NIH Public Access
Author Manuscript
Free Radic Biol Med. Author manuscript; available in PMC 2009 May 15.

Published in final edited form as:
Free Radic Biol Med. 2008 May 15; 44(10): 1787–1794.

Oxidative and Nitrative Protein Modifications in Parkinson's Disease

Steven R. Danielson[*] and **Julie K. Andersen**[*]

[*]*Buck Institute For Age Research, 8001 Redwood Blvd., Novato, CA, USA*

Abstract

Parkinson's disease (PD) is a complex neurodegenerative syndrome likely involving contributions from various factors in individuals including genetic susceptibility, exposure to environmental toxins, and the aging process itself. Increased oxidative stress appears to be a common causative aspect involved in the preferential loss of dopaminergic neurons in a region of the brain prominently affected by the disorder, the substantia nigra (SN). Loss of dopaminergic SN neurons is responsible for the classic clinical motor symptoms associated with PD. Several oxidative and nitrative post-translational modifications (PTMs) have been identified on proteins pertinent to PD that may affect this or other aspects of disease progression. In this review, we discuss several examples of such PTMs to illustrate their potential consequences in terms of initiation or progression of PD neuropathophysiology.

Keywords

Oxidation; nitration; HNE; 3NT; post-translational modification; Parkinson's disease

Introduction

While oxygen in its many states is a requirement for life, dismutation of molecular oxygen leads to the formation of more reactive oxygen species that can lead to extensive damage to various cellular macromolecules including DNA, lipids and proteins contributing to the general process of aging as well as to various age-related diseases. Oxidative stress has indeed emerged as a common causative factor in the pathology of several neurodegenerative disorders including Parkinson's disease (PD) [1].

PD, the second most common neurodegenerative disease after Alzheimer's, is characterized by the preferential loss of dopaminergic neurons in a region of the midbrain known as the substantia nigra (SN) and the presence of α-synuclein-containing inclusion bodies (referred to as Lewy bodies) in the cytoplasm of neurons in various regions of the brain including the SN, olfactory bulb, neocortex, and brain stem [2]. Whether oxidative/nitrative stress is a primary trigger for PD or merely a byproduct of cellular dysfunction is currently unknown, however several related PTMs identified on proteins with relevance to PD suggest that such modifications may indeed contribute to disease neuropathology. These oxidative and nitrative PTMs and their significance for PD are discussed in this review and summarized in Table 1.

Address Correspondence to: Dr. Julie K. Andersen, Buck Institute For Age Research, 8001 Redwood Blvd., Novato, CA 94945, USA, Email: jandersen@buckinstitute.org Tel: 415 209-2070, Fax: 415 209-2231.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Evidence for Oxidative and Nitrative Stress in PD

Extensive evidence has accumulated particularly from postmortem brain tissues which suggests that oxidative and nitrative stress is an integral factor in neurodegeneration of the SN associated with PD [3]. Decreased levels of the antioxidant glutathione (GSH) in the SN, for instance, is one of the earliest biochemical changes observed in the disease [4–6]. The PD SN also displays increased levels of lipid peroxidation as indicated by decreased levels of polyunsaturated fatty acids (substrates for lipid peroxidation) and increased levels of malondialdehyde (MDA) [7] and 4-hydroxy-2-nonenal (HNE) [8]. Another marker of oxidative stress, the nucleic acid oxidation product 8-hydroxyguanosine (8OHG), is elevated in affected neurons compared to surrounding brain regions in the PD brain as well as in comparison to the SN of age-matched controls [9,10]. A widely used assay to measure general protein oxidation involves the reaction of 2,4-dinitrophenylhydrazine with carbonyl groups on proteins which can then be quantified spectrophotometrically. This method has been used to demonstrate the presence of increased levels of oxidized protein carbonyls in the SN of PD patients [11,12]. Oxidative damage to proteins also occurs through the nitration of tyrosine residues by peroxynitrite formed from the reaction of nitric oxide with superoxide radicals or through nitrite-dependent peroxidase activity [13]. Antibodies specific for 3-nitrotyrosine (3NT) have been developed and used to demonstrate a qualitative increase in the nitration of proteins within Lewy bodies in the PD brain [14,15]. Increased 3NT levels have also been detected in baboons [16] and mice [17] following systemic administration of the PD-inducing toxin 1-methyl-4-phenyl-1,2,3,6-tetrapyridine (MPTP).

Some Possible Sources of Oxidative/Nitrative Stress in PD

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) can be generated by many biological processes. Nitric oxide (NO) can be formed by the enzyme nitric oxide synthase (NOS). Inhibition of the neural isoform NOS1 in baboons or the inducible isoform NOS2 (expressed in glial cells) in mice has been found to enhance resistance to MPTP toxicity [18–20], indicating that NO is required for MPTP-induced cell death. Iron, which can drive the formation of the reactive hydroxyl radical via Fenton chemistry, is increased in the SN of PD patients [21–23]. If this iron is present in a free state it can contribute to ROS/RNS production and subsequent neurodegeneration. Environmental toxins such as rotenone, MPTP and paraquat, all known environmental risk factors for PD, are capable of generating oxidative stress in several ways, either directly or indirectly by inhibiting complex I [24,25], activation of microglia, or through formation of α-synuclein aggregates [26].

The Possible Role of Dopamine Oxidation in PD

Scientific evidence from as early as 1895 suggested that the SN was likely involved in PD [27]. This finding was followed by the discovery of proteinacious inclusion bodies in the brains of PD patients [28] and lesions of the SN [29]. In the 1960's it was determined that there is a severe decrease in the levels of neurotransmitter dopamine in the corpus striatum and SN of PD patients [30]. Subsequently, this dopamine deficiency was connected with the loss of dopaminergic cells in the SN [31]. This led to the use of levodopa (L-DOPA, a dopamine precursor) as a treatment for PD [32,33]. Dopamine is chemically reactive and is the most predisposed of the catacholamines towards oxidation [34]. It can, for example, be metabolized by monoamine oxidase (MAO) which also creates H_2O_2 as a byproduct of the reaction [35]. Dopamine oxidation can also create dopamine quinone which can directly modify proteins [36]. Various catecholamine oxidation products including DOPAC (3,4-dihydroxyphenylacetic acid), formed via monoamine oxidase-B-mediated dopamine oxidation to 3'-4'-dihydrophenylaldehyde and further conversion via an aldehyde dehydrogenase, readily form adducts with GSH and cysteine. Increased levels of free 5-S-cysteinyl-

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

catecholamine adducts have been found in the SN of PD patients and have been reported to result in inhibition of mitochondrial complex I, a hallmark of the disease [37]. Rat nigral cells treated with 5-S-cysteinyl-catecholamines were reported to have elevated levels of DNA damage including 8OHG [38]. Another product of dopamine oxidation, 7-(2-aminoethyl)-3,4-dihydro-5-hydroxy-2H-1,4benzothiazine-3-carboxylic acid or DHBT-1, has also been reported to be an irreversible inhibitor of complex I activity, resulting in subsequent oxidative stress [39]. Dopamine oxidation and reaction with electrophiles such as cysteine and glutathione however is not necessarily neurotoxic as it constitutes the pathway for neuromelanin production which is formed as a function of the normal aging process.

Specific Oxidative/Nitrative PTMs of Proteins Associated with PD

α-Synuclein

The function of α-synuclein and its role in PD has been heavily studied since the discovery of a dominant mutation in the α-synuclein gene in a number of Greek and Sicilian families with a familial form of the disorder [40]. This discovery was soon followed by its identification as a major component of Lewy bodies in sporadic cases of the disease [41]. The exact function of α-synuclein and the mechanism by which it aggregates to form soluble oligomers, soluble protofibrils and insoluble fibrils (the form contained in Lewy bodies) is unknown. It is also still controversial as to whether the fibrils contribute to disease pathology or whether this form of the protein acts as a sink to eliminate toxic soluble oligomers and/or protofibrils. Rotenone, paraquat, nitrating agents as well as expression of the human familial mutation A53T have all been reported to result in increased aggregation of α-synuclein [42–45].

Lewy bodies appear to qualitatively contain high levels of nitrated tyrosine residues as indicated by antibodies specific for 3NT [14]. Nitrated α-synuclein has also been detected in the SN and ventral midbrain of mice treated with MPTP [46]. Recombinant α-synuclein treated with nitrating agents (peroxynitrite/CO_2 myeloperoxidase/H_2O_2/nitrite) generates highly stable nitrated α-synuclein oligomers as a consequence of oxidation resulting in the formation of crosslinked o-o'-ditryrosine [47]. This form of aggregated α-synuclein is resistant to proteolysis, perhaps due to its structural stabilization. Further work from Virginia Lee and collaborators using antibodies specific for nitrated tyrosine residues on α-synuclein demonstrated that α- synuclein in particular is a target for nitrating agents and that nitrated α-synuclein is present in the Lewy bodies of postmortem tissues from PD patients as well as other neurodegenerative synucleinopathies [48]. Human α-synuclein contains four tyrosine residues (Tyr39, Tyr125, Tyr133, Tyr136), each of which when independently mutated to phenylalanine (particularly Tyr125), play a vital role in α-synuclein aggregation induced by peroxynitrite [49]. α-Synuclein has been suggested to function in the regulation of synaptic vesicles and its 3NT modification at Tyr39 has been demonstrated to significantly decrease the binding of α-synuclein to synthetic vesicles and to decrease the rate at which it is degraded [50]. PTMs identified on α-synuclein in rotenone-treated cultured PC12 cells include 3NT as well as aminotyrosines at Tyr133 and Tyr136 [51]. A recent paper from Benner *et. al.* has suggested the possibility that nitrated α-synuclein may escape the immune privileged area of the brain into lymphoid tissue and elicit an immune response. Mice treated with MPTP were found to generate antibodies against both un-modified and nitrated α -synuclein. A strong immune response was observed when mice were immunized with the nitrated c-terminal epitope of α-synuclein but not upon immunization with the un-modified protein. Nitrated α-synuclein activated T cells transferred to MPTP-treated mice enhancing microglial activation and degeneration of dopaminergic neurons. Mice with low levels of B and T cells were found to have decreased MPTP-induced neurodegeneration. This suggests that oxidative/nitrative PTMs may cause an adaptive immune response that augments the neuropathology in PD [52].

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

In addition to tyrosine nitration, rotenone treatment of cultured cells was also found to result in the formation of a methionine sulfone at Met127 of α-synuclein. Methionine residues can be readily oxidized to methionine sulfoxide through the addition of oxygen to the reactive sulfur atom. The reverse reduction reaction is catalyzed by a family of enzymes called methionine sulfoxide reductases (MSRs). In a α-synuclein *Drosophila* model, overexpression of MSRA was found to prevent the decreased locomotor activity and shortened lifespan caused by elevated α-synuclein [53].

Other identified PTMs of α-synuclein may also be a consequence of increased oxidative stress. The most common PTM found on fibrils of α-synuclein within Lewy bodies is phosphorylation of serine-129 [54]. This phosphorylation likely occurs as a consequence of oxidative kinase induction, as its levels are found to be increased after exposure of cultured SH-SY5Y cells to H_20_2 [55]; phosphorylation of this serine also appears to promote α-synuclein fibril formation *in vitro* [56].

Dopamine adducts on α-synuclein have been reported to stabilize protofibril formation possibly enhancing toxicity and progression of PD [57]. In addition to being degraded by the proteasome, wildtype α-synuclein can also be degraded by lysosomes through chaperone-mediated autophagy (CMA). Mutations in α-synuclein have been found to inhibit degradation of α-synuclein by the CMA. Dopamine-modified α-synuclein not only inhibited its own degradation by the CMA but also impairs the degradation of other proteins suggesting why dopaminergic cells may be particularly sensitive to various forms of stress leading to selective SN neurodegeneration in PD patients [58]. More recent data has suggested that dopamine oxidation products on alpha-synuclein may not be derived by adduct formation but rather via noncovalent interaction of oxidized catechols with the protein which may result in its inappropriate C-terminal cleavage and/or conformational changes that impact on its oligomerization [59,60].

A final PTM of α-synuclein is a byproduct of lipid peroxidation malondialdehyde (MDA) which can conjugate with lysine residues of proteins (MDA-Lys). α-Synuclein isolated post-mortem from the frontal cortex and SN of PD patients has been shown to contain this modification. The extent to which this is a PD specific modification is unknown as one control cortex sample and one control SN sample were found to have α-synuclein containing MDA-Lys [61].

Parkin

Parkin is a ubiquitin E3 ligase encoded by the PARK2 gene [62]. Recessive mutations in the gene are responsible for approximately half of all familial juvenile and early-onset cases of parkinsonism [2]. Overexpression of mutant forms of parkin in NT-2 or SK-NMC cell lines have been shown to increase levels of oxidative damage to proteins and lipids specifically protein carbonyl, lipid peroxidation and 3NT levels. Interestingly, increased oxidative stress did not change the anti-oxidant activity of SOD1, SOD2, catalase, glutathione peroxidase or glutathione reductase however glutathione levels did decrease [63]. Parkin −/− mice exhibited decreased serum antioxidant capacity, increased protein carbonyl levels and an age-dependent increase of HNE immunostaining in the parkin −/− brain [64]. Parkin has been suggested to be S-nitrosylated in MPTP-treated mice and in human PD and DLBD patients. S-nitrosylation results in decreased autoubiquitination of Parkin as well as decreased ubquitination of synphilin-1 in HEK293 cells [65]. Further work will need to be done to establish what proportion of Parkin is S-nitrosylated and the functional impact of this modification on other substrates.

It has been demonstrated that when dopaminergic MES23.5 neuronal cells or cell lysates are incubated with 250 uM ^{14}[C]-dopamine, parkin but not DJ-1 (see below) becomes modified

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

by dopamine resulting in a decrease of Parkin's E3 ligase activity. Catechol-modified parkin was also found in the SN of normal human brain; whether levels are altered in the SN of PD patients is currently not clear [34].

DJ-1

Mutations and deletions in the PARK7 gene that encodes DJ-1 [66] account for less than 1% of all familial cases of PD [2]. Several potential functions have been attributed to DJ-1 including roles in mRNA regulation, as a chaperone, a protease and antioxidant [67]. Its role as an antioxidant has garnered the most interest since it had previously been shown that DJ-1 is converted to variants with a lower pI after treatment with hydroperoxides [68]. It was subsequently reported in a cell culture model that the downregulation of DJ-1 enhanced cell death via oxidative stress [69]. DJ-1 exists as a homodimeric protein with an approximate MW of 39 kD but migrates as a monomer (~20 kD) after 1D SDS-PAGE due to denaturation by the detergent. As many as 10 different isoforms of DJ-1 may exist, 6 monomeric versions with isoelectric points ranging from 5.5 to 6.4 and 4 SDS-resistant dimeric forms with isoelectric points ranging from 7.0 to 8.4 [70]. In one study all monomeric forms of DJ-1 were found to be widely expressed in human frontal cortex, particularly in astrocytes although the most alkaline isoform was reduced in abundance in the cortex of PD brains [71]. Subsequent reports indicated that the acidic monomer isoforms of DJ-1 (pI 5.5 and 5.7) and the basic dimer isoforms (pI 8.0 and 8.4) were increased in the frontal cortex of PD and Alzheimer's disease patients as compared to controls. Overall the total expression of DJ-1 appears to be elevated in PD and AD brains [70]. Similarly Meulener *et al.* found levels of the most acidic isoforms of DJ-1 increase (~10–30%) with age in flies, mice and humans [72]. General measurements of carbonyl levels in samples only show an overall increase in oxidative stress but do not indicate if there was a specific target with higher sensitivity to oxidative stress. By first isolating DJ-1 from post mortem PD brains and then assaying for carbonyl levels, Choi *et. al.* was able to determine that there was a significant increase in carbonyl levels in the one monomeric isoform (pI 6.4) and three dimeric isoforms (pI 7.4, 8.0 and 8.4) of DJ-1 [70].

Employing a mass spectrometry approach, Kinumi *et al.* analyzed the structural modifications of the acidic isoform of DJ-1 taken from whole cell extract of H_2O_2 treated human umbilical vein endothelial cells run on 2D gels. DJ-1 contains 3 cysteines (amino acid numbers 46, 53, and 106). All 3 cysteines were found to be oxidized to cysteine sulfonic (SO_3H) acid to various extents. Treatment with 100 μM H_2O_2 for 1 hour oxidized a small percentage of Cys46 and Cys53 while Cys106 was found to be completely oxidized. This suggests that Cys106 is the most sensitive cysteine to oxidation and thus may be important for the acidification and function of DJ-1 [73]. Oxidation of Cys106 has been suggested to amongst other things predispose DJ-1 to cleavage between glycine 157 and proline 158 residues [74]. The Drosophila homolog (DJ-1b) of human DJ-1 also functions as an antioxidant and contains a cysteine at amino acid 104 which is analogous to Cys106 in human DJ-1. It has been determined that this cysteine (Cys104) is similarly oxidized to sulfinic (SO_2H) and sulfonic acid in Drosophila [72]. Oxidation of Cys53 to sulfonic acid was detected in both control and PD frontal cortex brain samples from age-matched individuals (5 controls 65–87 and 5 PD 66–79 years old) indicating that some level of age-related oxidation is normal. This same study also identified the presence of an oxidized methionine Met133 to methionine sulfone in DJ-1 which was detected in PD but not control samples via mass spectrometry [70]. The relative levels of oxidized and unoxidized DJ-1 may be informative for determining if some threshold of oxidized DJ-1 levels must be overcome to induce oxidative stress.

By systematically mutating the 3 DJ-1 cysteine residues Canet-Aviles *et al.* confirmed that Cys106 is the critical residue oxidized to cysteine-sulfinic acid resulting in the acidic shift in pI of DJ-1. They also demonstrated in a separate PD model (MPP^+ treatment of neuroblastoma

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

cells) that acidification of DJ-1 occurred [75] consistent with earlier findings that acidification of DJ-1 by MPP^+ is caused by increased oxidation of Cys106 [76]. Interestingly, targeted mutagenesis in SH-SY5Y cells treated with 0.25 or 1 mM S-nitroglutathione identified S-nitrosylation of DJ-1 at Cys46 and Cys53, but not Cys106 suggesting these residues are differentially sensitive to nitrosylation. S-nitrosylation of Cys46 decreased the levels of homodimeric isoforms of DJ-1 [77] but the consequences of these modifications in terms of DJ-1 function as an antioxidant or any other function are unknown.

PINK1

PTEN-induced kinase 1 (PINK1) is a widely expressed and mitochondrially-targeted protein whose function is still unclear. Mutations in PINK1 account for about 1–2% of early onset PD [2]. Homozygous expression of the PD-associated G302D mutation in PINK1 results in increased levels of lipid peroxidation and GSH levels and a mild decrease in complex I activity [78]. Inhibition of the *Drosophila* homolog of PINK1 (dPINK1) using RNAi resulted in a progressive loss of dopaminergic neurons that could be abrogated by expressing human PINK1 or through the addition of antioxidants [79]. This suggests that PINK1 may play a functional role in preventing neural loss through oxidative stress. Work from Pridgeon *et al*. identified a mitochondrial heat shock protein (Hsp75 or TNF receptor-associated protein 1, TRAP1) as a substrate for PINK1. Phosphorylation of TRAP1 by PINK1 protected cells from oxidative stress by preventing cytochrome c release from mitochondria suggesting that the serine/threonine kinase activity of PINK1 may be involved in preventing apoptotic neuronal cell death in PD [80].

Mitochondrial complex I

MPTP can selectively inhibit mitochondrial complex I activity [81,82]. Since this discovery the role of complex I in PD has been intensely studied. Post mortem SN samples from PD patients have a complex I deficiency indicating a link between PD and reduced complex I activity [83]. The cause of the selective complex I inhibition is unknown.

Several antioxidant molecules exhibit altered expression levels in PD. Total glutathione (GSH + GSSG) depletion is the earliest known indication of oxidative stress in PD, occurring prior to decreases in mitochondrial complex I activity and dopamine levels [4,5,84]. We previously demonstrated *in vitro* that decreased glutathione levels resulted in selective inhibition of complex I activity. Complex I activity could be restored to normal levels by adding the reducing agent dithiothreitol (DTT) suggesting that the reversible oxidation of cysteine residues could be the cause [85]. Previous studies have demonstrate that oxidation of GSH to GSSG can result in glutathionylation and inhibition of complex I [86,87]. However, given that both GSH and GSSG are decreased in PD, glutathionylation of complex I is unlikely to be the cause of complex I inhibition in the disease. In order to determine the oxidative or nitrative species involved in the observed CI inhibition, we used scavengers specific for various ROS or RNS [88,89]. Data from these studies indicate that following either acute or chronic glutathione depletion, complex I inhibition appears to be dependent on nitric oxide (NO)-related mechanisms. Since complex I inhibition was found in our system to be reversible via either DTT or restoring glutathione levels to normal in our cells, this suggests an S-nitrosylation event. Direct treatment of isolated mitochondria with peroxynitrite had previously been shown to lead to significant reductions on the activities of several components of the electron transport chain including complex I, none of which were not found to be DTT-reversible [90]. By isolating complex I from peroxynitrite-treated mitochondria via sucrose gradient separation Murray *et al.* demonstrated that at least 5 subunits of complex I are modified by 3NT. Subsequent analysis using mass spectrometry identified those subunits and in several cases the specific modified residues as NDUFS2, NDUFS8, NDUFB4 (Tyr46 3NT, Tyr50 3NT, Tyr51 3NT, Tyr128 3NT), NDUFA12 (Tyr48 3NT, Tyr49 3NT, Trp61 *N*-formylkynurenine), and

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NDUFA6 (Tyr122 3NT) [90]. While this demonstrates that 3NT modifications can occur in complex I as a consequence of peroxynitrite exposure, it does not prove that these modifications result in the observed complex I inhibition. Indeed, we also observe the presence of 3NT modifications in complex I subunits following glutathione depletion but as in our case enzymatic inhibition is reversible, this would argue against them playing a functional role in glutathione-mediated effects [91]. In further *in vivo* studies, we were able to demonstrate that inducible dopaminergic glutathione depletion in the adult animal results in S-nitrosylation of complex I subunits which coincides with a selective decrease in complex I activity and an age-dependent neurodegeneration of dopaminergic SN neurons [92]. Coupled with our *in vitro* data, this strongly implies that a possible mechanism of the hallmark complex I inhibition in these cells could be a consequence of early glutathione depletion.

Complex I immunocaptured from the frontal cortex of PD patients shows increased carbonyl levels as compared to age-matched controls [93]. This increase in carbonylation of complex I could be replicated by treating mitochondria from control frontal cortex samples with rotenone. Here again, the presence of such modifications does not prove whether or not they are functionally significant.

Inhibition of complex I results in two critical predicaments which can impede cell survival, decreased ATP production and increased ROS production. These two factors can augment and aggravate each other in a cyclic fashion amplifying the detrimental effect on cells and tissues. Using three models (SK-N-MC human neuroblastoma cells, rat midbrain slice cultures, and *in vivo* rat), chronic rotenone treatment was shown to cause oxidative damage (via increased total carbonyl levels) and dose-dependent cell death independent of ATP depletion [94]. ATP levels decreased only about 1/3 in SK-NMC cells treated with 100 nM rotenone, a concentration which was completely toxic after 2 days. Cells treated with 1 mM 2-deoxyglucose (2-DG) had a similar decrease in ATP levels with no corresponding toxicity or increase in carbonyl levels. Systemic rotenone infusion *in vivo* caused a slight increase in total protein carbonyl levels in the cortex and striatum but no increase in the cerebellum and hippocampus. The largest increase in carbonyl levels due to rotenone treatment occurred in the olfactory bulb and midbrain, the dopaminergic areas of the brain [94]. Cell type may be an important factor in terms of vulnerability to rotenone. Dopaminergic (MN9D) cells were found to be more sensitive than non-dopaminergic (MN9X) cells following chronic exposure to nanomolar levels of rotenone [95]. Enhanced sensitivity of dopaminergic cells to rotenone could be explained by either increased vulnerability to oxidative stress or the higher energy demand of dopaminergic cells.

Proteasome

Increased levels of oxidatively or nitratively damaged proteins may be due to either increased oxidative/nitrative stress levels or decreased elimination of modified proteins. The proteasome is a large protein complex that degrades a wide variety of proteins in an ATP-dependent fashion, after proteins have be targeted for proteolysis by the addition of several ubiquitin molecules via E3 ubiquitin ligases such as parkin [96]. Decreased activity of the proteasome has been proposed as a mechanism involved in the neurodegeneration associated with PD due to the accumulation of damaged proteins. This theory is supported by evidence that demonstrates that proteins modified by the lipid peroxidation product HNE are more slowly degraded by the proteasome [97]. High levels of HNE can decrease proteasomal activity [98] however, such high levels of HNE may never occur physiologically [99]. Mutations in α-synuclein associated with familial forms of PD also have decreased rates of elimination as compared to wildtype α-synuclein [100]. Selective inhibition of the proteasome by lactacystin or epoxomicin results in increased oxidative stress through increased protein carbonyls, lipid peroxidation and DNA damage [101]. Whether decreased proteasomal activity has a causative role or is a consequence of other mechanisms in PD is still unclear.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Tyrosine hydroxylase

One of the biochemical changes evident in PD patients is the severe decrease in dopamine levels in neurites emanating from the SN and innervating the striatum. Any detrimental effects on the activity of tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine biosynthesis, would be of obvious importance in terms of disease development. Work from Harry Ischiropoulos and colleagues has indicated that TH can be selectively nitrated by either peroxynitrite or MPP^+ at tyrosine residues resulting in the inhibition of TH activity [102]. Subsequent work from the laboratory has demonstrated that nitration of tyrosine residue 423 in particular is critical for the observed reduction in TH activity [103]. Conversely, work from Donald Kuhn and associates suggest that while tyrosine residues are nitrated by peroxynitrite, the reduced TH activity is instead due to the oxidation of cysteine residues [104]. Subsequently Kuhn's lab has demonstrated that tyrosine nitration is prevented when TH is treated with peroxynitrite in the presence of excess cysteine or glutathione and that TH activity is repressed by Sthiolation [105]. Work from both laboratories clearly indicate that tyrosine residues on TH are nitrated after exposure to peroxynitrite. However it is not clear to what extent nitration versus cysteine thiolation has on the activity of TH; further experiments will be needed to clarify this issue.

Conclusions

Increased oxidative/nitrative stress has been linked with PD-associated neurodegeneration as well as several oxidative and nitrative PTMs on proteins pertinent to PD that may affect disease progression. The ever growing number of examples of such PTMs suggests that oxidative/nitrative stress is likely to play a major role in PD initiation or progression. Whether a common source exists for the various oxidative and nitrative modifications observed on the proteins discussed here is unknown. The identification of modifications that impact protein function and in doing so contribute to Parkinson's disease pathologies may prove valuable for diagnoses and preventative or therapeutic targets.

References

1. Andersen JK. Iron dysregulation and Parkinson's disease. J Alzheimers Dis 2004;6:S47–S52. [PubMed: 15665414]
2. Farrer MJ. Genetics of Parkinson disease: paradigm shifts and future prospects. Nat Rev Genet 2006;7:306–318. [PubMed: 16543934]
3. Jenner P, Dexter DT, Sian J, Schapira AH, Marsden CD. Oxidative stress as a cause of nigral cell death in Parkinson' and incidental Lewy body disease. The Royal Kings and Queens Parkinson' Disease Research Group. Ann Neurol 1992;32:S82–S87. [PubMed: 1510385]
4. Perry TL, Godin DV, Hansen S. Parkinson's disease: a disorder due to nigral glutathione deficiency? Neurosci Lett 1982;33:305–310. [PubMed: 7162692]
5. Perry TL, Yong VW. Idiopathic Parkinson's disease, progressive supranuclear palsy and glutathione metabolism in the substantia nigra of patients. Neurosci Lett 1986;67:269–274. [PubMed: 3737015]
6. Pearce RK, Owen A, Daniel S, Jenner P, Marsden CD. Alterations in the distribution of glutathione in the substantia nigra in Parkinson's disease. J Neural Transm 1997;104:661–677. [PubMed: 9444566]
7. Dexter DT, Carter CJ, Wells FR, Javoy-Agid F, Agid Y, Lees A, Jenner P, Marsden CD. Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. J Neurochem 1989;52:381–389. [PubMed: 2911023]
8. Yoritaka A, Hattori N, Uchida K, Tanaka M, Stadtman ER, Mizuno Y. Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson disease. Proc Natl Acad Sci U S A 1996;93:2696–2701. [PubMed: 8610103]
9. Alam ZI, Jenner A, Daniel SE, Lees AJ, Cairns N, Marsden CD, Jenner P, Halliwell B. Oxidative DNA damage in the parkinsonian brain: an apparent selective increase in 8-hydroxyguanine levels in substantia nigra. J Neurochem 1997;69:1196–1203. [PubMed: 9282943]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

10. Zhang J, Perry G, Smith MA, Robertson D, Olson SJ, Graham DG, Montine TJ. Parkinson's disease is associated with oxidative damage to cytoplasmic DNA and RNA in substantia nigra neurons. Am J Pathol 1999;154:1423–1429. [PubMed: 10329595]
11. Alam ZI, Daniel SE, Lees AJ, Marsden DC, Jenner P, Halliwell B. A generalised increase in protein carbonyls in the brain in Parkinson's but not incidental Lewy body disease. J Neurochem 1997;69:1326–1329. [PubMed: 9282961]
12. Floor E, Wetzel MG. Increased protein oxidation in human substantia nigra pars compacta in comparison with basal ganglia and prefrontal cortex measured with an improved dinitrophenylhydrazine assay. J Neurochem 1998;70:268–275. [PubMed: 9422371]
13. Turko IV, Murad F. Protein nitration in cardiovascular diseases. Pharmacol Rev 2002;54:619–634. [PubMed: 12429871]
14. Good PF, Hsu A, Werner P, Perl DP, Olanow CW. Protein nitration in Parkinson's disease. J Neuropathol Exp Neurol 1998;57:338–342. [PubMed: 9600227]
15. Duda JE, Giasson BI, Chen Q, Gur TL, Hurtig HI, Stern MB, Gollomp SM, Ischiropoulos H, Lee VM, Trojanowski JQ. Widespread nitration of pathological inclusions in neurodegenerative synucleinopathies. Am J Pathol 2000;157:1439–1445. [PubMed: 11073803]
16. Ferrante RJ, Hantraye P, Brouillet E, Beal MF. Increased nitrotyrosine immunoreactivity in substantia nigra neurons in MPTP treated baboons is blocked by inhibition of neuronal nitric oxide synthase. Brain Res 1999;823:177–182. [PubMed: 10095024]
17. Pennathur S, Jackson-Lewis V, Przedborski S, Heinecke JW. Mass spectrometric quantification of 3-nitrotyrosine, ortho-tyrosine, and o,o'-dityrosine in brain tissue of 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine-treated mice, a model of oxidative stress in Parkinson's disease. J Biol Chem 1999;274:34621–34628. [PubMed: 10574926]
18. Hantraye P, Brouillet E, Ferrante R, Palfi S, Dolan R, Matthews RT, Beal MF. Inhibition of neuronal nitric oxide synthase prevents MPTP-induced parkinsonism in baboons. Nat Med 1996;2:1017–1021. [PubMed: 8782460]
19. Liberatore GT, Jackson-Lewis V, Vukosavic S, Mandir AS, Vila M, McAuliffe WG, Dawson VL, Dawson TM, Przedborski S. Inducible nitric oxide synthase stimulates dopaminergic neurodegeneration in the MPTP model of Parkinson disease. Nat Med 1999;5:1403–1409. [PubMed: 10581083]
20. Wu DC, Jackson-Lewis V, Vila M, Tieu K, Teismann P, Vadseth C, Choi DK, Ischiropoulos H, Przedborski S. Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. J Neurosci 2002;22:1763–1771. [PubMed: 11880505]
21. Youdim MB, Ben-Shachar D, Riederer P. Is Parkinson's disease a progressive siderosis of substantia nigra resulting in iron and melanin induced neurodegeneration? Acta Neurol Scand Suppl 1989;126:47–54. [PubMed: 2618593]
22. Dexter DT, Wells FR, Lees AJ, Agid F, Agid Y, Jenner P, Marsden CD. Increased nigral iron content and alterations in other metal ions occurring in brain in Parkinson's disease. J Neurochem 1989;52:1830–1836. [PubMed: 2723638]
23. Sofic E, Paulus W, Jellinger K, Riederer P, Youdim MB. Selective increase of iron in substantia nigra zona compacta of parkinsonian brains. J Neurochem 1991;56:978–982. [PubMed: 1704426]
24. Turrens JF, Boveris A. Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. Biochem J 1980;191:421–427. [PubMed: 6263247]
25. Hasegawa E, Takeshige K, Oishi T, Murai Y, Minakami S. 1-Methyl-4-phenylpyridinium (MPP+) induces NADH-dependent superoxide formation and enhances NADH-dependent lipid peroxidation in bovine heart submitochondrial particles. Biochem Biophys Res Commun 1990;170:1049–1055. [PubMed: 2167668]
26. Ischiropoulos H, Beckman JS. Oxidative stress and nitration in neurodegeneration: cause, effect, or association? J Clin Invest 2003;111:163–169. [PubMed: 12531868]
27. Brissaud E. Nature et pathogenie de la maladie de Parkinson. Lecons sur les maladies nerveuses 1895:488–501.
28. Lewy, F. Paralysis agitans. I. Pathologiishe anatomie. In: Lewandowsky, M., editor. Handbuch der neurology. Springer: Berlin Germany; 1912. p. 920-933.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

29. Greenfield JG, Bosanquet FD. The brain-stem lesions in Parkinsonism. J Neurol Neurosurg Psychiatry 1953;16:213–226. [PubMed: 13109537]
30. Ehringer H, Hornykiewicz O. Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system. Klin Wochenschr 1960;38:1236–1239. [PubMed: 13726012]
31. Poirier LJ, Sourkes TL. Influence of the Substantia Nigra on the Catecholamine Content of the Striatum. Brain 1965;88:181–192. [PubMed: 14280273]
32. Cotzias GC, Papavasiliou PS, Gellene R. Modification of Parkinsonism--chronic treatment with L-dopa. N Engl J Med 1969;280:337–345. [PubMed: 4178641]
33. Savitt JM, Dawson VL, Dawson TM. Diagnosis and treatment of Parkinson disease: molecules to medicine. J Clin Invest 2006;116:1744–1754. [PubMed: 16823471]
34. LaVoie MJ, Ostaszewski BL, Weihofen A, Schlossmacher MG, Selkoe DJ. Dopamine covalently modifies and functionally inactivates parkin. Nat Med 2005;11:1214–1221. [PubMed: 16227987]
35. Adams RN, Murrill E, McCreery R, Blank L, Karolczak M. 6-Hydroxydopamine, a new oxidation mechanism. Eur J Pharmacol 1972;17:287–292. [PubMed: 4337349]
36. Stokes AH, Hastings TG, Vrana KE. Cytotoxic and genotoxic potential of dopamine. J Neurosci Res 1999;55:659–665. [PubMed: 10220107]
37. Spencer JP, Jenner P, Daniel SE, Lees AJ, Marsden DC, Halliwell B. Conjugates of catecholamines with cysteine and GSH in Parkinson's disease: possible mechanisms of formation involving reactive oxygen species. J Neurochem 1998;71:2112–2122. [PubMed: 9798937]
38. Spencer JP, Whiteman M, Jenner P, Halliwell B. 5-s-Cysteinyl-conjugates of catecholamines induce cell damage, extensive DNA base modification and increases in caspase-3 activity in neurons. J Neurochem 2002;81:122–129. [PubMed: 12067224]
39. Li H, Shen XM, Dryhurst G. Brain mitochondria catalyze the oxidation of 7-(2-aminoethyl)-3,4-dihydro-5-hydroxy-2H-1,4-benzothiazine-3-carboxyli c acid (DHBT-1) to intermediates that irreversibly inhibit complex I and scavenge glutathione: potential relevance to the pathogenesis of Parkinson's disease. J Neurochem 1998;71:2049–2062. [PubMed: 9798930]
40. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 1997;276:2045–2047. [PubMed: 9197268]
41. Baba M, Nakajo S, Tu PH, Tomita T, Nakaya K, Lee VM, Trojanowski JQ, Iwatsubo T. Aggregation of alpha-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies. Am J Pathol 1998;152:879–884. [PubMed: 9546347]
42. Lee HJ, Shin SY, Choi C, Lee YH, Lee SJ. Formation and removal of alpha-synuclein aggregates in cells exposed to mitochondrial inhibitors. J Biol Chem 2002;277:5411–5417. [PubMed: 11724769]
43. Manning-Bog AB, McCormack AL, Li J, Uversky VN, Fink AL, Di Monte DA. The herbicide paraquat causes up-regulation and aggregation of alpha-synuclein in mice: paraquat and alpha-synuclein. J Biol Chem 2002;277:1641–1644. [PubMed: 11707429]
44. Ostrerova-Golts N, Petrucelli L, Hardy J, Lee JM, Farer M, Wolozin B. The A53T alpha-synuclein mutation increases iron-dependent aggregation and toxicity. J Neurosci 2000;20:6048–6054. [PubMed: 10934254]
45. Paxinou E, Chen Q, Weisse M, Giasson BI, Norris EH, Rueter SM, Trojanowski JQ, Lee VM, Ischiropoulos H. Induction of alpha-synuclein aggregation by intracellular nitrative insult. J Neurosci 2001;21:8053–8061. [PubMed: 11588178]
46. Przedborski S, Chen Q, Vila M, Giasson BI, Djaldatti R, Vukosavic S, Souza JM, Jackson-Lewis V, Lee VM, Ischiropoulos H. Oxidative post-translational modifications of alpha-synuclein in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease. J Neurochem 2001;76:637–640. [PubMed: 11208927]
47. Souza JM, Giasson BI, Chen Q, Lee VM, Ischiropoulos H. Dityrosine cross-linking promotes formation of stable alpha -synuclein polymers. Implication of nitrative and oxidative stress in the pathogenesis of neurodegenerative synucleinopathies. J Biol Chem 2000;275:18344–18349. [PubMed: 10747881]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

48. Giasson BI, Duda JE, Murray IV, Chen Q, Souza JM, Hurtig HI, Ischiropoulos H, Trojanowski JQ, Lee VM. Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesions. Science 2000;290:985–989. [PubMed: 11062131]

49. Takahashi T, Yamashita H, Nakamura T, Nagano Y, Nakamura S. Tyrosine 125 of alpha-synuclein plays a critical role for dimerization following nitrative stress. Brain Res 2002;938:73–80. [PubMed: 12031537]

50. Hodara R, Norris EH, Giasson BI, Mishizen-Eberz AJ, Lynch DR, Lee VM, Ischiropoulos H. Functional consequences of alpha-synuclein tyrosine nitration: diminished binding to lipid vesicles and increased fibril formation. J Biol Chem 2004;279:47746–47753. [PubMed: 15364911]

51. Mirzaei H, Schieler JL, Rochet JC, Regnier F. Identification of rotenone-induced modifications in alpha-synuclein using affinity pull-down and tandem mass spectrometry. Anal Chem 2006;78:2422–2431. [PubMed: 16579629]

52. Benner EJ, Banerjee R, Reynolds AD, Sherman S, Pisarev VM, Tsiperson V, Nemachek C, Ciborowski P, Przedborski S, Mosley RL, Gendelman HE. Nitrated alpha-Synuclein Immunity Accelerates Degeneration of Nigral Dopaminergic Neurons. PLoS ONE 2008;3:e1376. [PubMed: 18167537]

53. Wassef R, Haenold R, Hansel A, Brot N, Heinemann SH, Hoshi T. Methionine sulfoxide reductase A and a dietary supplement S-methyl-L-cysteine prevent Parkinson's-like symptoms. J Neurosci 2007;27:12808–12816. [PubMed: 18032652]

54. Anderson JP, Walker DE, Goldstein JM, de Laat R, Banducci K, Caccavello RJ, Barbour R, Huang J, Kling K, Lee M, Diep L, Keim PS, Shen X, Chataway T, Schlossmacher MG, Seubert P, Schenk D, Sinha S, Gai WP, Chilcote TJ. Phosphorylation of Ser-129 is the dominant pathological modification of alpha-synuclein in familial and sporadic Lewy body disease. J Biol Chem 2006;281:29739–29752. [PubMed: 16847063]

55. Smith WW, Margolis RL, Li X, Troncoso JC, Lee MK, Dawson VL, Dawson TM, Iwatsubo T, Ross CA. Alpha-synuclein phosphorylation enhances eosinophilic cytoplasmic inclusion formation in SH-SY5Y cells. J Neurosci 2005;25:5544–5552. [PubMed: 15944382]

56. Fujiwara H, Hasegawa M, Dohmae N, Kawashima A, Masliah E, Goldberg MS, Shen J, Takio K, Iwatsubo T. alpha-Synuclein is phosphorylated in synucleinopathy lesions. Nat Cell Biol 2002;4:160–164. [PubMed: 11813001]

57. Conway KA, Rochet JC, Bieganski RM, Lansbury PT Jr. Kinetic stabilization of the alpha-synuclein protofibril by a dopamine-alpha-synuclein adduct. Science 2001;294:1346–1349. [PubMed: 11701929]

58. Martinez-Vicente M, Talloczy Z, Kaushik S, Massey AC, Mazzulli J, Mosharov EV, Hodara R, Fredenburg R, Wu DC, Follenzi A, Dauer W, Przedborski S, Ischiropoulos H, Lansbury PT, Sulzer D, Cuervo AM. Dopamine-modified alpha-synuclein blocks chaperone-mediated autophagy. J Clin Invest. 2008

59. Norris EH, Giasson BI, Hodara R, Xu S, Trojanowski JQ, Ischiropoulos H, Lee VM. Reversible inhibition of alpha-synuclein fibrillization by dopaminochrome-mediated conformational alterations. J Biol Chem 2005;280:21212–21219. [PubMed: 15817478]

60. Mazzulli JR, Armakola M, Dumoulin M, Parastatidis I, Ischiropoulos H. Cellular oligomerization of alpha-synuclein is determined by the interaction of oxidized catechols with a C-terminal sequence. J Biol Chem 2007;282:31621–31630. [PubMed: 17785456]

61. Dalfo E, Ferrer I. Early alpha-synuclein lipoxidation in neocortex in lewy body diseases. Neurobiol Aging. 2006

62. Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 1998;392:605–608. [PubMed: 9560156]

63. Hyun DH, Lee M, Hattori N, Kubo S, Mizuno Y, Halliwell B, Jenner P. Effect of wild-type or mutant Parkin on oxidative damage, nitric oxide, antioxidant defenses, and the proteasome. J Biol Chem 2002;277:28572–28577. [PubMed: 12034719]

64. Palacino JJ, Sagi D, Goldberg MS, Krauss S, Motz C, Wacker M, Klose J, Shen J. Mitochondrial dysfunction and oxidative damage in parkin-deficient mice. J Biol Chem 2004;279:18614–18622. [PubMed: 14985362]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

65. Chung KK, Thomas B, Li X, Pletnikova O, Troncoso JC, Marsh L, Dawson VL, Dawson TM. S-nitrosylation of parkin regulates ubiquitination and compromises parkin's protective function. Science 2004;304:1328–1331. [PubMed: 15105460]

66. Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, Krieger E, Dekker MC, Squitieri F, Ibanez P, Joosse M, van Dongen JW, Vanacore N, van Swieten JC, Brice A, Meco G, van Duijn CM, Oostra BA, Heutink P. Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. Science 2003;299:256–259. [PubMed: 12446870]

67. Cookson MR. The biochemistry of Parkinson's disease. Annu Rev Biochem 2005;74:29–52. [PubMed: 15952880]

68. Mitsumoto A, Nakagawa Y, Takeuchi A, Okawa K, Iwamatsu A, Takanezawa Y. Oxidized forms of peroxiredoxins and DJ-1 on two-dimensional gels increased in response to sublethal levels of paraquat. Free Radic Res 2001;35:301–310. [PubMed: 11697128]

69. Yokota T, Sugawara K, Ito K, Takahashi R, Ariga H, Mizusawa H. Down regulation of DJ-1 enhances cell death by oxidative stress, ER stress, and proteasome inhibition. Biochem Biophys Res Commun 2003;312:1342–1348. [PubMed: 14652021]

70. Choi J, Sullards MC, Olzmann JA, Rees HD, Weintraub ST, Bostwick DE, Gearing M, Levey AI, Chin LS, Li L. Oxidative damage of DJ-1 is linked to sporadic Parkinson and Alzheimer diseases. J Biol Chem 2006;281:10816–10824. [PubMed: 16517609]

71. Bandopadhyay R, Kingsbury AE, Cookson MR, Reid AR, Evans IM, Hope AD, Pittman AM, Lashley T, Canet-Aviles R, Miller DW, McLendon C, Strand C, Leonard AJ, Abou-Sleiman PM, Healy DG, Ariga H, Wood NW, de Silva R, Revesz T, Hardy JA, Lees AJ. The expression of DJ-1 (PARK7) in normal human CNS and idiopathic Parkinson's disease. Brain 2004;127:420–430. [PubMed: 14662519]

72. Meulener MC, Xu K, Thomson L, Ischiropoulos H, Bonini NM. Mutational analysis of DJ-1 in Drosophila implicates functional inactivation by oxidative damage and aging. Proc Natl Acad Sci U S A 2006;103:12517–12522. [PubMed: 16894167]

73. Kinumi T, Kimata J, Taira T, Ariga H, Niki E. Cysteine-106 of DJ-1 is the most sensitive cysteine residue to hydrogen peroxide-mediated oxidation in vivo in human umbilical vein endothelial cells. Biochem Biophys Res Commun 2004;317:722–728. [PubMed: 15081400]

74. Ooe H, Maita C, Maita H, Iguchi-Ariga SM, Ariga H. Specific cleavage of DJ-1 under an oxidative condition. Neurosci Lett 2006;406:165–168. [PubMed: 16935423]

75. Canet-Aviles RM, Wilson MA, Miller DW, Ahmad R, McLendon C, Bandyopadhyay S, Baptista MJ, Ringe D, Petsko GA, Cookson MR. The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization. Proc Natl Acad Sci U S A 2004;101:9103–9108. [PubMed: 15181200]

76. Andres-Mateos E, Perier C, Zhang L, Blanchard-Fillion B, Greco TM, Thomas B, Ko HS, Sasaki M, Ischiropoulos H, Przedborski S, Dawson TM, Dawson VL. DJ-1 gene deletion reveals that DJ-1 is an atypical peroxiredoxin-like peroxidase. Proc Natl Acad Sci U S A 2007;104:14807–14812. [PubMed: 17766438]

77. Ito G, Ariga H, Nakagawa Y, Iwatsubo T. Roles of distinct cysteine residues in S-nitrosylation and dimerization of DJ-1. Biochem Biophys Res Commun 2006;339:667–672. [PubMed: 16316629]

78. Hoepken HH, Gispert S, Morales B, Wingerter O, Del Turco D, Mulsch A, Nussbaum RL, Muller K, Drose S, Brandt U, Deller T, Wirth B, Kudin AP, Kunz WS, Auburger G. Mitochondrial dysfunction, peroxidation damage and changes in glutathione metabolism in PARK6. Neurobiol Dis 2007;25:401–411. [PubMed: 17141510]

79. Wang D, Qian L, Xiong H, Liu J, Neckameyer WS, Oldham S, Xia K, Wang J, Bodmer R, Zhang Z. Antioxidants protect PINK1-dependent dopaminergic neurons in Drosophila. Proc Natl Acad Sci U S A 2006;103:13520–13525. [PubMed: 16938835]

80. Pridgeon JW, Olzmann JA, Chin LS, Li L. PINK1 Protects against Oxidative Stress by Phosphorylating Mitochondrial Chaperone TRAP1. PLoS Biol 2007;5:e172. [PubMed: 17579517]

81. Nicklas WJ, Vyas I, Heikkila RE. Inhibition of NADH-linked oxidation in brain mitochondria by 1-methyl-4-phenyl-pyridine, a metabolite of the neurotoxin, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. Life Sci 1985;36:2503–2508. [PubMed: 2861548]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

82. Heikkila RE, Nicklas WJ, Vyas I, Duvoisin RC. Dopaminergic toxicity of rotenone and the 1-methyl-4-phenylpyridinium ion after their stereotaxic administration to rats: implication for the mechanism of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity. Neurosci Lett 1985;62:389–394. [PubMed: 3912685]

83. Schapira AH, Cooper JM, Dexter D, Jenner P, Clark JB, Marsden CD. Mitochondrial complex I deficiency in Parkinson's disease. Lancet 1989;1:1269. [PubMed: 2566813]

84. Jenner P. Altered mitochondrial function, iron metabolism and glutathione levels in Parkinson's disease. Acta Neurol Scand Suppl 1993;146:6–13. [PubMed: 8333254]

85. Jha N, Jurma O, Lalli G, Liu Y, Pettus EH, Greenamyre JT, Liu RM, Forman HJ, Andersen JK. Glutathione depletion in PC12 results in selective inhibition of mitochondrial complex I activity. Implications for Parkinson's disease. J Biol Chem 2000;275:26096–26101. [PubMed: 10846169]

86. Taylor ER, Hurrell F, Shannon RJ, Lin TK, Hirst J, Murphy MP. Reversible glutathionylation of complex I increases mitochondrial superoxide formation. J Biol Chem 2003;278:19603–19610. [PubMed: 12649289]

87. Beer SM, Taylor ER, Brown SE, Dahm CC, Costa NJ, Runswick MJ, Murphy MP. Glutaredoxin 2 catalyzes the reversible oxidation and glutathionylation of mitochondrial membrane thiol proteins: implications for mitochondrial redox regulation and antioxidant DEFENSE. J Biol Chem 2004;279:47939–47951. [PubMed: 15347644]

88. Hsu M, Srinivas B, Kumar J, Subramanian R, Andersen J. Glutathione depletion resulting in selective mitochondrial complex I inhibition in dopaminergic cells is via an NO-mediated pathway not involving peroxynitrite: implications for Parkinson's disease. J Neurochem 2005;92:1091–1103. [PubMed: 15715660]

89. Chinta SJ, Kumar JM, Zhang H, Forman HJ, Andersen JK. Up-regulation of gamma-glutamyl transpeptidase activity following glutathione depletion has a compensatory rather than an inhibitory effect on mitochondrial complex I activity: implications for Parkinson's disease. Free Radic Biol Med 2006;40:1557–1563. [PubMed: 16632116]

90. Murray J, Taylor SW, Zhang B, Ghosh SS, Capaldi RA. Oxidative damage to mitochondrial complex I due to peroxynitrite: identification of reactive tyrosines by mass spectrometry. J Biol Chem 2003;278:37223–37230. [PubMed: 12857734]

91. Bharath S, Andersen JK. Glutathione depletion in a midbrain-derived immortalized dopaminergic cell line results in limited tyrosine nitration of mitochondrial complex I subunits: implications for Parkinson's disease. Antioxid Redox Signal 2005;7:900–910. [PubMed: 15998245]

92. Chinta SJ, Kumar MJ, Hsu M, Rajagopalan S, Kaur D, Rane A, Nicholls DG, Choi J, Andersen JK. Inducible alterations of glutathione levels in adult dopaminergic midbrain neurons result in nigrostriatal degeneration. J Neurosci 2007;27:13997–14006. [PubMed: 18094238]

93. Keeney PM, Xie J, Capaldi RA, Bennett JP Jr. Parkinson's disease brain mitochondrial complex I has oxidatively damaged subunits and is functionally impaired and misassembled. J Neurosci 2006;26:5256–5264. [PubMed: 16687518]

94. Sherer TB, Betarbet R, Testa CM, Seo BB, Richardson JR, Kim JH, Miller GW, Yagi T, Matsuno-Yagi A, Greenamyre JT. Mechanism of toxicity in rotenone models of Parkinson's disease. J Neurosci 2003;23:10756–10764. [PubMed: 14645467]

95. Kweon GR, Marks JD, Krencik R, Leung EH, Schumacker PT, Hyland K, Kang UJ. Distinct mechanisms of neurodegeneration induced by chronic complex I inhibition in dopaminergic and non-dopaminergic cells. J Biol Chem 2004;279:51783–51792. [PubMed: 15469939]

96. Halliwell B. Hypothesis: proteasomal dysfunction: a primary event in neurogeneration that leads to nitrative and oxidative stress and subsequent cell death. Ann N Y Acad Sci 2002;962:182–194. [PubMed: 12076974]

97. Friguet B, Szweda LI. Inhibition of the multicatalytic proteinase (proteasome) by 4-hydroxy-2-nonenal cross-linked protein. FEBS Lett 1997;405:21–25. [PubMed: 9094417]

98. Okada K, Wangpoengtrakul C, Osawa T, Toyokuni S, Tanaka K, Uchida K. 4-Hydroxy-2-nonenal-mediated impairment of intracellular proteolysis during oxidative stress. Identification of proteasomes as target molecules. J Biol Chem 1999;274:23787–23793. [PubMed: 10446139]

99. Grune T, Davies KJ. The proteasomal system and HNE-modified proteins. Mol Aspects Med 2003;24:195–204. [PubMed: 12892997]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

100. Bennett MC, Bishop JF, Leng Y, Chock PB, Chase TN, Mouradian MM. Degradation of alpha-synuclein by proteasome. J Biol Chem 1999;274:33855–33858. [PubMed: 10567343]

101. Lee MH, Hyun DH, Jenner P, Halliwell B. Effect of proteasome inhibition on cellular oxidative damage, antioxidant defences and nitric oxide production. J Neurochem 2001;78:32–41. [PubMed: 11432971]

102. Ara J, Przedborski S, Naini AB, Jackson-Lewis V, Trifiletti RR, Horwitz J, Ischiropoulos H. Inactivation of tyrosine hydroxylase by nitration following exposure to peroxynitrite and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Proc Natl Acad Sci U S A 1998;95:7659–7663. [PubMed: 9636206]

103. Blanchard-Fillion B, Souza JM, Friel T, Jiang GC, Vrana K, Sharov V, Barron L, Schoneich C, Quijano C, Alvarez B, Radi R, Przedborski S, Fernando GS, Horwitz J, Ischiropoulos H. Nitration and inactivation of tyrosine hydroxylase by peroxynitrite. J Biol Chem 2001;276:46017–46023. [PubMed: 11590168]

104. Kuhn DM, Sadidi M, Liu X, Kreipke C, Geddes T, Borges C, Watson JT. Peroxynitrite-induced nitration of tyrosine hydroxylase: identification of tyrosines 423, 428, and 432 as sites of modification by matrix-assisted laser desorption ionization time-of-flight mass spectrometry and tyrosine-scanning mutagenesis. J Biol Chem 2002;277:14336–14342. [PubMed: 11834745]

105. Sadidi M, Geddes TJ, Kuhn DM. S-thiolation of tyrosine hydroxylase by reactive nitrogen species in the presence of cysteine or glutathione. Antioxid Redox Signal 2005;7:863–869. [PubMed: 15998241]

Glossary

Abbreviations

α-syn
: α-synuclein

PD
: Parkinson’s disease

SN
: substantia nigra

PTM
: post-translational modification

GSH
: glutathione (reduced)

GSSG
: glutathione (oxidized)

MDA
: malondialdehyde

HNE
: 4-hydroxy-2-nonenal

8OHG
: 8-hydroxyguanosine

3NT
: 3-nitrotyrosine

MPTP
: 1-methyl-4-phenyl-1,2,3,6-tetrapyridine

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

ROS
reactive oxygen species

RNS
reactive nitrogen species

NO
nitric oxide

NOS
nitric oxide synthase

MAO
monoamine oxidase

DOPAC
3,4-dihydroxyphenylacetic acid

DHBT-1
7-(2-aminoethyl)-3,4-dihydro-5-hydroxy-2H-1, 4benzothiazine-3-carboxylic acid

Tyr
tyrosine

Cys
cysteine

Met
methionine

Trp
tryptophan

Ser
serine

MSR
methionine sulfoxide reductase

CMA
chaperone-mediated autophagy

MDA-Lys
malondialdehyde-lysine

SOD
superoxide dismutase

DTT
dithiothreitol

TH
tyrosine hydroxylase

MPP^+
1-methyl-4-phenylpyridinium

Phos

	phosphorylation
pI	
	isoelectric point

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 1

Protein	Source of Modification	PTM	Proposed Consequence	Reference
α-synuclein				
	unknown kinase	Phosphorylated ser129	Promotes α-syn fibrils	[53–55]
	Lewy bodies/ nitrating agents/MPTP	Several 3NTs	Stable α-syn oligomers	[13,45–47]
	peroxynitrite	Tyr125 3NTs	α-syn dimerization	[48]
	peroxynitrite	Tyr39 3NTs	Decreased vesicle binding	[49]
	dopamine	Dopamine adduct formation	Stabilizes α-syn protofirils/ inhibits degradation of proteins by CMA	[56,57]
	rotenone	Aminotyrosine at Tyr133 and Tyr136	Unknown	[50]
	α-synuclein overexpression	Met127 sulfone formation	Prevents fibril formation	[52]
	MPTP	Nitration	Immune response	[51]
parkin				
	overexpression of mutant parkin	Increased lipid peroxidation, protein carbonyls and 3NTs in total protein	Decreased GSH, increased oxidative stress	[60]
	parkin −/− mice	Increased HNE adducts and carbonyl levels	Decreased antioxidant capacity	[61]
	dopamine	Dopamine adduct formation	Decreased parkin ligase activity	[33]
	MPTP	S-nitrosylation	Decreased parkin ligase activity	[62]
DJ-1				
	DJ-1 expression decreased	Oxidation	Cell death, oxidative stress	[66]
	Hydroperoxides/H_2O_2	Cys46, Cys53 and Cys106 oxidized to sulfonic acid	Acidification of DJ-1, oxidative stress, DJ-1 cleavage	[70,71,65]
	PD brain tissues	Cys53 sulfonic acid, increased carbonyl levels on several isoforms of DJ-1	Acidification of DJ-1, oxidative stress	[67]
	MPTP	Met133 sulfone, Cys106 sulfinic acid	Acidification of DJ-1, oxidativestress	[72,73]
	S-nitrosylation	S-nitrosylation of Cys46 and Cys53	Decreased homodimer DJ-1 formation	[74]
PINK1				
	G302D-PINK1 mutant	Increased lipid peroxidation	Decreased complex I activity, Increased SOD and GSH levels	[75]
	PINK1 kinase activity	Phosphorylation of Trap1	Prevention of cytochrome-c release and apoptosis	[77]
mitochondrial complex I				
	GSH depletion	Elevated cysteine oxidation, Decreased sulfhydryls, mitochondrial HNE adducts, and S-nitrosylation levels increased	Complex I activity inhibited	[82,85,86, 88,89]
	Peroxynitrite	Complex I subunit oxidation (several 3NTs)	Complex I activity inhibited	[87]
	PD brain, frontal cortex	Increased carbonyl levels particularly in dopaminergic areas	Complex I activity inhibited	[90]
	Rotenone	Increased carbonyl levels in dopaminergic areas of brain	Oxidative stress	[91]
proteasome				
	HNE	HNE adduct formation	Proteasome Inhibition	[94,95]
	Lactacystin or epoxomicin	Increased carbonyl, lipid peroxidation, and DNA damage	Oxidative stress	[98]
tyrosine hydroxylase				
	Peroxynitrite or MPTP	Tyr423 (3NT)	TH activity inhibited	[99,100]
	Peroxynitrite	Cysteine oxidation	TH activity inhibited	[101,102]