

Review Article

Targeted proteolytic products of τ and α -synuclein in neurodegeneration

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CNS pathological inclusions comprising τ or α -synuclein (α Syn) define a spectrum of neurodegenerative diseases, and these can often present concurrently in the same individuals. The aggregation of both proteins is clearly associated with neurodegeneration and the deleterious properties of each protein is further supported by mutations in each gene (*MAPT* and *SNCA*, respectively) resulting in disease. The initiating events in most sporadic neurodegenerative diseases are still unclear but growing evidence suggests that the aberrant proteolytic cleavage of τ and α Syn results in products that can be toxic and/or initiate aggregation that can further spread by a prion-like mechanism. The accumulation of some of these cleavage products can further potentiate the progression of protein aggregation transmission and lead to their accumulation in peripheral biofluids such as cerebrospinal fluid (CSF) and blood. The future development of new tools to detect specific τ and α Syn abnormal cleavage products in peripheral biofluids could be useful biomarkers and better understand of the role of unique proteolytic activities could yield therapeutic interventions.

Introduction

τ and α -synuclein (α Syn) form toxic brain inclusions as either neurofibrillary tangles (NFTs) in Alzheimer's disease (AD) or Lewy bodies (LBs) in Parkinson's disease (PD), respectively [1,2]. Natively, both proteins are intrinsically disordered and can adopt multiple conformations [3,4]. The progressive accumulation of τ and α Syn-laden pathological inclusions each defines a spectrum of neurodegenerative diseases; however, the affected brain regions, the involved neuronal populations and contribution of other brain cells, such as astrocytes and oligodendrocytes, can be disease-specific [1,2,5,6]. Prion-like conformational mechanisms have been implicated in the propagation of τ and α Syn protein aggregates associated with the insidious nature of neurodegenerative diseases [1,2,5,6]. Both τ and α Syn can undergo extensive post-translational modifications that may promote their prion-like seeding potency and toxic spread [7–9]. Increasing evidence shows that τ and α Syn can lead to the formation of hybrid fibrils and aggregates [10,11]. For example, in many neurodegenerative diseases, pathological inclusions containing τ and α Syn often present concurrently and even co-localize within the same cells [10,12]. *In vitro* studies and experimental models have demonstrated that α Syn can initiate τ aggregation, and that both types of protein aggregation can be synergistic [10,13,14]. Here, we present the concept that similarities in the proteolysis of τ protein and α Syn could be closely linked and significantly contribute to the development of neurodegeneration in a variety of different diseases.

τ protein and disease-associated proteolytic fragments

Microtubule-associated protein τ is encoded in the *MAPT* gene and binds to microtubules (MTs), promoting their assembly and stability [5,8]. τ 's protein structure is composed of an amino (N)-terminal

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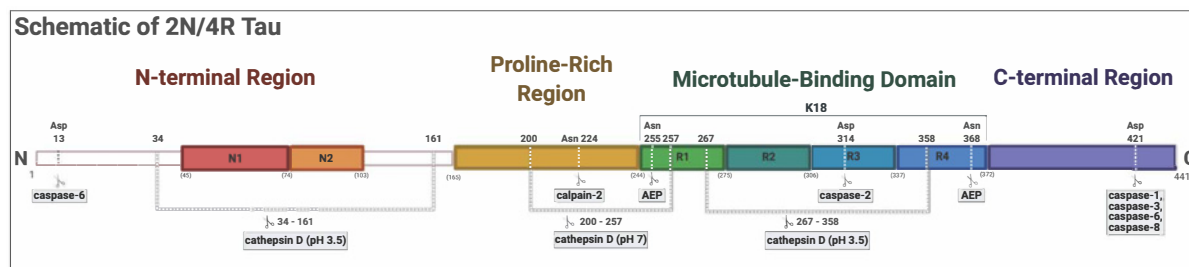


Figure 1. Schematic of 2N/4R τ and some of the major proteolytic cleavage sites

The 2N/4R τ isoform is 441 amino acids long and is composed of an N-terminal region, a proline-rich region, an MTBD with four MT binding repeats (R1–R4), and a C-terminal region. Due to alternative splicing of exons 2 and 3, the N1 and N2 segments within the N-terminus may not be present. Similarly, due to alternative splicing of exon 10, the R2 repeat in the MT binding repeat may not be present resulting in τ protein variant with only three MT binding repeats. τ 's N-terminus can be cleaved by caspase-6 at Asp¹³ [65] and cathepsin D, in acidic conditions, cleaves a fragment of τ consisting of amino acids 34–161 [49]. The proline-rich region is a target for calpain-2 at Asn²²⁴ [47], while cathepsin D, at a neutral pH, cleaves a fragment that cuts into the MTBD (200–257) [49]. The MTBD is also susceptible to AEP, which cleaves at Asn²⁵⁵, and cathepsin D which, under acidic conditions, excises the τ fragment, 267–358 [49]. Caspase-2 and AEP also target this domain at Asp³¹⁴ [36] and Asn³⁶⁸ [62], respectively. The C-terminus region can be cleaved at Asp⁴²¹ by caspases 1, 3, 6, and 8 [33,34]. Created with BioRender.com. Abbreviation: AEP, asparagine endopeptidase.

region, a proline-rich region, a microtubule-binding domain (MTBD), and a carboxy (C)-terminal region (Figure 1) [5,8]. In the human central nervous system, τ can be alternatively spliced into six isoforms, based on the presence or absence of exons, 2, 3, and 10 [5,8], and are grouped into 3R or 4R isoforms, which contain three or four MT-associated binding repeats, respectively. Hyperphosphorylated τ is the major component of insoluble pathological inclusions found in many tauopathies including, AD, frontotemporal dementia (FTD), progressive supranuclear palsy (PSP), corticobasal degeneration, and chronic traumatic encephalopathy (CTE) [5,8]. Frontotemporal dementia with parkinsonism linked to chromosome 17 with τ inclusions (FTDP-17t) is caused by missense, silent or intronic *MAPT* mutations, establishing a direct causal role of τ in neurodegeneration [15,16].

In the human brain, proteolysis of τ can result in a wide variety of τ fragments found in different cortical regions, hippocampus, and cerebellum in people with ages from 18 to 104 years [17]. Most of these τ fragments have not been studied; however, many fragments are more likely to be physiologic or benign, and only some are correlated with disease processes. τ fragmentation of specific regions of τ such as the central region may be more likely to be pathologic. For example, deletion of the first 150 or 230, but not the first 50 amino acids of the τ protein was correlated with an escalation of its pathological propensity, defined as increased phosphorylation density, accelerated aggregation, and increased interactions with τ oligomers isolated from AD brains [18]. Similarly, C-terminal truncation of the last 50 amino acids increased these pathological properties, while deletion of the last 20 amino acids was correlated with suppressed phosphorylation at Ser¹⁹⁹, Ser²⁶², Thr²³¹, Ser³⁹⁶, and Ser⁴⁰⁴ and enhanced phosphorylation at Ser²³⁵ [18]. This suggests that significant proteolysis of τ at either the N- or C-terminus may play a role in regulating its phosphorylation status, which in turn can alter its pathological properties.

τ fragments containing only the MTBD can also lead to increased pathologic properties such as τ aggregation. The expression of the K18 τ fragment, which comprises only the MTBD, with the Δ K280 τ mutation in N2A cells leads to increased τ phosphorylation, τ aggregation, and generation of additional different τ fragments [19]. Different steps of N- and C-terminal cleavage of K18 Δ K280 by endogenous proteases further increase its ability to form τ aggregates [20]. Additionally, pre-aggregated τ fragments, such as K19, which consists of the 3R τ MT-binding domain, or K18, can act as effective inducers of prion-like seeding and promote nucleation-driven τ aggregation in both cell and animal models [15,21,22].

Importantly, multiple τ fragments could be secreted extracellularly, allowing for detection within the cerebrospinal fluid (CSF) or blood—reservoirs which can potentially serve as clinical biomarkers [23,24]. Many proteases, including extracellular enzymes [25] have been implicated in the formation of proteolytic τ fragments; below, we will discuss some of the major intracellular activity that have been studied.

Caspase: generation of τ protein fragments

Caspases are a group of cysteine-dependent proteases that cleave substrates after aspartic residues and are involved in apoptotic cell death and inflammatory pathways [26]. Many caspases, such as caspase-3, caspase-6, caspase-8, and caspase-9, are found to be elevated, with increased activity, in AD brains, and can be found in τ inclusions [27–30]. Downstream products of caspase-induced apoptotic pathways, such as fodrin, are also correlated with the density of τ inclusions in AD [31]. Caspases can also regulate τ phosphorylation, for instance, caspase-3 proteolytically cleaves protein kinase B (also called Akt), which leads to activation of glycogen synthase kinase 3 β (GSK-3 β) and increased levels of τ phosphorylation [32].

A C-terminally truncated τ fragment at Asp⁴²¹ (Figure 1) can be generated by multiple protein caspases including caspase-1, caspase-3, caspase-6, caspase-7, and caspase-8 [33]. Asp⁴²¹ τ has increased propensity to form τ fibrillar filaments, as has been detected in NFTs of AD brains [33] and its formation is an early event in τ pathology as it can adopt a pathological conformation [34]. Furthermore, τ truncated at Asp⁴²¹, is found in greater quantities in AD, compared with control brains, and correlates with cognitive decline as determined by the mini-mental state examination (MMSE) for cognition, attention, and memory [34]. τ transgenic mice that overexpress Asp⁴²¹-truncated τ (τ C3) develop significant learning and memory impairment, decreased synaptic density, and formation of τ oligomers and aggregates [35].

Δ tau314 (Figure 1) is a soluble τ fragment generated by cleavage at Asp³¹⁴ by caspase-2 [36]. In patients with mild cognitive impairment (MCI), both Δ tau314 and caspase-2 are elevated compared with controls and have mild predictive value for the future development of dementia [37]. In patients with Lewy body dementia (LBD), Δ tau314 levels are elevated compared with PD [38]. Furthermore, PD and LBD patients have decreased total soluble τ and β -tubulin levels, which suggest increased loss of axons [38].

In rTg4510 τ transgenic mice, which overexpress the P301L τ mutation, Δ tau314 was found to be elevated [36]. Interestingly, Δ tau314 does not increase τ fibrillization or aggregation and is primarily related to functional impairment by promoting τ missorting in dendritic spines and synaptic impairment, which contributes to the progression of cognitive and memory deficits in this model [36]. An introduced τ mutation resistant to Asp³¹⁴ cleavage (D314E) suppressed the development of cognitive and memory deficits, which suggests that the Δ tau314 fragment is essential in functional impairment [36]. Likewise, suppression of caspase-2 expression by anti-caspase-2 oligonucleotides alleviated memory deficits [36]. These results support the role of Δ tau314 fragment in promoting functional impairment in various neurodegenerative diseases.

Calpains and τ cleavage

Calpains are highly conserved, calcium-dependent cysteine proteases that have been linked to neurodegeneration in AD [39]. Current evidence suggests that calpain-1 activity is important for physiologic development of synaptic plasticity, learning and memory, while calpain-2 is related to synaptic plasticity and neuronal death [40]. However, dysregulation of both calpain-1 and calpain-2 activity can lead to improper processing and proteolysis of τ protein, which generates different τ fragments. In postmortem AD brains with early stage Braak stage II–III τ pathology, increased calpain I activity is associated with activation of multiple τ kinases including GSK-3 β and cyclin-dependent kinase-5 (cdk5), which can lead to τ hyperphosphorylation [41]. Calpain-I can cleave different τ kinases themselves such as GSK-3 β , which further increases its catalytic kinase activity and indirectly lead to τ hyperphosphorylation [42].

Similarly, calpain-2 colocalizes with hyperphosphorylated τ inclusions in multiple neurodegenerative diseases including AD, Down Syndrome, PSP, corticobasal degeneration, and LBD [43]. In JNPL3 transgenic mice that overexpress the Pro³⁰¹Leu τ mutation [44], calpain activation leads to increased cdk5 activity and formation of τ oligomers and fragments [45]. The overexpression of calpastatin, an endogenous calpain inhibitor, partially alleviated these pathogenic processes and extended the lifespan of JNPL3 mice by 3 months [45]. This suggests that therapeutic inhibition of calpain activity could be effective in slowing neurodegeneration. Measurement of calpain activity could also be useful as a biomarker since its activity is elevated within the CSF of AD patients compared with healthy controls [46]. Calpain-2 can generate an N-terminal τ fragment cleaved at amino acid 224 (Asn²⁴⁴), which is specifically increased in CSF of AD patients [47].

Cathepsins

Cathepsins are a class of proteases involved in protein and aggregate degradation primarily in the low pH environment of lysosomes but also in endocytic and autophagic pathways [48]. Cathepsins have been shown to cleave α Syn and τ [9,49], but their precise role in AD-related proteostasis remains unclear. For example, cleavage by lysosomal

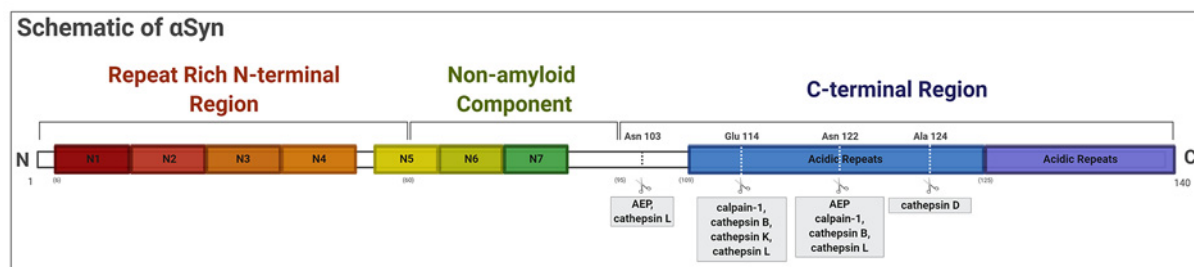


Figure 2. Schematic of α Syn and some of the major proteolytic cleavage sites

α Syn can be divided into three regions: the repeat rich N-terminal region, the hydrophobic, aggregation-prone, non-amyloid component (NAC), both of which contain imperfect KTKEGV repeat regions (N1–N7), and the highly charged, intrinsically disordered, C-terminal region. This latter region can be a hotspot for proteolytic cleavage. Asn¹⁰³ can be cleaved by AEP and cathepsin L [58,61], Glu¹¹⁴ is a target for calpain-1, cathepsin B, cathepsin K, and cathepsin L [55,59,66,67], Asn¹²² can be cleaved by AEP, calpain-1, cathepsin B, and cathepsin L [55,59,66,67], while cleavage after Ala¹²⁴ is a target for cathepsin D [59]. Created with BioRender.com.

Cathepsin D can result in isoform and pH-dependent τ fragments, specifically, fragments 34–161 and 267–358 at pH 3.5 or fragment 200–257 at pH of 7 and above [49]. Postmortem analysis of AD brains has revealed a correlation between up-regulated levels of cathepsin D in the neocortex with increased τ inclusion density and increased Ser³⁹⁶ phosphorylation of full-length τ within τ inclusions [50]. Cathepsin D and its elevated activity may serve as a potential biomarker in the CSF of AD patients [51].

α Syn proteolytic truncations associated with neurodegenerative diseases

α Syn is a relatively small (140 amino acid) but abundant, presynaptic neuronal protein whose functions largely revolve around vesicular trafficking [4,52]. Due to its highly charged and natively unstructured properties (Figure 2), α Syn is a very soluble protein [4,9], however, these same properties render it a prime target for prion-like, conformational templating and progressive accumulation in a spectrum of neurodegenerative diseases, including PD and LBD [2,6,9]. Similar to τ , missense mutations in the α Syn gene *SNCA* can result in neurodegeneration with protein aggregation [2,53]. However, the abundant accumulation of α Syn C-truncated species is an invariant finding in all synucleinopathies, and these products are associated with initiating and promoting α Syn aggregation while enhancing its toxicity [9]. Caspase-1 [54], calpain-1 [55], and various cathepsins [56–59] can be implicated in the C-cleavage of α Syn at specific sites (Figure 2). Cathepsin L and the lysosomal protease asparagine endopeptidase (AEP) can cleave α Syn after residue 103, increasing the polymerization templating activity of α Syn [59–61] while propagating the unique twisted fibril morphology on to full-length α Syn consistent with prion-like strain behavior [59,60]. The cleavage of α Syn after Asn¹⁰³ is associated with both increased aggregation and neurotoxicity in disease models [61]. Furthermore, AEP can also cleave τ after residue Asp³⁶⁸ and this truncated form of τ can robustly interact with α Syn Asn¹⁰³ [62]. These copolymers can propagate more efficiently from the gut to CNS and appear to display increased toxicity [62].

Clinical applications as biomarkers

Many recent clinical studies have demonstrated that τ protein is elevated and enriched in the CSF and blood, which can be utilized as an effective biomarker to track disease progression in AD, FTD, and other tauopathies [8]. There is a wide range of various proteolytic τ fragments that have been discovered within the CSF, many of which are also widely phosphorylated or post-translationally modified [23,24]. In the CSF, most τ fragments lack the C-terminal region and are cleaved within the proline-rich domain at approximately residues 222–225 [63]. These truncated forms of τ could be the result of up-regulated protease activity, including, but not limited to, calpains, cathepsins, and caspases.

More recent studies have shown that CSF τ fragments containing the MTBD and beginning at the approx. residue 243 are correlated with τ pathology PET imaging and cognitive symptoms in AD patients [64]. However, most τ CSF fragments have not been widely characterized and could be a source of future biomarkers and important predictors of cognitive decline. In the field of neurodegeneration, a significant limitation is the lack of truncation-specific

monoclonal antibodies that can detect both τ and α Syn fragments that are uniquely elevated in the disease state. Future studies could focus on development of these tools for clinical applications of diagnosis and tracking disease progression.

Conclusion

In conclusion, partial proteolysis/truncation is a shared post-translational modification of both τ and α Syn, and can contribute to aggregation and/or neurodegeneration. Both of these proteins are involved in the formation of insoluble protein inclusions and can coexist in multiple neurodegenerative disorders. Additionally, they are largely cleaved by similar families of proteases, including caspases, calpains and cathepsins, which are closely associated with cell death and neuroinflammation. Activation of various proteases can generate a wide variety of truncated protein fragments and species that can be found to be significantly elevated in CSF and blood, which require further investigation. In the future, the generation of novel truncation-specific antibodies for both τ and α Syn can be utilized as useful tools for clinical diagnosis and elucidation of different pathways and mechanisms.

Summary

- τ and α Syn proteins form toxic brain inclusions in a variety of neurodegenerative diseases and can occur concurrently.
- Both τ and α Syn are proteolytically processed by similar classes of proteases.
- Multiple τ and α Syn proteolytic fragments can be produced, which can end up into the CSF and blood; detection of these protein fragments can be used as clinical biomarkers to track disease onset and progression.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contribution

Y.X., G.M.L. and B.I.G. wrote and edited the manuscript.

Abbreviations

α Syn, α -synuclein; AD, Alzheimer's disease; AEP, asparagine endopeptidase; cdk5, cyclin-dependent kinase-5; CSF, cerebrospinal fluid; FTD, frontotemporal dementia; GSK-3 β , glycogen synthase kinase 3 β ; LBD, Lewy body dementia; MT, microtubule; MTBD, microtubule-binding domain; NFT, neurofibrillary tangle; PD, Parkinson's disease; PSP, progressive supranuclear palsy.

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