revealed striking similarities to Aβ aggregation; tau aggregates via either a nucleation-dependent mechanism (*21*) or the formation of intermediates (*22*).

In vitro, amyloid fibrils can accelerate the aggregation of the same protein via a nucleation-dependent mechanism, i.e., "seeding" (*23*−*25*). Seeding refers to the addition of a substoichiometric amount of fibrils, intact or sonicated, to a monomeric solution of the same protein, thus increasing the rate of conversion to amyloid fibrils. Lately, we and others have reported methods for preparing homogeneous Aβ and α-synuclein amyloid species (e.g., oligomers and fibrils) (*26*, *27*). These techniques provide an opportunity to test the effectiveness of different amyloid species as seeds. We have observed that amyloid oligomers, similar to fibrils, can seed and induce monomer aggregation and oligomer formation (*28*, *29*). It is well established that aggregated Aβ makes an important contribution to tau phosphorylation and aggregation in animal models and cell cultures. In primary neuronal cultures, Aβ is capable of inducing tau phosphorylation (*30*). Aβ42 fibrils induce formation of NFT in P301L tau transgenic mice (*31*), and pre-aggregated Aβ42 induces the formation of tau paired helical filaments in cells that overexpress human tau (*32*, *33*). These experiments have used aggregated Aβ, which is likely to contain different prefibrillar and fibrillar Aβ aggregates. To test the possibility of oligomer cross-seeding in vitro and the effects of different amyloid species on tau aggregation, we used homogeneous preparations of Aβ42 and α-synuclein oligomers and fibrils as seeds to promote tau aggregation. Here, we report that preformed Aβ42 and α-synuclein oligomer seeds induce the conversion of unstructured, monomeric human recombinant tau into β-sheet rich toxic tau oligomers.

Full-length human recombinant tau protein [tau-441 (Protein Data Bank entry 2N4R; molecular mass of 45.9 kDa)] was expressed and purified as described previously (*34*, *35*). Aliquots of a monomeric tau solution (1 mg/mL) were prepared in 1× PBS buffer (pH 7.4) (see the Supporting Information). Purity was assessed using fast protein liquid chromatography (FPLC). Aβ and α-synuclein oligomers were prepared as previously described (*26*, *36*, *37*) (see the Supporting Information). Seven microliters of Aβ42 or α-synuclein oligomers (0.3 mg/mL) was added as seeds to 1000 μL of the tau stock (0.3 mg/mL) in 1× PBS at a ratio of 1:140 (w/w). The sample was mixed by pipetting for 1 min and then incubated at room temperature for 1 h on an orbital shaker. The resulting tau oligomers were purified by FPLC and used to seed a fresh sample of monomeric tau. By two rounds of seeding of monomeric tau with purified tau oligomers, Aβ oligomer seeds have been diluted below the detection limit; after three rounds, the Aβ:tau ratio was estimated to be less than 1:2470000.

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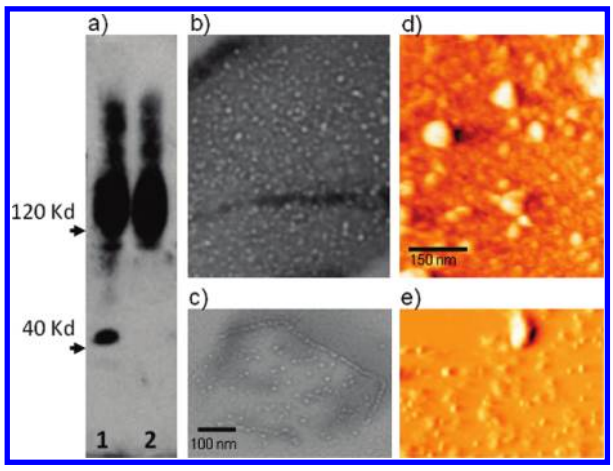

FIGURE 1: Characterization of tau oligomers prepared by cross-seeding with preformed oligomers in 1× PBS (pH 7.4) at an oligomer:tau ratio of 1:140 (w/w). (a) Western blot of tau oligomers prepared using α-synuclein oligomer seeds (lane 1) or Aβ42 oligomer seeds (lane 2), probed with Tau-5 antibody, which recognizes all forms of tau. (b and c) TEM images of tau oligomers prepared by seeding with Aβ42 oligomers. (d and e) AFM images of oligomers prepared by seeding with α-synuclein oligomers.

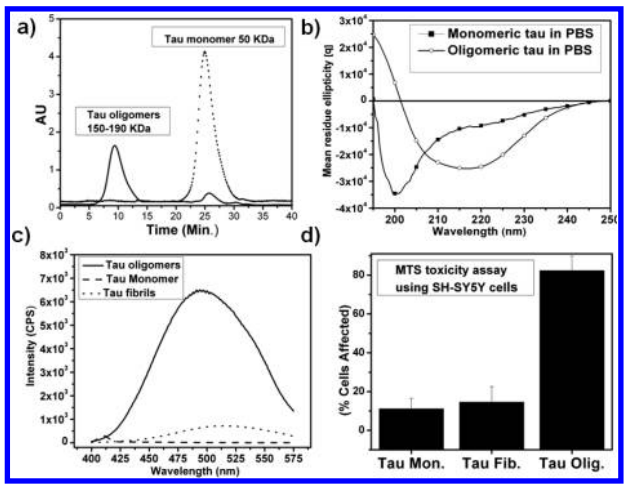

FIGURE 2: (a) FPLC chromatogram of tau oligomers. The main peak is at ≈150−190 kDa, which probably represents a tau trimer, consistent with Western blot data (Figure 1a). (b) Circular dichroism (CD) spectra of tau oligomers (○) and monomer (■), at a concentration of 0.3 mg/mL in 1× PBS. CD spectroscopy confirms that tau oligomers are β-sheet rich, unlike unordered monomeric tau. (c) Bis-ANS binds strongly to tau oligomers and weakly to tau fibrils and does not bind to tau monomer. Fluorescence emission spectra (400−575 nm) confirm that tau oligomers contain hydrophobic surfaces. (d) Tau oligomer toxicity shown by an MTS assay. Tau oligomers were toxic to SH-SY5Y cells at the final concentration of 1 μM, while tau monomer and fibrils were significantly less toxic.

ELISA and Western blots failed to detect Aβ or α-synuclein oligomers using 4G8 and 4D6, respectively. We used a 1:140 Aβ:tau ratio in the first round for cross-seeding, in line with the standards reported in the literature for fibrillar seeding (*23*−*25*).

Tau oligomers prepared by this novel method were largely SDS-stable apparent trimers based on Western blot analysis using Tau-5 (Figure 1a). Tau oligomers display a spherical morphology when characterized by transmission electron microscopy (EM) (Figure 1b,c) or atomic force microscopy (AFM) (Figure 1d,e), similar to oligomers formed by other amyloidogenic proteins (*26*, *36*). Tau oligomers were homogeneous and easily purified by gel filtration (Figure 2a). When shaken in PBS buffer for longer periods of time, tau oligomers prepared by this method continue to aggregate and eventually form tau filaments (Figure S1 of the Supporting Information). For controls, tau samples were incubated with shaking under the same conditions with Aβ42 fibrils, Aβ42 monomer, α-synuclein fibrils, and α-synuclein monomer; surprisingly, we found neither monomer nor fibrils promoted oligomerization or aggregation of tau.

Biophysical characterization of tau oligomers by circular dichroism (CD) spectroscopy at 0.3 mg/mL in PBS demonstrates that tau oligomers are β-sheet rich with minimal ellipticity [≈215 nm (Figure 2b)] as compared with the natively unfolded monomeric tau, which shows a random coil CD spectrum with minimal ellipticity [≈205 nm (Figure 2b)]. Although tau oligomers contain β-sheet structure, they did not bind thioflavin T or Congo red (*38*) dyes known for their affinity for amyloid. Nevertheless, tau oligomers bind strongly to bis-ANS (Figure 2c), with maximal emission at ≈485 nm after excitation at 360 nm, indicative of the presence of surface-exposed hydrophobic patches (*39*, *40*).

To investigate the toxicity of tau oligomers, SY5Y human neuroblastoma cells were treated for 4 h with varying concentrations of tau oligomers, monomer, and fibrils (see the Supporting Information). Toxicity was as determined using a colorimetric tetrazolium-based assay [MTS (Figure 2d)] and confirmed using a fluorescence cell viability kit [AlamarBlue (Figure S2 of the Supporting Information)]. Similar to oligomers formed by other amyloidogenic proteins (*26*), tau oligomers were significantly more toxic than monomer or fibrils (Figure 2d).

Tau and tau oligomers in particular hold promise as a therapeutic target for neurodegeneration (*8*, *9*, *41*). Our findings provide a better understanding of tau aggregation protocols for preparation of tau oligomers under physiological conditions that are critical for understanding their toxicity and the evaluation of therapeutic approaches (*42*). A central issue in AD pathogenesis is the relationship between amyloid deposition and NFT formation. Although recent evidence suggests that amyloid pathology lies upstream of or parallel to tau pathology (*43*, *44*), the underlying pathways and mechanistic details are still unclear. How does the Aβ peptide induce tau pathology in vivo, and which aggregation state of Aβ is most significant for tau aggregation? Our data suggest that amyloid oligomers specifically are capable of inducing tau aggregation in vitro and perhaps in vivo. This interaction may explain the synergies between Aβ and tau in AD, as well as between tau and other proteins such as α-synuclein in PD (*45*, *46*). In addition, the ability of tau oligomers to seed monomeric tau may play a role in disease progression; this intriguing phenomenon was recently discovered and elegantly studied in cell culture (*47*). This study show that tau oligomers enter cultured cells, seed the aggregation of intracellular tau, and transfer between cells in a prion-like mechanism (*47*, *48*). Moreover, recent data suggest that this phenomenon is not Aβ-specific but rather oligomer-specific, as demonstrated by the ability of soluble oligomers from a non-disease-related protein, hen egg white lysozyme, to mimic tau hyperphosphorylation induced by Aβ aggregates (*49*).

Dynamic oligomers represent a toxic amyloid species that is conformationally distinct from fibrils and monomer. Targeting oligomers is a challenge that requires reliable protocols and reagents such as those described here. Further investigations and analysis are needed to elucidate the contribution of amyloid oligomers to the induction of tau aggregation and to understand fully the role of tau oligomers in tauopathies.

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SUPPORTING INFORMATION AVAILABLE

Experimental procedures and Figures S1 and S2. This material is available free of charge via the Internet at http://pubs.acs.org.

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