α-Synuclein Phosphorylation and Nitration in Parkinson’s Disease

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Summary

Parkinson’s disease (PD) is the second most common neurodegenerative disease, affecting six million people worldwide. PD results from the specific loss of substantia nigra dopaminergic neurons. Aggregation of one protein, α-synuclein, is characteristic of PD. This aggregation is thought to be a critical step in the etiology of the disease. While the molecular mechanism of α-synuclein aggregation remains unknown, nitrate stress and phosphorylation have been implicated in α-synuclein modification and aggregation. In fact, nitration of α-synuclein tyrosine residues 39, 125, 133 or 136, may be an early event in aggregates, Lewy bodies, seen in PD. Furthermore, nitrate stress leads to the induction of α-synuclein aggregation at a higher rate than seen in other PD mutants. This aggregation may result from a stabilization of pre-assembled α-synuclein filaments, which, upon nitration, may withstand denaturing conditions and enhance formation of SDS-insoluble, heat-stable high mass aggregates. Phosphorylation of α-synuclein also appears to play a critical role in the formation of aggregates. Extensive studies indicate that α-synuclein found in PD patient brains is extensively phosphorylated. Phosphorylation of ser-129 may enhance formation of aggregates reminiscent of Lewy bodies in vitro and in vivo. Nitration and phosphorylation of α-synuclein may be key to the mechanisms underlying the formation of Lewy bodies in PD.

Introduction

Parkinson’s disease (PD) is a common, fatal neurodegenerative disease that affects millions worldwide. This disease, characterized by postural instability, resting, and bradykinesia affects 1 in 50 individuals over the age of 60 (Olanow and Tatton, 1999; Periquet et al., 2007). There are two forms of PD: sporadic and familial. The sporadic form of PD is the most common, constituting ~90-95 of PD cases. The remaining 5-10% of PD cases are familial (Dauer et al., 2003). Although there is an increasing understanding of PD and its causes, there is still much to be investigated. Our knowledge is still very limited, and because of that, there is unfortunately still no cure for this tragic affliction.

Pathology

Both familial and sporadic forms of PD are linked to the death of midbrain dopaminergic substantia nigra neurons, which accumulate as misfolded and aggregated protein α-synuclein (Lee et al., 2004). As these cells die, there is diminished release of the neurotransmitter dopamine, which regulates the activity of parts of the brain that control movement initiation and coordination (Parkinson’s disease, 2007). This diminished amount of dopamine renders patients with a decreased ability to control and initiate movement, resulting in the clinical manifestations of PD.

Upon autopsy, neurons of the substantia nigra are found to contain large filamentous aggregates called Lewy bodies. The major component of Lewy bodies is a protein called α-synuclein (Periquet et al., 2007).

Characteristics of α-Synuclein

This natively unfolded protein of 140 amino acids has three major regions. The first major region of this protein is an N-terminal amphipathic region consisting of amino acids 1-61, while the central region consists of amino acids 61-95 (Periquet et al., 2007). Deletion of this region prevents α-synuclein aggregation in vivo and in vitro, suggesting that this region is essential for aggregation of the protein (Periquet et al., 2007). Finally, the third region, a highly acidic C-terminal region consists of amino acids 95-140. This C-terminal region may have an inhibitory role in the aggregation of α-synuclein, as C-terminally truncated forms of α-synuclein are found to aggregate into filaments more readily than full-length wild-type α-synuclein (Periquet et al., 2007). About 15% of Lewy bodies contain C-terminally truncated α-synuclein (Gaisson et al., 2000).

This highly conserved protein of unknown function is found throughout the central nervous system and is abundant in neurons, especially in pre-synaptic terminals (Gaisson et al., 2000). As previously stated, the aggregation and misfolding of this protein contributes to the formation of Lewy bodies, which are hallmarks of the disease.

Recent evidence has shed light on several post-translational events that can influence protein folding and function. This review will focus on the nitration and phosphorylation of α-synuclein. An emphasis will be made on the affects of the previously mentioned post-translational modifications on α-synuclein and the link of these modifications on pathology of the disease.

Phosphorylation of α-synuclein

Much research has gone into understanding the mechanism of Lewy Body formation and PD pathogenesis. While it is known that phosphorylation plays a key role in the functional properties of several proteins, there is limited knowledge on phosphorylation on PD-associated α-synuclein (Okoshi et al., 2000).

Initially, specific post-translational modifications that underlie the aggregation of α-synuclein were not known. More recently, however, our knowledge has increased in regards to these post-translational modifications due to several studies that have been conducted using a variety of models.
α-Synuclein or Synphilin-1?

While many studies focus on α-synuclein phosphorylation and its consequences, another study points to another protein’s phosphorylation as playing a critical role in aggregate formation.

α-Synuclein, along with the protein synphilin-1, are found as components of Lewy bodies. The interaction between these two proteins is found to be phosphorylation dependent, as inhibition of CK-2, a kinase previously established to phosphorylate α-synuclein, results in an inhibition of binding of the two proteins. Furthermore, the inhibition of α-synuclein and synphilin-1 binding results in a significant decrease in the percentage of cells that contain cytoplasmic aggregates (Lee et al., 2004). Interestingly, mutations in α-synuclein that result in no α-synuclein phosphorylation have no effect on α-synuclein/synphilin-1 interaction or aggregate formation. These results suggest that the interaction of these two proteins is phosphorylation dependent and that phosphorylation of synphilin-1, not α-synuclein is critical for the formation of cytoplasmic aggregates (Lee et al., 2004).

While one study implicates synphilin-1 phosphorylation as key to aggregate formation, α-synuclein, the major component of Lewy bodies, is still of major interest and many studies still focus on this protein in an effort to understand the pathogenesis of PD. Using neuroblastoma cells, Smith and colleagues examined the effect of α-synuclein phosphorylation on aggregate formation. Co-expression of mutant S129A of α-synuclein, which abolishes phosphorylation and synphilin-1, results in few or no aggregates forming. Furthermore, co-immunoprecipitation assays reveal that there is a decreased interaction between α-synuclein and synphilin-1. Together, these findings suggest that α-synuclein phosphorylation is important for α-synuclein/synphilin interaction, as well as aggregate formation (Smith et al., 2005).

Previous attempts at uncovering mechanisms for aggregate formation have been done in different
cells in vivo and in vitro. One study, however, uses a Drosophila model in order to examine the role of α-synuclein phosphorylation on specifically the phosphorylation of serine 129, on neurotoxicity and aggregate formation. When serine 129 is altered to a negatively charged residue, aspirate, mimicking phosphorylation, significant α-synuclein toxicity is observed (Chen et al., 2005). Other studies, however, show that phosphorylation and nitrosylation-deficient mutants are toxic to yeast (Herrera et al., 2005).

In vivo, α-synuclein is phosphorylated by G-protein receptor kinase (Gprk2). Moreover, blocking the phosphorylation of α-synuclein results in a progressive accumulation of cytoplasmic aggregates resembling Lewy bodies (Chen et al., 2005).

α-synuclein Nitration

Nitration’s Link to Formation of Aggregates

While it is known that α-synuclein is nitrated in PD aggregates, the effects of this nitration remained to be elucidated (Duda et al., 2000). Initial studies focused on attempting to determine the effects of α-synuclein nitration on the protein and its aggregation. Exposure of human recombinant α-synuclein to nitrate and oxidative agents results in the formation of α-synuclein aggregates (Souza et al., 2000; Paxinou et al., 2001). These aggregates are in the form of dimers, trimers and oligomers, suggesting cross-linking between nitrated tyrosine residues (Souza et al., 2000; Takahashi et al., 2002.)

To examine how concentration of nitrating agent affects the formation of aggregates, Souza and colleagues expose the cells to an increasing proportion of nitrating agent (Souza et al., 2000). Increasing the ratio of nitrating agent, such as peroxynitrite/CO2, to protein not only results in increased nitration of dimers and trimers, but also in an augmentation in insoluble oligomers. Further examination of the protein reveals that it is nitrated at tyrosine 39, as well as tyrosine 125, 133, and 136. Tyrosine-125, however, appears to have a critical role in α-synuclein dimerization, as a lack of this residue significantly decreases dimerization compared to α-synuclein lacking tyrosine 39, 133 or 136 (Souza et al., 2000).

Effects of Nitrative Stress

Previous studies have established that nitrative and oxidative stress lead to the formation of aggregates in α-synuclein transfected cells. It has been hypothesized that tyrosine cross-linking may be the cause of this event. To test this hypothesis, Norris et al. mutated tyrosine residues and examined fibril formation in these mutant proteins. Results showed that one or more tyrosine residues are required for cross-linking resulting from the exposure to nitrating agents. Tyrosine residues are not required, however, for cross-linking resulting from the exposure to oxidative agents. Furthermore, the formation of aggregates after nitrative and oxidative stress in cells requires tyrosine residues (Norris et al., 2003).

Norris et al. propose a model to explain the role of nitration and oxidation in the formation of aggregates of α-synuclein. It is proposed that soluble-α-synuclein can be formed as a result of both nitration and oxidation. Nitration-induced oligomers and nitrated monomers, however, are incapable of assembling fibrils, the intermediate to aggregates. α-synuclein monomers and oligomers that have been modified due to oxidation, however, are capable of forming fibrils, as they undergo a structural change. These monomers and oligomers go from an α-helix or random coil conformation to a β-sheet conformation. Sheets are then capable of assembling into protofibrils and eventually fibrils. Both nitrative and oxidative modifications resulting in cross-links can stabilize the filaments, which can then aggregate with other intracellular proteins to form aggregates.

Further examination of the modification of α-synuclein upon nitration indicates that nitration leads to α-synuclein’s formation of a rapidly folded conformation of the protein with an increased secondary structure. This modification is a critical step in the formation of fibrils, as a non-modified protein does not have the ability to fibrillize.

Lipid Binding

Nitration has been found to modify α-synuclein’s structure and aggregate formation. Recent studies also implicate nitration as the cause of diminished lipid binding to vesicles. This is important because the interaction of α-synuclein with lipid vesicles is believed to be critical in the regulation of neurotransmission at presynaptic terminals. Examination of the protein in the presence of lipid vesicles shows that purified monomer-nitrated α-synuclein did bind to lipid vesicles at a diminished rate.

Furthermore, association of α-synuclein with biological membranes protects the protein from oxidation and nitration, thereby decreasing the formation of molecules able to form aggregates.

Summary: Effects of α-synuclein modification

Several studies have focused on examining Lewy bodies. PD aggregates are phosphorylated at several residues; however, ser-129 is the major phosphorylation site of α-synuclein in Lewy bodies (Fujisawa et al., 2002). CK1, CK2 and Gprk2 and Src protein-tyrosine kinase family members phosphorylate the protein in vitro (Chen et al., 2005; Ellis et al., 2001; Okochi et al., 2000). The interaction between α-synuclein and another protein synphilin-1 is critical to aggregate formation and is phosphorylation dependent (Lee et al., 2005). The question of which protein’s phosphorylation is crucial to this aggregation is still under debate (Lee et al., 2004; Smith et al., 2005).

Nitrated α-synuclein has also been shown to be a major component of Lewy bodies. The protein is nitrated at tyrosine 39, as well as tyrosine 125, 133, and 136. Tyrosine-125 though, appears to have a critical role in α-synuclein dimerization, as a lack of this residue significantly decreases dimerization compared to α-synuclein lacking tyrosine 39, 133 or 136 (Souza et al., 2000).

α-synuclein has been found to be modified as a result of nitration and phosphorylation. Modifications include the change in conformation seen in the protein,
which allows it to aggregate more readily. The nitration and phosphorylation of α-synuclein result in the increased aggregation of the protein (Chen et al., 2005; Norris et al., 2003; Smith et al., 2005; Fujiwara et al., 2002).

The relation of aggregation to toxicity has yet to be discovered. There are still conflicting studies concerning aggregate formation and its correlation to toxicity. While some studies suggest that phosphorylation of the protein increases aggregate formation and increases toxicity (Chen et al., 2000).

Future Research
As previously stated, the link between aggregation and toxicity in post-translation mutants is still under debate. Post-translational modification, such as phosphorylation and nitration, increase the rate of fibrillation and aggregate formation of α-synuclein. If this increased aggregation is linked to diminished toxicity, then perhaps these modifications serve a protective purpose.

Moreover, it is necessary to understand if there is a relationship between post-translational modifications and neurodegeneration, as this relationship is not yet fully understood. Nitrosylation-deficient mutants exhibit significant toxicity (Herrera et al., 2005).

Conclusion
Parkinson’s disease is the second most common neurodegenerative disease in the world, affecting millions of people. Extensively phosphorylated and nitrated α-synuclein has been found in PD aggregates known as Lewy Bodies. Recent studies show that these post-translational modifications of α-synuclein increase the rate of fibril formation and increase the rate of aggregate formation. Examinations of the role of phosphorylation and nitration on toxicity have not come to conclusive results, therefore, future research is necessary to understand these post-translational modifications’ role in neurodegeneration.

Research has shed light on the effects of phosphorylation and nitration of α-synuclein on this protein’s aggregation and misfolding, providing important evidence of this protein’s role in the pathogenesis of PD.

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References


