Alpha-synuclein truncation and disease

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ABSTRACT

Alpha-synuclein is the major component of Lewy bodies, insoluble protein aggregates, found in patients with Parkinson's disease, diffuse Lewy body disease, and the Lewy body variant of Alzheimer's disease. Alpha-synuclein has been found within Lewy bodies to contain many different modifications, including nitration, phosphorylation, ubiquitination, and truncation. C-terminally truncated forms of alpha-synuclein aggregate faster than the full-length protein in vitro, and are thus believed to play a role in Lewy body formation and disease progression. Pathological studies of post mortem brain tissue and the generation of transgenic mouse models further support a role of C-terminally truncated forms of alpha-synuclein in disease. Several enzymes, some of which function extracellularly, have been implicated in the production of these C-terminally truncated forms of alpha-synuclein. However, the enzymes responsible for alpha-synuclein truncation in vivo have not yet been firmly established.

Keywords: Alpha-Synuclein; Neurodegeneration; Parkinson's Disease; Lewy Body; Proteasome; Truncation; Degradation; Aggregation; Protease

1. INTRODUCTION

Lewy bodies are insoluble, predominantly cytoplasmic, protein aggregates located in the brain that are characteristic of a group of neurological diseases. Lewy body diseases include Parkinson's disease (PD), diffuse Lewy body disease (DLBD), and the Lewy body variant of Alzheimer’s disease (LBV). No single event has been shown to cause Lewy body diseases, yet all of these diseases result in similar pathological and physiological characteristics. Lewy body diseases are all pathologically defined by the accumulation of cytoplasmic protein deposits and neuronal cell death (reviewed in [1]). Physiological effects include an increase in cellular oxidative damage [2] and inflammation ([3], reviewed in [4]). These diseases are all progressive, and Lewy body formation correlates with a decline in motor and cognitive functions, and eventual fatality.

2. MAIN BODY

2.1. Lewy Body Diseases

The major component of Lewy bodies is alpha-synuclein ([Figure 1]). Before the identification of alpha-synuclein, Lewy bodies were characterized by the presence of ubiquitin and hyper-ubiquitinated proteins [6]. In addition to alpha-synuclein and ubiquitin, Lewy bodies have been found to contain a plethora of other protein components, but not all of these components are present in every Lewy body in every patient. Other proteins that play a role in the UPS have been identified.
in Lewy bodies, and these include dorfin [7], Nub1 [8], and p62 [9,10]. Other proteins identified in Lewy bodies include microtubule-associated proteins [11-14] and protein kinases [15]. The mechanism by which these proteins co-aggregate with alpha-synuclein and the significance of their aggregation is unknown.

While a vast amount of information has been generated about Lewy body diseases in the past two decades, many questions remain about the cause and progression of these diseases. One such question is whether the alpha-synuclein-containing protein aggregates are harmful or if they are merely a mechanism by which the cell sequesters individual protein components that might otherwise be toxic. Recent results suggest that the alpha-synuclein-containing inclusions may not be toxic themselves, but that the intermediate, oligomeric species along the aggregation pathway might be responsible for proteotoxicity [17,18]. The factors that initiate this aggregation pathway are unknown. Several factors, however, have been shown to contribute to the development and progression of Lewy body diseases, including both genetic and environmental factors. In the case of Parkinson’s disease (PD), three independent missense mutations in alpha-synuclein (A30P, E46K, and A53T) have been shown to cause early-onset forms of the disease [19-21]. Duplications [22] and triplications [23] of the alpha-synuclein gene, and, thus, overexpression of the protein, also cause early-onset forms of PD. Mutations in other genes, such as LRRK2 [24,25], PARK2 [26], PINK1 [27], DJ-1 [28], and ATP13A2 [29] are linked to familial forms of PD; however, these monogenic forms of PD only account for about 5% of all cases. Interestingly, the other 95% of cases have the same pathological hallmarks as the monogenic forms of disease. No mutations in the SCNA gene encoding for alpha-synuclein have been found to cause other Lewy body diseases, yet these diseases exhibit alpha-synuclein pathology similar to that found in PD patients. These observations suggest that a common mechanism of pathogenesis exists in all Lewy body diseases, but that the initiation of pathogenesis may vary.

The most prevalent risk factor for Lewy body diseases is age. Many physiological processes are altered as an organism ages. Some of the age-related processes that have been correlated with Lewy body diseases include an increase in oxidative damage to cellular components (reviewed in [30]), dysfunction of the mitochondria (reviewed in [31]), and the long-term exposure to environmental toxins [32]. Additionally, many studies have aimed to understand the effects of aging on the cellular protein degradation machinery. Proteolysis offers an alternative mechanism for reduction in levels of the presumptive cytotoxic protomer. Changes in both major pathways of protein degradation in the cell, the UPS and autophagy, have been observed with age. While effects on the trypsin- and chymotrypsin-like activities of the proteasome with age have been inconclusive [33-36], the PGPH-like activity of the proteasome has consistently been shown to decrease with age [37,38]. Additionally, gene expression studies have indicated a change in proteasomal subunit expression patterns with age in both murine muscle [39] and human fibroblasts [40]. A decline in autophagy function was observed in rats and human fibroblasts, through a decrease in both substrate binding and transport to lysosomes [41].

With these wide-ranging physiological alterations that occur with age, it is reasonable to hypothesize that proteins could be modified over time, leading to enhanced aggregation propensity, and the possible initiation of disease. Lewy body diseases might be the result of the failing protein degradation pathways being unable to compensate for the buildup of damaged proteins. Certain combinations of variables or specific genetic backgrounds may yield an individual more susceptible to these alterations and the lack of compensatory mechanisms, explaining why some individuals succumb to Lewy body diseases while others do not. A further study of each of these processes will allow for a more complete understanding of disease pathogenesis and the generation of targeted therapeutics to slow progression or prevent these diseases altogether.

### 2.2. Alpha-Synuclein Structure

Alpha-synuclein is a 140-amino acid protein that is paralogous to two other nervous system proteins, referred to as beta- and gamma-synuclein. Alpha-, beta-, and gamma-synuclein are similar in sequence, with most of the similarity lying within the N-terminus of the proteins. The N-terminal portion of alpha-synuclein includes seven imperfect repeats of 11 residues containing the KTKQEV consensus sequence, while the C-terminus contains many acidic residues and is, thus, negatively-charged (Figure 2). Residues 61 - 95 encompass many hydrophobic residues, and a peptide corresponding to this region of the protein (referred to as the NAC region)

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**Figure 2.** Human alpha-synuclein sequence. The sequence of the 140-amino acid protein (accession number P37840.1) is shown. Underlined regions are the seven imperfect 11-residue repeats and the sequence in red is the amyloidogenic NAC (Non-αβ Component) region of the protein.
Alpha-synuclein is among the increasing number of proteins recognized as an intrinsically-disordered protein (IDP), a class of proteins characterized by their lack of a defined structure in the native state (reviewed in [43]). Upon binding to negatively-charged vesicles, alpha-synuclein adopts a conformation that has a high alpha-helical propensity [44,45], and a fraction of neuronal alpha-synuclein has been found to associate with membranes in vivo [46]. Structural studies aimed at understanding the membrane-bound form of alpha-synuclein have relied on nuclear magnetic resonance (NMR) spectroscopy. A structure of the full-length, membrane-bound form of alpha-synuclein (Figure 3) reveals a conformation in which the N-terminal two-thirds of the protein forms a broken, amphipathic alpha-helix [47,48]. This structured portion of the protein is responsible for membrane binding, and residues at the very N-terminus are essential for this process [49]. In the NMR structure of alpha-synuclein, the negatively-charged C-terminal tail remains flexible and disordered [47,48].

Structural studies aimed at understanding the unbound state of alpha-synuclein have relied on molecular dynamics (MD) simulations and more complicated NMR techniques, such as residual dipolar coupling (RDC) and paramagnetic relaxation enhancement (PRE). These techniques have produced results suggesting that alpha-synuclein adopts several thousand structurally distinct conformations, many of which are more compact than expected for a random coil [50]. Many of these conformations include long-range (15 Å to 20 Å) interactions between the C-terminus and both the N-terminus and central portion of the protein [50-52].

Several observations suggest that disruption of these long-range interactions facilitates aggregation of the protein. In one study, spermine (a polyaniline that has been shown to interact with the acidic C-terminus of alpha-synuclein) was shown to disrupt these long-range interactions while simultaneously promoting in vitro aggregation [52]. A similar result was observed when temperature was increased [52]. Additionally, studies have shown that the PD-causing A30P and A53T alpha-synuclein mutations both have decreased propensity for these long-range interactions [53]. A disruption of these intramolecular long-range interactions and increased aggregation propensity of the protein may serve as a mechanism by which these two point mutations cause Lewy body formation and disease pathogenesis.

2.3. Alpha-Synuclein Physiology and Function

Alpha-synuclein is a protein expressed in all vertebrates. Homology of alpha-synuclein across species is greater at the N-terminus of the protein, with more variability in sequence located toward the C-terminus. Alpha-synuclein is expressed predominantly in the central nervous system and localizes to presynaptic terminals [54]. Expression of alpha-synuclein is quite high, consisting of up to 1% of the total protein in certain regions of the brain [54]. Within neuronal cells, alpha-synuclein has been detected in both the cytoplasm and nucleus [55]. Studies utilizing fractionated rat brains revealed that about 15% of alpha-synuclein is membrane-bound [56], and the protein was recently found associated with mitochondrial membranes in normal dopaminergic neurons [57,58]. The relative subcellular distribution of alpha-synuclein varies among different neuronal cell populations [58,59].

While the function of alpha-synuclein has not been clearly established, observations related to the protein’s cellular localization have provided clues to its function. Studies aimed directly at establishing a role for alpha-synuclein have relied on mammalian cell culture and animal models. Alpha-synuclein knockout mice have been generated in several laboratories and these mice are viable, suggesting that other proteins might play a redundant role in the cell. Deletion of alpha-synuclein in mice causes only mild phenotypes including defects in presynaptic vesicles [60], synaptic transmission [61], and the trafficking [62] and metabolism [63,64] of fatty acids. Additionally, mice lacking alpha-synuclein are protected from the changes in cellular morphology and cell death caused by exposure to MPTP (an inhibitor of mitochondrial complex 1) that are observed in wild-type mice [65]. The levels of striatal dopamine were also less affected by MPTP-treatment in alpha-synuclein knockout mice than wild-type mice [66].

Alpha-synuclein has been shown to play a role in neurotransmitter release, as studied by neuronal cell lines expressing alpha-synuclein [67], knockout mice [68], and mice overexpressing alpha-synuclein [67]. Alpha-synuclein has also been shown to exhibit a non-classical chaperone activity that plays a role in SNARE complex assembly [69]. Both the N- and C-termini of alpha-synuclein play a role in this process, as the N-terminus of alpha-synuclein has been found to colocalize with Aβ plaques in Alzheimer’s disease [42].

Figure 3. Structure of alpha-synuclein bound to lipid vesicles. The structure of the full-length human protein was determined by NMR (PDB ID: 1XQ8) and image was rendered in PyMol. The structure is colored from blue (N-terminus) to red (C-terminus).
the protein binds to phospholipids while the C-terminus is necessary for synaptobrevin-2 binding [69]. Alpha-, beta-, and gamma-synuclein, triple knockout mice showed an age-dependent decrease in SNARE complex assembly [69], indicating that other members of the synuclein family may serve roles that are similar to, or possibly overlap with, those of alpha-synuclein.

While neuronally-expressed alpha-synuclein has been the focus of most studies, many other tissues have been found to express the protein. These tissues include muscle [70], cerebral blood vessels [71], red blood cells [72], plasma [73], and blood cells of the immune system [74]. The function of alpha-synuclein in these tissues has not been elucidated. An investigation of alpha-synuclein expression in human fetuses revealed that alpha-synuclein is expressed throughout fetal tissue; however, expression in most of these tissues is reduced in adulthood [75], suggesting that alpha-synuclein might also play a role in development.

3. DISCUSSION

3.1. Alpha-Synuclein Truncation and Disease

Wild-type and the three PD-causing missense mutations of alpha-synuclein were the primary focus of early studies related to alpha-synuclein aggregation and disease; however, recent studies have acknowledged post-translational modifications of alpha-synuclein and the role that these forms of the protein might play in disease. Within Lewy bodies, alpha-synuclein has been found to exist with several modifications (Table 1). These modifications include phosphorylation [76-78], nitration [79], and ubiquitination [80,81]. In addition to full-length alpha-synuclein, truncated forms of alpha-synuclein have been identified in pathological aggregates [82,83]. These truncations have been found to occur from either the C-terminus or both the N-terminus and C-terminus in patient samples [84,85] and transgenic mouse models of PD [84,86]. The C-terminus of alpha-synuclein is negatively-charged, and truncating the protein to remove this terminus produces species that are more prone to aggregation in vitro [84,87,88]. Notably, truncated alpha-synuclein can facilitate aggregation of the full-length protein in vitro [84] and in vivo [89]. A truncated form of alpha-synuclein, the NAC region, accumulates in Alzheimer’s disease patients [90], suggesting a role for alpha-synuclein truncation in the pathogenesis of multiple diseases.

Transgenic mice have recently been generated that overexpress truncated forms of alpha-synuclein. These mice exhibit physiological and pathological similarities to patients with Lewy body diseases [95-97]. The three different models generated expressed the human alpha-synuclein (residues 1 - 120) on a mouse alpha-synuclein null background [95], human A53T alpha-synuclein (residues 1 - 130) on an endogenous mouse alpha-synuclein background [96], and human alpha-synuclein (residues 1 - 119) on an endogenous mouse alpha-synuclein background [97]. The mice expressing human A53T alpha-synuclein (residues 1 - 130) exhibited loss of dopaminergic neurons in the substantia nigra, lower levels of striatal dopamine, and an alteration in spontaneous locomotor activities [96]. Mice expressing human alpha-synuclein (residues 1 - 119) showed a similar loss of striatal dopamine [97]. The expression of truncated alpha-synuclein also led to a greater susceptibility to stress [95].

The identification of truncated forms of alpha-synuclein that are both prone to aggregation and capable of cross-seeding aggregation of the full-length protein, suggests that alpha-synuclein truncation is a mechanism that contributes to the progression of Lewy body diseases. Some reports have indicated that truncation of alpha-synuclein is a natural process, and that truncated forms of alpha-synuclein are detectable in the brain of healthy individuals [98]. However, expression of the disease-causing A30P and A53T alpha-synuclein leads to enhanced production of these C-terminally truncated species and faster aggregation [86]. These results, in addition to the transgenic mice studies described previously [95-97], suggest that truncated alpha-synuclein species might lead to the development of clinical and pathological features if expression exceeds a certain level. In individuals with disease, the amount of truncated alpha-synuclein species generated might have reached a threshold that can no longer be tolerated by the cell. The mechanism by which these truncated forms of alpha-synuclein are produced and accumulated in the cell is unknown.

Table 1. Modifications of alpha-synuclein identified in Lewy bodies and their effects on in vitro aggregation. The identification of alpha-synuclein modifications that are present in Lewy bodies might provide insight into disease progression.

<table>
<thead>
<tr>
<th>Protein Modification</th>
<th>Source of Identification</th>
<th>Effect on In Vitro Aggregation</th>
<th>Source of In Vitro Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitration of Tyr residues</td>
<td>[79]</td>
<td>decreased aggregation</td>
<td>[91,92]</td>
</tr>
<tr>
<td>S87 phosphorylation</td>
<td>[78]</td>
<td>decreased aggregation</td>
<td>[78]</td>
</tr>
<tr>
<td>S129 phosphorylation</td>
<td>[76,77]</td>
<td>inconclusive</td>
<td>[76,93]</td>
</tr>
<tr>
<td>Truncation (C-terminal)</td>
<td>[84-86]</td>
<td>increased aggregation</td>
<td>[84,87,88]</td>
</tr>
<tr>
<td>Truncation (N-terminal)</td>
<td>[84]</td>
<td>increased aggregation</td>
<td>[94]</td>
</tr>
<tr>
<td>Mono-, di-, tri-ubiquitination</td>
<td>[80,81]</td>
<td>unknown</td>
<td>-</td>
</tr>
</tbody>
</table>

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3.2. Degradation of Alpha-Synuclein by the 20S Proteasome

Several *in vitro* studies have shown that alpha-synuclein can be degraded by the 20S proteasome in a ubiquitin-independent manner [84,99,100]. While degradation of alpha-synuclein by the 20S proteasome has not been established in an animal model, several *in vivo* observations support a role for the 20S proteasome in alpha-synuclein truncation and disease. C-terminally truncated forms of alpha-synuclein isolated from A53T alpha-synuclein transgenic mice were identified by mass spectrometry, and some species were identical to those produced by the 20S proteasome *in vitro* [84,86]. Follow-up studies, in which antibodies were generated to specifically recognize the C-terminus of truncated forms of alpha-synuclein, revealed that two C-terminally-truncated alpha-synuclein species, residues 1 - 110 (syn110) and residues 1 - 119 (syn119) are present at much higher levels in patients with Lewy body diseases than in age-similar controls (Figure 4) [85]. Additionally, it was shown that these truncated species are not always colocalized within the same cell [85], hinting at a mechanism by which their production may be regulated.

3.3. Roles of Other Enzymes in Alpha-Synuclein Degradation

While many independent laboratories have shown that alpha-synuclein can be degraded by the 20S proteasome *in vitro* [99-101], other studies have implicated different enzymes in the cleavage and degradation of alpha-synuclein [102-115]. These enzymes are different in their activities, their cellular localization, and their regulation. It is possible that more than one of these enzymes works in concert to produce truncated forms of alpha-synuclein that promote Lewy body formation and disease progression. Understanding these processes and their cooperativity in normal physiological processes and in disease progression is essential to the understanding and treatment of Lewy body diseases.

Calpain 1 is a calcium-dependent cysteine protease [116,117]. In *in vitro* assays, the monomeric form of alpha-synuclein is predominantly cleaved by calpain 1 after residue 57, while fibrillar forms of alpha-synuclein are degraded at the C-terminus, specifically after residues 114 and 122 [102]. Another study by the same group revealed that the cleavage products produced by calpain 1-mediated degradation of soluble alpha-synuclein inhibited aggregation of the full-length protein, while cleavage products produced by calpain 1-mediated degradation of fibrillar forms of alpha-synuclein were aggregation-prone and capable of cross-seeding aggregation of full-length, monomeric alpha-synuclein [103]. It has also been reported that the activities of calpain I and the 20S proteasome may act in a concerted manner in producing aggregation-prone C-terminally truncated forms.

![Figure 4](image url)
of alpha-synuclein [104]. In this study, a product of calpain 1-mediated degradation that was resistant to further degradation by the enzyme, was able to enhance the degradation of full-length alpha-synuclein by the 20S proteasome and this enhancement was specific for alpha-synuclein, as no enhancement was observed for the degradation of azocasein or peptide substrates [104].

Another protein that has been suggested to play a role in the accumulation of truncated forms of alpha-synuclein is cathepsin D. Cathepsin D is a lysosomal protease that has been shown to cleave alpha-synuclein both in vitro and in the lysosomal fraction of cells expressing alpha-synuclein [105]. In one study, reduced proteasomal activity was observed in cathepsin D-deficient mice [106], implicating a connection between these two mechanisms of proteolysis. Additionally, RNAi knockdown of cathepsin D in a mammalian cell culture model [105] and cathepsin D knockout mice [106] exhibit alpha-synuclein accumulations. Another study, utilizing Drosophila [107], suggests that this enzyme might play a role in the normal clearance of alpha-synuclein.

In light of recent reports suggesting that alpha-synuclein is found extracellularly [108,118-120], proteases that might act on these extracellular forms of alpha-synuclein might also play a role in this process. One such enzyme is neurosin, a serine protease that is highly expressed in the nervous system [121]. Neurosin has been shown to co-aggregate with alpha-synuclein in Lewy bodies [109] and, in in vitro assays, specific cleavage products of alpha-synuclein were produced in which the protein was cleaved in the NAC region and at several sites within the C-terminus [110]. A recent study has revealed that neurosin-mediated cleavage of alpha-synuclein can only occur extracellularly, once neurosin is activated upon secretion [111].

Matrix metalloproteases (MMPs) are a class of enzymes that are secreted, and they are known to play a role in the degradation of extracellular and membrane-bound proteins (reviewed in [122]). Several studies have implicated MMPs in the cleavage and aggregation of alpha-synuclein. In one study, a dopaminergic neuronal cell line was transfected with alpha-synuclein, and the overexpression of alpha-synuclein led to its secretion [112]. Additionally, when these transfected cells were subjected to oxidative stress, the expression of matrix metalloprotease-3 (MMP-3) was increased and alpha-synuclein fragments were observed in the media. Generation of alpha-synuclein fragments was blocked by pre-incubation with a matrix metalloprotease inhibitor. In addition, results from this study showed that alpha-synuclein can be cleaved at several positions, and that the products generated facilitate aggregation and cell toxicity. Other studies have also shown that matrix metalloproteases can cleave alpha-synuclein, and cleavage by both MMP-1 and MMP-3 was shown to increase aggregation propensity [113]. Another study showed C-terminal cleavage by MMP-3, and found that MMP-3 cleavage of the disease-causing A53T mutation of alpha-synuclein resulted in an increased number of degradation products [114]. By analyzing post mortem brain tissue from PD patients, the authors reported that over 50% of Lewy bodies contain MMP-3 [121]. Recently, plasmin, a serine protease in the blood, was also implicated in alpha-synuclein degradation and disease pathogenesis [115].

4. CONCLUSION

These in vitro and in vivo results reveal that the formation of truncated alpha-synuclein species is a complex process that likely plays a role in disease. The major goal of studying these diseases is to develop therapeutics to halt or slow down the progression of the disease. Whereas truncation of alpha-synuclein is correlated with accelerated disease progression, interference in this process may have therapeutic benefit. Elucidating the mechanism by which these enzymes produce partially-truncated and aggregation-prone alpha-synuclein cleavage products is an initial step in identifying relevant therapeutic targets. Considering the large number of enzymes that have been shown to produce truncated alpha-synuclein species in vivo, it is likely that alpha-synuclein degradation is the result of a combination of enzymes that either work independently or together to produce specific aggregation-prone species.

REFERENCES


