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Chapter 15
Redox Proteomics Identification of Oxidatively Modified Proteins in Alzheimer's Disease Brain and in Brain from a Rodent Model of Familial Parkinson's Disease: Insights into Potential Mechanisms of Neurodegeneration

Rukhsana Sultana, H. Fai Poon, and D. Allan Butterfield

Introduction

Oxidative stress, an imbalance between the oxidant and antioxidant systems, has been implicated in the pathogenesis of numerous neurodegenerative diseases [1]. Among all the body organs, the brain is particularly vulnerable to oxidative damage because of its high utilization of oxygen, increased levels of polyunsaturated fatty acids, and relatively high levels of redox transition metal ions in certain brain regions; in addition, the brain has relatively low levels of antioxidants [2–6]. The presence of iron ion in an oxygen-rich environment can further lead to enhanced production of superoxide radicals and ultimately to a cascade of oxidative events. Either the oxidant directly or the products of oxidative stress could trigger the oxidative modification of a number of cellular macromolecular targets, including proteins, lipids, DNA, RNA, and carbohydrates, which may lead to impairment of cellular functions [2,3,5,7–9].

Among the earliest of these changes following an oxidative insult are increased levels of toxic carbonyls, 3-nitrotyrosine (3-NT), and 4-hydroxy-2-trans-nonenal (HNE) [2,4,7,10–13]. HNE is derived from free radical attack on unsaturated acyl chains of phospholipids, particularly arachidonic acid. Oxidation leads to introduction of carbonyl groups to proteins [14]. Carbonyl groups are incorporated into proteins by direct oxidation of certain amino acid side chains, peptide backbone scission, or Michael addition reactions with products of lipid peroxidation or glycol oxidation [4,15,16]. Protein carbonyls can be detected by the derivatization of the carbonyl group with 2,4-dinitrophenylhydrazine (DNPH), followed by immunochemical detection of the hydrazone product [14]. Oxidative stress can stimulate additional damage via overexpression of inducible nitric oxide synthase (iNOS) and the action of constitutive

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neuronal NOS (nNOS), which leads to increased levels of 3-NT. The levels of thiobarbituric acid reactive substance (TBARS), free fatty acid release, HNE and acrolein formation, and iso- and neuroprostane formation are the most commonly used parameters to index lipid peroxidation. DNA and RNA oxidation are measured by formation of 8-OH-2α-deoxyguanosine and other oxidized bases as well as altered DNA repair mechanisms.

Proteomics

Oxidatively modified brain proteins were initially identified using immunoprecipitation methods [17,18]. However, there are serious limitations to the use of this technique. For example, prior knowledge about the identity of the protein of interest is required, the availability of the particular antibody for the protein of interest is necessary, and the time-consuming and laborious nature of the process is a hindrance. In addition, posttranslational modification of protein may change the structure of proteins, thereby preventing the formation of the appropriate antigen–antibody complex. Redox proteomics has enabled us to identify a large number of oxidatively modified proteins in cells, tissues, and other biological samples that were previously undetected by other methods such as immunoprecipitation [19–21]. Unlike gene analysis and mRNA analysis, proteomics provides a broad spectrum of information that allows insights into the mechanisms of disease and identification of disease-associated markers and may also help to identify selected targets for specific therapy (Fig. 1).

Redox proteomics couples two-dimensional (2D) gel electrophoresis separation of proteins and 2D Western blots with mass spectrometric techniques that

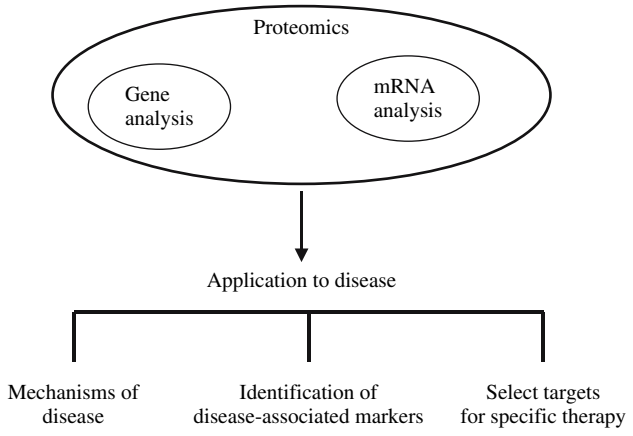

Fig. 1 Proteomics: global analysis of cellular proteins

allow facile identification of oxidatively modified proteins without consuming as much time and effort as immunochemical methods [19–21]. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) allows the investigator to analyze complex protein mixtures based on two important physicochemical properties: isoelectric focusing (IEF), which separates proteins based on their isoelectric points (pI); and separation of proteins based on their relative mobility (Mr) on sodium dodecyl sulfate (SDS)-PAGE in the second dimension [22]. Normally a single spot on the 2D gel represents a single protein [23]. This property allows separation of thousands of different protein spots on one gel. In addition, 2D-PAGE is used to catalog proteins and create databases [24].

2D-PAGE is a sensitive, reliable method with high reproducibility, although many challenges still exist. The first serious limitation of 2D is the solubilization process for membrane proteins [25] as ionic detergents would introduce a charge to the protein, thereby interfering with IEF. The inability to detect low-abundance proteins is the second limitation of 2D-PAGE; and the third limitation is the insensitivity to proteins of high lysine and arginine content (which leads to small tryptic peptides that could be lost on a gel). The use of chaotropic agents such a urea and thiourea coupled with nonionic or zwitterionic detergents can solubilize proteins and also avoid protein precipitation during the IEF and the SDS gel processes [26]. The use of immobilized pH IEF strips (immobilized pH gradient, or IPG, strips) improves the reproducibility of proteins maps and also eliminates the typical cathodic drift associated with previously used tube gels [27]. The use of narrow-range IPG strips enables protein separation over a wide pH range but within 1 pH unit. However, the normally employed IEF strip pH range (i.e., 3–10) limits the identification of highly basic proteins. If a protein from a low-abundance protein group were involved in the pathogenesis of a disease, it would be difficult to use this technique for detection.

In our laboratory we coupled redox proteomics techniques with immunochemical detection of protein carbonyls derivatized by 2,4-dinitrophenylhydrazine (DNPH), nitrated proteins indexed by 3-NT, and protein adducts of HNE, followed by mass spectrometric (MS) analysis (as shown in Fig. 2) to identify oxidatively modified proteins from Alzheimer's disease (AD) brain and related models. With this method we employ a parallel analysis: The 2D Western blots and 2D gel images are matched by computer-assisted image analysis, and the anti-DNP/nitrotyrosine/HNE immunoreactivity of individual proteins are normalized to their content, obtained by measuring the intensity of colloidal Coomassie Blue staining or SYPRO ruby-stained spots (Figs. 3, 4). Such analysis allows comparison of levels of oxidatively modified brain proteins in experimental versus control subjects. Once the protein is identified as oxidatively modified, it is digested in gel with a protease (e.g., trypsin) that not only cleaves the protein into small peptides but produces sequence-specific proteolysis. These mass fingerprints are modified proteins from AD brain and related models; they are characteristic of a particular protein, which facilitates the identification of a particular protein using a

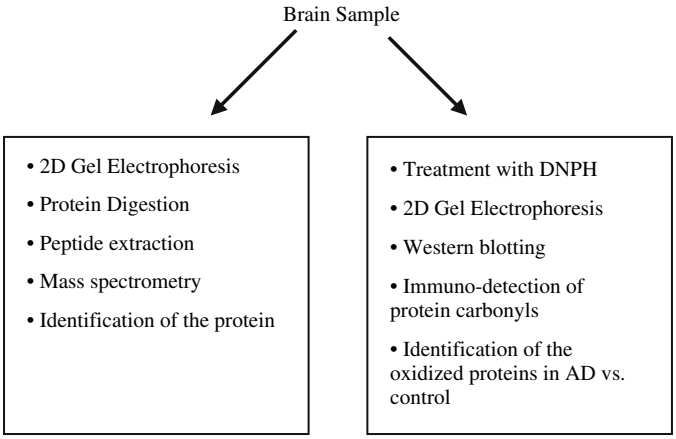

Fig. 2 Redox proteomics to identify oxidatively modified brain proteins in Alzheimer's disease

suitable database (Table 1) that compares the experimental masses with theoretical masses of trypsin-generated protein sequences.

Mass spectrometry determines the peptide masses and can determine the amino acid sequence for the proteins of interest. Modern MS instruments use softer ionization techniques than previously, and they can provide a precise peptide mass. The two most commonly employed MS techniques are MALDI

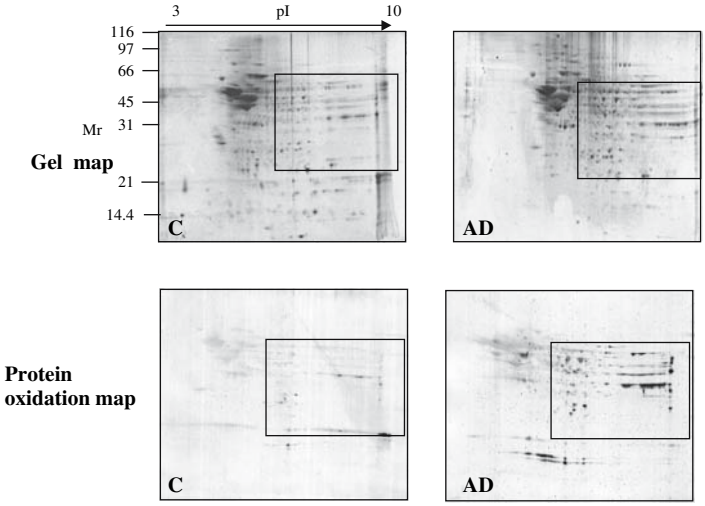

Fig. 3 Two-dimensional maps of brain proteins from controls (C) and Alzheimer's disease patients (AD)

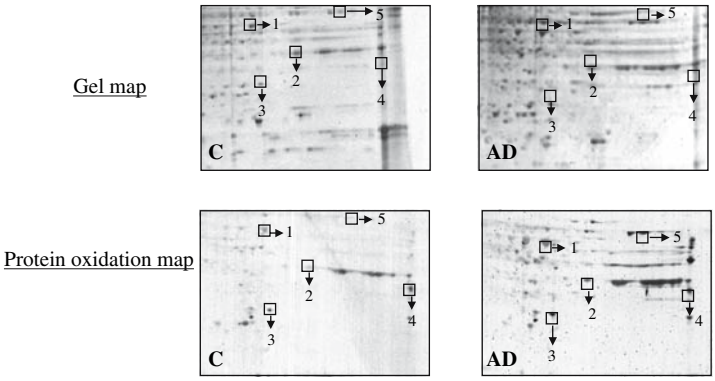

Fig. 4 Oxidatively modified proteins in Alzheimer's disease brain (AD) identified by redox proteomics using expanded two-dimensional oxyblots from Figure 3. 1, enolase; 2, glyceraldehyde 3-phosphate dehydrogenase; 3, carbonic anhydrase II; 4, voltage-dependent anion channel protein-1; 5, ATP synthase α chain

(matrix-assisted laser desorption/ionization) and ESI (electrospray ionization). With MALDI analysis the peptide sample is mixed with a matrix, usually α-cyano-4-hydroxycinnamic acid or 2,5-dihydroxybenzoic acid, and deposited on a plate that is subjected to laser radiation. The matrix absorbs the energy, which is then transferred to the peptides. The peptides then vaporize as detectable MH^+ ions by an unknown mechanism related to the acidic nature of the matrix.

In contrast to MALDI MS, ESI permits direct MS analysis of the samples from high-performance liquid chromatography (HPLC) columns for characterization. towing to the high potential difference between the capillary and the MS instrument, the inlet sample is dispersed as small droplets. These droplets undergo solvent evaporation until droplet fission occurs, because of the high charge-to-surface tension ratio, finally leading to the formation of a single detectable ion per droplet. The best online preseparation of peptides with HPLC and MS requires low salt concentration. In addition, reducing the flow time to nanoliters per minute can increase the time for analysis. Tandem MS/MS provides better isolation and fragmentation of a specific ion. This tandem MS/MS technique provides further information about the sequence

Table 1 Mass spectrometry search engines for peptide mass fingerprinting

Search engine and URL
Mascot—http://www.matrixscience.com
MOWSE—http://www.hgmp.mrc.ac.uk/Bioinformatics/Webapp/mowse
Profound—http://www.prowl.rocketfeller.edu/sgi-bin/profound
MS-fit—http://www.prospector.ucsf.edu/ucsfhtlm3.4/msfit.htm
Peptident—http://www.expasy.ch.ch/tools/peptident.html

of the protein [28]. With MS/MS analysis, the isolation of a single ion is achieved by scanning all of the ions that were generated from a sample, followed by application of a wide range of frequencies, except for the resonating frequency of the ion of interest. Fragmentation of the isolated ion, which provides additional information for protein identification or for evaluation of possible protein modification, is the final step in MS/MS.

The identity of proteins is determined by employing online databases following MS analysis. SwissProt, the most commonly used database for protein identification, is based on computer algorithms [29]. SwissProt and other databases are available through the Internet; they are listed in Table 1. These search engines provide theoretical protease digestion of the proteins contained in the database, to which are compared the experimental masses obtained by MS. The successful protein identification using these databases also accounts for several factors, such as protein size and the probability that a single peptide occurs in the whole database. The search engine produces a probability score for each entry, which is calculated by a mathematical algorithm specific for each search engine. Any hit with a score higher that the one specific for significance of the particular search engine is considered statistically significant and has a valid chance to be the protein cut from a given spot. In addition, the molecular weight and pI of the protein is calculated based on the position in the 2D map to avoid any false identification. In many cases, validation of protein identification is achieved by immunochemical means [13,18,30–34].

In this chapter, we review the redox proteomics identification of oxidatively modified proteins in AD and Parkinson's disease (PD), two age-related neurodegenerative disorders that involve deposition of aggregated proteins (Aβ, synuclein, and parkin) as pathological hallmarks of the respective disorders.

Alzheimer's Disease

Alzheimer's disease is an age-related neurodegenerative disorder characterized by progressive loss of memory and cognition, accumulation of extracellular amyloid plaques (Aβ) and intracellular neurofibrillary tangles (NFTs), and loss of synaptic connections in selective brain regions. NFTs consist of aggregates of hyperphosphorylated microtubule-associated protein tau that form paired helical filaments and related straight filaments [35]. Amyloid α-peptide (Aβ), a 40- to 42-amino acid peptide derived from proteolytic cleavage of an integral membrane protein known as amyloid precursor protein (APP) by the action of β- and γ-secretases, is the main amyloid component of senile plaque (SP). Ab is thought to play a casual role in the development and progression of AD [36]. Furthermore, a number of studies suggest that the small oligomers of Aβ are the actual toxic species of this peptide rather than Aβ fibrils [37–40].

Several mechanisms have been proposed to explain AD pathogenesis. These mechanisms include amyloid cascade, excitoxicity, oxidative stress, and

inflammation. We previously showed that regions of AD brain rich in β have increased protein oxidation, whereas Aβ-poor cerebellum does not [41]. Protein carbonyls, HNE, and 3-nitrotyrosine levels were found to be elevated in AD brain and cerebrospinal fluid (CSF) [9,37,41], results that support the oxidative stress hypothesis of AD. Moreover, the observation that vitamin E in cell culture diminishes Aβ(1-42)-induced oxidative stress and neurotoxicity further supports a role of oxidative stress in AD pathology [2,42,43]. Aβ-induced lipid peroxidation leads to increased formation of HNE in vitro and also was observed in AD brain and CSF [8,16,17,44]. Using immunoprecipitation techniques, Lauderback et al. showed the HNE-mediated oxidative modification of glutamate transporter (GLT-1) in AD brain. GLT-1 is involved in regulating the levels of glutamate outside the neuron. These researchers also observed that synaptomes treated with β(1-42) demonstrated HNE-modified GLT-1 [17]. This oxidative modification leads to altered structure [45] and loss of function of the transport protein, which could eventually lead to excitotoxic neuronal death (Fig. 5) [46].

One of the mechanisms for removal of HNE from neurons is by conjugation to GSH, followed by the action of glutathione S-transferase (GST) and the multidrug resistant protein-1 (MRP-1) to efflux this conjugate from the cell [47]. However, in AD brain, GST and MRP-1 were demonstrated to have excessively bound HNE and showed reduced activity, supporting the idea that oxidative modification leads to loss of functionality [18].

As noted above, there are several serious limitations to the use of immunoprecipitation to identify proteins, including the requirement of prior identification of the protein of interest, the availability of a specific antibody for this protein, and the extensive time needed for this process. Moreover, sometimes a posttranslational modification can change the structure of proteins, thereby preventing the formation of the appropriate antigen–antibody complex. Redox

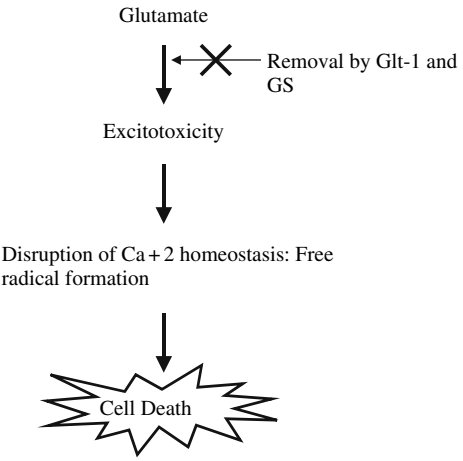

Fig. 5 The glutamate transporter Glt-1 and glutamine synthase (GS) modulate glutamate-induced excitotoxicity. Conversely, if Glt-1 and GS are oxidatively modified and lose functionality, neuronal death can occur

Table 2 Proteomic identification of specifically oxidatively modified proteins in AD brain

- Energy dysfunction—creatine kinase; α-enolase; γ-enolase; triose phosphate isomerase; phosphoglycerate mutase 1
- Excitotoxicity—glutamine synthase; glutamate transported by EAAT2
- Proteasomal dysfunction—ubiquitin carboxy-terminal hydrolase L-1
- Lipid abnormalities and cholinergic dysfunction—neuropolypeptide h3
- Neuritic abnormalities—dihydropyrimidinase-related protein 2; β-actin
- Tau hyperphosphorylation—peptidyl prolyl *cis*-trans isomerase
- Synaptic abnormalities—γ-soluble *N*-ethylmaleimide-sensitive factor attachment protein
- pH buffering and CO_2 transport—carbonic anhydrase II

proteomics, which couples 2D gel electrophoresis separation of proteins and 2D Western blots with MS techniques, is highly successful in identifying oxidatively modified brain proteins [19–21]. Proteomics has enabled us to identify a large number of oxidatively modified proteins in AD brain and models thereof.

The identification of oxidatively modified proteins in the AD inferior parietal lobule (IPL) and hippocampus was accomplished by proteomics [10,13,33,34,48,49]. This research has provided insights into the role of oxidative stress in AD and has helped to unravel the mechanisms associated with AD pathology [10,13,33,34,48,49]. Oxidatively modified brain proteins identified by our redox proteomics approach include creatine kinase BB (CK), glutamine synthase (GS), ubiquitin carboxy-terminal hydrolase L-1 (UCH L-1), triose phosphate isomerase (TPI), neuropolypeptide h3, dihydropyrimidinase-related protein 2 (DRP2), α-enolase, phosphoglycerate mutase 1 (PGM1), γ-soluble NSF attachment protein (SNAP), carbonic anhydrase II (CA-II), and peptidyl prolyl cis-trans isomerase (Pin 1). No oxidatively modified proteins were identified in cerebellum [34], confirming earlier studies [41].

The oxidatively modified proteins in AD brain are involved in known dysfunctional processes in AD. The identified oxidatively modified proteins were grouped based on their functions (Table 2) and were linked to AD pathology, symptomatology, and loss of enzyme activity, consistent with a plausible mechanism of neurodegeneration [19,20].

Energy Dysfunction

Creatine kinase, TPI, ATP synthase-α, GAPDH, VDAC-1, PGM1, and α-enolase are enzymes involved in energy metabolism and were identified as oxidized proteins with reduced activity in AD brain, that could be linked to the observed decreased ATP production in AD [50] and could be detrimental to neurons [10,13,30,34,48,49,51]. Decreased ATP production would lead to impaired ion-motive ATPases, altered protein synthesis, and maintenance of synaptic transmission, all of which are hallmarks of AD [10,13,30,34,48,49,52]. Decreased ATP production could induce hypothermia, leading to abnormal tau hyperphosphorylation through differential inhibition of kinase and

phosphatase activities, ion pumps, electrochemical gradients, cell potential, and voltage-gated ion channels [53].

Excitotoxicity

Glutamine synthase and EAAT2 (GLT-1) are involved in regulating extraneuronal glutamate levels and neurotransmission. GS and EAAT2 oxidation could lead to accumulation of glutamate in the synaptic cleft, leading to influx of calcium into the cell via activation of *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors and causing neuronal excitotoxic death [54]. As noted above, HNE, a lipid peroxidation product, has been shown to modify oxidatively the glutamate transporter EAAT2 in AD brain and synaptosomes treated with Aβ(1-42) [17].

Proteasomal Dysfunction

When proteins are damaged or aggregated, they become ubiquitinylated, as a polyubiquitin chain. Such poly (ubiquitin) chains can be as large as 70 units [55]. The poly (ubiquitin) chain is a marker that targets the damaged protein to the 26S proteasome for subsequent degradation. UCH L-1 removes ubiquitin from the poly (ubiquitin) chain, one ubiquitin unit at a time from the carboxyl terminal end before insertion of the damaged protein into the core of the proteasome [56]. This has the effect of maintaining the pool of ubiquitin in the brain. Oxidative modification of UCH L-1 was found in AD brain [34,48,57]. Presumed resultant decreased UCH L-1 activity in AD brain could lead to depletion of the free pool of ubiquitin or cause saturation of the proteasome with polyubiquitin chains and accumulation of damaged proteins, leading to synaptic deterioration and degeneration. Decreased activity of UCHL-1 would lead to increased protein ubiquitinylation, decreased proteasomal activity, and accumulation of damaged and aggregated proteins, all of which are observed in AD brain [20,58]. A recent in vitro study showed that HNE decreases hydrolase activity of recombinant UCH-L1 [54,59], and that the HNE-bound protein and crosslinked proteins could impair proteasomal function [59]. Others recently confirmed the oxidative modification of UCH L-1 in AD brain using proteomics [57]. Interestingly, if UCH L-1 is dysfunctional, as it is in the gracile axonal dystrophic mouse, oxidative modification of important brain proteins occurs [60].

Lipid Abnormalities and Cholinergic Dysfunction

Neuropolypeptide h3 (also known as phosphatidylethanolamine-binding protein, or PEBP) may play an important role in maintaining phospholipid asymmetry of the membrane [61]. Oxidative modification of neuropolypeptide h3

has been observed in AD brain [10]. Because this protein is indirectly involved in the production of choline acetyltransferase, oxidative modification of neuropolypeptide h3 could lead to altered choline acetyltransferase levels. Moreover, in its role as PEBP, its oxidative modification could lead to apoptosis by the exposure of phosphatidylserine to the outer bilayer leaflet of the membrane, leading to cell death and observed cognitive decline in AD [62]. Aβ(1-42) and HNE added to synaptosomes lead to a loss of phospholipid asymmetry [63,64].

Neuritic Abnormalities

DRP-2 is involved in axonal outgrowth and pathfinding through transmission and modulation of extracellular signals, [65–67] and β-actin is involved in cell integrity. Decreased expression of DRP-2 protein was observed in AD, adult Down's syndrome (DS) [68], fetal DS, [69] schizophrenia, and affective disorders.70 Oxidation of DRP-2 and β-actin, as observed in AD brain, [10,37,49] could be related to the observed shortening of dendrites and synapse loss in AD brain.71 Shortened dendrites would be predicted to lead to less efficient interneuronal communication, which could be important in a cognitive and memory disorder.

Tau Hyperphosphorylation

Peptidyl-prolyl isomerases (PPIases or Pin 1) catalyze the conversion of the cis to trans conformation and vice versa of proteins between given amino acids and a proline [72]. Also, PPIases have been shown to be necessary for entry into mitosis, and they interact with cell cycle regulating proteins (e.g., p53, Myt1, Wee1, Cdc25C). We determined by proteomics that PPIase (Pin 1) is oxidized in AD brain [33,34]. This modification conceivably could cause dramatic structural modifications, which could affect the properties of targeted proteins. One target for Pin1 is tau, a neuronal cytoskeletal protein, which is hyperphosphorylated in AD [73]. Recent studies show an inverse relation of Pin 1 activity and co-localization with phosphorylated tau in AD brain [74–76]. In addition, the cell cycle machinery of AD neurons was reported to be altered in AD brain [77,78]. Pin 1 oxidation and decreased activity could therefore be involved in the initial events that trigger tangle formation, cell cycle-related abnormalities, and oxidative damage [33,34,79]. All these effects can lead to memory loss.

Synaptic Abnormalities and LTP

Oxidation of γ-SNAP, a member of the *N*-ethylmaleimide-sensitive factor (NSF) attachment proteins (SNAPs), could impair vesicular transport in the constitutive secretory pathway as well as in neurotransmitter release, hormone

secretion, and mitochondrial organization [34,80,81]. This, in turn, could lead to impaired learning and memory processes and altered neurotransmitter systems in AD brain.

pH Buffering and CO_2 Transport

Carbonic anhydrase II plays an important role in regulating cellular pH, CO_2 and HCO_3^- transport, and maintaining H_2O and electrolyte balance [82]. CA-II deficiency leads to cognitive defects, varying from disabilities to severe mental retardation, in addition to osteoporosis, renal tubular acidosis, and cerebral calcification. Oxidation and decreased activity of this protein was observed in AD brain compared to age-matched controls [13,34,83]. Oxidization of CA-II may lead to an imbalance of both the extracellular and intracellular pH in the cell, mitochondrial alterations in oxidative phosphorylation, and impaired synthesis of glucose and lipids. Moreover, altered neuronal pH could contribute to the known protein aggregation in AD brain.

AD Models for Aβ(1-42)

Identification of oxidatively modified AD brain proteins was substantially recapitulated in vitro and in vivo by action of human Aβ(1-42) in neuronal cell cultures, synaptosomes, intracerebral injection into rat basal forebrain, and expression in Caenorhabditis elegans [31,84–87]. These findings are consistent with the notion that Aβ(1-42) (Fig. 6) contributes to the observed oxidative stress and oxidative modification of proteins in AD brain [4,19,20].

Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder affecting the population of age 65 and older [88]. Clinical symptoms of PD, such as bradykinesia, resting tremor, cogwheel rigidity, and postural instability, result from loss of dopaminergic neurons in the substantia nigra compacta. Mutations in α-synuclein, parkin, DJ-1, and PINK1 contribute to early-onset familial PD [89]. Four mutations of α-synuclein have been identified in familial PD: A53T, A30P, E46A, and genomic duplication [90,91].

Oxidative damage is a well known pathological change in PD brains [92–95]. Overexpression of wild-type or mutant α-synuclein induces toxicity that is associated with oxidative stress [96]. Moreover, oxidative stress in PD is linked to cell death in PD brains by mitochondrial dysfunction, excitotoxicity, and the toxic effects of nitric oxide [94].

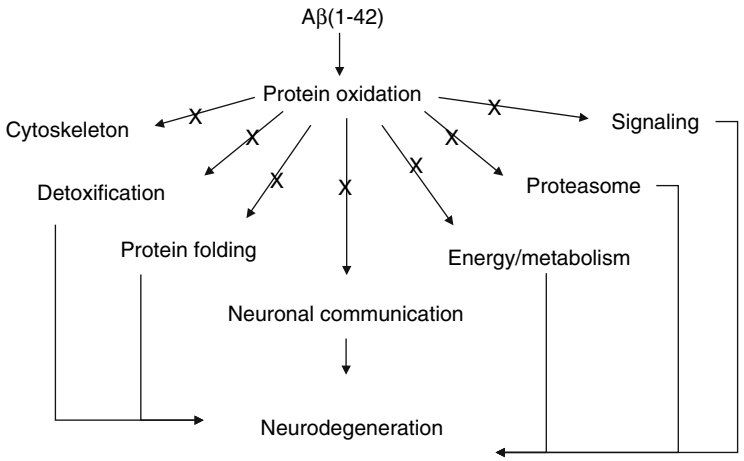

Fig. 6. Potential dysfunction of proteomics-identified β(1-42)-induced oxidized proteins. Protein oxidative modification is similar to that in Alzheimer's disease brain

Redox Proteomics in PD

α-Synuclein has a strong tendency to aggregate, leading to neurotoxicity. Expression of mutant α-synuclein in cells produces increased oxidative parameters and accelerated cell death in response to oxidative insult [97]. Symptoms in A30P α-synuclein transgenic mice occur in parallel with the aggregation of α-synuclein, [98] and these mice develop an age-dependent accumulation of α-synuclein in neurons of the brain stem [99,100], suggesting that α-synuclein aggregation-associated oxidative stress is involved in the pathology in A30P α-synuclein transgenic mice.

Using redox proteomics, several significantly oxidatively modified brain stem proteins were identified in symptomatic mice with overexpression of a A30P mutation in α-synuclein compared to the brain proteins from the nontransgenic mice. These proteins were identified as carbonic anhydrase 2 (CA-II), α-enolase (ENO1), and lactate dehydrogenase 2 (LDH2) [101]. The activities of these enzymes were also significantly decreased in the A30P α-synuclein transgenic mice brains when compared to the brain proteins from nontransgenic control [101]. This observation is consistent with the notion that oxidative modification of proteins leads to loss of their activities [16–18,41].

Carbonic anhydrase II, which, as noted above, is an oxidatively modified protein in AD brain, is a Zn^{2+} metalloenzyme that catalyzes reversible hydration of CO2 to bicarbonate (HCO3–). CA-II shares high (68%) similarity to the mitochondrial counterpart carbonic anhydrase 5a (CA-5a) and 5b (CA-5b), implicating the potential coupling or interaction with each other to function in

metabolic processes, cellular transport, gluconeogenesis, and mitochondrial metabolism [102,103]. Oxidative modification of CA-II may lead to loss of the buffering system in brains with resultant aggregation of synuclein and subsequent neurodegeneration.

LDH2 is a subunit of lactate dehydrogenase (LDH), a glycolytic protein that catalyzes the reversible interconversion of pyruvate to lactate. Lactate is the predominant monocarboxylate oxidized by mitochondria for intracellular lactate transport [104]. Therefore, oxidative inactivation of LDH may contribute to mitochondrial dysfunction in PD patients.

Eno1 is a subunit of enolase that interconverts 2-phosphoglycerate and phosphoenolpyruvate during glycolysis. Enolase was identified in an intermembrane space/outer mitochondrial membrane fraction [105]. These studies suggest that enolase is present in mitochondria and contributes to mitochondrial function. Therefore, oxidative inactivation of enolase may alter normal glycolysis and mitochondrial function in brains and may contribute to the alteration of energy metabolism in PD. Interestingly, LDH2 and ENO1 (possibly CA II) are associated with mitochondrial function. Increasing data implicate mitochondrial dysfunction and oxidation in PD [94,106–108]. Furthermore, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and rotenone lead to mitochondrial dysfunction with increased oxidative modification of proteins and α-synuclein aggregation [109–112]. Moreover, DJ-1, PINK1, and parkin all appear to modulate mitochondrial function [113–115]. The observation that each of the oxidatively modified brain proteins in A30P mutant synuclein mice is associated with mitochondria provides strong evidence for the notion that mitochondrial dysfunction contributes to the toxicity of PD and implicates mitochondrial pathology in toxicity associated with aggregated synuclein. This suggests that the oxidative stress-mediated mitochondrial dysfunction may be responsible, at least partially, for the neurodegeneration in the brains of A30P α-synuclein transgenic mice. Furthermore, this oxidative stress-mediated impaired energy metabolism and mitochondrial dysfunction is contributed by the oxidative inactivation of ENO1, LDH2, and CA-II. Therefore, the mitochondria dysfunction in familial PD may be associated with the oxidative inactivation of ENO1, LDH2, and CA-II.

Conclusions

The application of redox proteins to AD brain revealed important targets of protein oxidation. The use of in vivo and in vitro models of AD with human Aβ(1-42), which led to the identification of oxidatively modified proteins similar to those found in AD brain, provided strong evidence of the oxidative stress and neurotoxicity associated with Aβ(1-42) in AD brain. The use of relevant models for AD could be a powerful tool to investigate the role and mechanisms of Aβ(1-42) in the pathogenesis of AD. Furthermore, the use of animal models

together with redox proteomics approaches have provided potential insights into the mechanisms of neurodegeneration in AD and PD and may also be of value in the development of therapeutic approaches to prevent or delay these neurodegenerative disorders.

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