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Review

Methionine oxidation, α-synuclein and Parkinson's disease

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Abstract

The aggregation of normally soluble α-synuclein in the dopaminergic neurons of the substantia nigra is a crucial step in the pathogenesis of Parkinson's disease. Oxidative stress is believed to be a contributing factor in this disorder. Because it lacks Trp and Cys residues, mild oxidation of α-synuclein in vitro with hydrogen peroxide selectively converts all four methionine residues to the corresponding sulfoxides. Both oxidized and non-oxidized α-synucleins have similar unfolded conformations; however, the fibrillation of α-synuclein at physiological pH is completely inhibited by methionine oxidation. The inhibition results from stabilization of soluble oligomers of Met-oxidized α-synuclein. Furthermore, the Met-oxidized protein also inhibits fibrillation of unmodified α-synuclein. The degree of inhibition of fibrillation by Met-oxidized α-synuclein is proportional to the number of oxidized methionines. However, the presence of metals can completely overcome the inhibition of fibrillation of the Met-oxidized α-synuclein. Since oligomers of aggregated α-synuclein may be cytotoxic, these findings indicate that both oxidative stress and environmental metal pollution could play an important role in the aggregation of α-synuclein, and hence possibly Parkinson's disease. In addition, if the level of Met-oxidized α-synuclein was under the control of methionine sulfoxide reductase (Msr), then this could also be factor in the disease.

Keywords: Methionine oxidation; α-Synuclein; Parkinson's disease

1. Introduction

Many studies have indicated that oxidative stress is a risk factor for dopamine cell degeneration in Parkinson's disease (PD) [1,13,38]. The healthy brain continuously generates high levels of reactive oxygen and nitrogen species. Paradoxically, it is least able to handle the high levels of reactive oxygen species (ROS) because of low levels of both antioxidant enzymes and cellular antioxidants [1,45]. The brain uses about 25% of respired oxygen even though it constitutes only 5% of body weight. Diseases involving oxidative stress can result from ineffective scavenger systems, insufficient concentrations of antioxidants, overproduction of free radicals or other oxidants, or a combination of all of these. Also, neurons have a very high membrane/volume ratio due to their unique shape, i.e., long thin extensions of axons and dendrites, and small cell bodies. Brain membranes have high levels of polyunsaturated fatty acids and thus are prime targets for oxygen and free radical damage. During normal respiration, the mitochondria generate water from oxygen, but also produce superoxide anion, hydrogen peroxide and hydroxyl free radical. In addition, the dopaminergic neurons in the substantia nigra, the region of the brain that is most affected in Parkinson's disease, have a special sensitivity to free radicals, due to their high levels of dopamine [9] and Fe^{2+} [43,65].

The deposition of protein fibrils (amyloid) is a prominent feature of a number of protein conformational diseases or protein deposition diseases, including Alzheimer's disease (AD), Parkinson's disease, motor neuron disease (ALS), the prion diseases and many others (reviewed in Refs. [2,15,46,80,103]). Under normal cellular conditions, misfolded proteins are rapidly sequestered

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or eliminated from the intracellular environment by two different pathways, molecular chaperones or the ubiquitin proteasomal system. However, under particular pathological conditions, misfolded proteins may aggregate, initiating a cascade of events that leads eventually to cell death [3,10,17,25,29,40,41,48,49,76]. The aggregation process may be a consequence of increased levels of protein misfolding, abnormalities in the ubiquitin–proteasome system for removing damaged proteins, or problems with the chaperone systems, or a combination of all three.

Parkinson's disease, the second most common neurodegenerative disease, results from death of dopaminergic neurons in the substantia nigra. Some surviving nigral dopaminergic neurons contain cytosolic filamentous inclusions known as Lewy bodies (LBs) and Lewy neurites (LNs) [21]. Besides the substantia nigra, LBs and LNs also are found in other brain regions, such as the dorsal motor nucleus of the vagus, the nucleus basalis of Meynert, and the locus coeruleus [21]. In addition, abundant LBs and LNs in the cerebral cortex are neuropathological hallmarks of dementia with LBs, a common late-life dementia that is clinically similar to Alzheimer's disease [75], in LB variant of Alzheimer's disease [100], diffuse LB disease [98], multiple system atrophy [60,100], and several other neurodegenerative disorders, collectively known as synucleinopathies [28,101]. The major fibrillar material of LBs and LNs is α-synuclein [90].

The discovery that a missense mutation in the α-synuclein gene resulted in autosomal dominantly inherited PD, and that this was accompanied by the accumulation of α-synuclein aggregates, sparked specific interest in the role of α-synuclein in this disease. Three different missense mutations in the α-synuclein gene, corresponding to A53T, A30P and E46K substitutions in α-synuclein, have now been identified in autosomal-dominantly inherited, early-onset Parkinson's disease [52,77,116]. The recent finding that triplication of the α-synuclein gene locus causes autosomal dominant PD with an average age of onset of 34 years [87] confirmed the critical role of α-synuclein aggregation in the etiology of PD [101]. The production of wild-type (WT) human α-synuclein in transgenic mice [62] or of WT, A30P, and A53T human α-synuclein in transgenic flies [20] leads to motor deficits and neuronal inclusions reminiscent of PD. All three proteins, as well as several C-terminal-truncated forms of recombinant α-synuclein, are able to assemble readily into filaments in vitro, with morphologies and staining characteristics similar to those extracted from disease-affected brain [11,84,90,112]. Furthermore, fibrils formed in vitro from α-synuclein and the familial mutant forms linked to Parkinson's disease are typical amyloid fibrils, i.e., structurally and morphologically they resemble fibrils formed by other amyloidogenic proteins. Interestingly, the peptide derived from the central hydrophobic region of α-synuclein represents a constituent of Alzheimer's plaques. This 35-amino-acid peptide, known as NAC (Non-Aβ Component of Alzheimer's disease amyloid), was shown to amount to about 10% of the amyloid plaque [102]. Recently, synergistic interactions in the fibrillation of tau (involved in Alzheimer's disease) and α-synuclein have been reported [26]. These observations indicate that α-synuclein is a key player in the pathogenesis of several neurodegenerative disorders.

α-Synuclein is a small, acidic, natively unfolded (intrinsically disordered) and soluble protein that is found both in the cytosol and associated with presynaptic vesicles [106,111]. It contains 140 amino acid residues, which can be grouped in three regions [61]: The N-terminal region consisting of 60 residues containing four 11-amino-acid imperfect repeats with a hexameric consensus motif (KTKEGV), a central region comprising the amyloidogenic NAC sequence (residues 61–95) with two additional repeats, and the C-terminal region (residues 96–140), rich in acidic residues and prolines, suggesting a disordered conformation and containing three conserved tyrosine residues.

The function of α-synuclein is unknown but its presence in presynaptic nerve terminals and its interaction with lipids and proteins suggest multiple functions, including lipid vesicle trafficking. Recent investigations of several disease-causing amyloidogenic proteins, including α-synuclein, suggest that the cytotoxic species is probably not the actual fibrils, but rather, some earlier oligomeric species [7,8,19,36,44,47,54,55,64,78,117]. It is likely that such oligomers lead to membrane permeability and hence cell death.

Numerous observations suggest that oxidative stress may be associated with Parkinson's disease; for example, inhibitors of mitochondrial function (such as MPTP), which lead to release of reactive oxygen species (ROS), result in neuronal degeneration and loss of dopaminergic neurons. All amino acids are susceptible to oxidation, although their reactivities vary greatly [92,93]. Methionine and cysteine are the most readily oxidized amino acids and are unique in that the products of oxidation can, under some circumstances, be reduced back to the native amino acid residues. For example, methionine is easily oxidized to methionine sulfoxide (MetO) by H_2O_2, hypochlorite, chloramines, and peroxynitrite; all these oxidants are produced in biological systems [108]. However, this modification can be repaired by methionine sulfoxide reductase (Msr), which catalyzes the thioredoxin-dependent reduction of MetO back to methionine, both in vitro [70,97] and in vivo [66,70]. The details of Msr action are described below in the section on Msr.

The nature of the relationship between protein oxidation, protein aggregation, and neurodegeneration in Parkinson's disease are still unclear. There is very good data to support the premise that the aggregation of α-synuclein is a critical component of the etiology of PD, and substantial evidence suggests that oxidative stress is associated with PD; however, there are as yet no unambiguous data regarding the connection between α-synuclein oxidation and its aggregation and cytotoxicity. In fact, there are many