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Redox proteomics identification of oxidatively modified proteins in Alzheimer's disease brain and in vivo and in vitro models of AD centered around Aβ(1–42)☆

Rukhsana Sultana[a,b], Marzia Perluigi[a,d], D. Allan Butterfield[a,b,c,*]

[a] Department of Chemistry, University of Kentucky, Lexington, KY 40506-0055, USA
[b] Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY 40506, USA
[c] Center of Membrane Sciences, University of Kentucky, Lexington, KY 40506, USA
[d] Department of Biochemical Sciences, University of Rome "La Sapienza", 00185 Rome, Italy

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Abstract

Alzheimer's disease is a progressive neurodegenerative disease associated with loss of memory and cognition. One hallmark of AD is the accumulation of amyloid β-peptide (Aβ), which invokes a cascade of oxidative damage to neurons that can eventually result in neuronal death. Several markers of oxidative stress have been identified in AD brain, thus providing greater understanding into potential mechanisms involved in the disease pathogenesis and progression. In the present article, we review the application of redox proteomics to the identification of oxidized proteins in AD brain and also our recent findings on amyloid β-peptide (Aβ)-associated in vivo and in vitro models of AD. Our redox proteomics approach has made possible the identification of specifically oxidized proteins in Alzheimer's disease (AD) brain, providing for the first time evidence on how oxidative stress plays a crucial role in AD-related neurodegeneration. The information obtained has great potential to aid in determining the molecular pathogenesis in and detecting disease markers of AD, as well as identifying potential targets for drug therapy in AD. Application of redox proteomics to study cellular events, especially related to disease dysfunction, may provide an efficient tool to understand the main mechanisms involved in the pathogenesis and progression of oxidative stress-related neurodegenerative disorders.

Keywords: Redox proteomics; Alzheimer's disease; Oxidative stress; Protein oxidation

1. Introduction

Alzheimer disease is an age-related neurodegenerative disorder, the leading cause of dementia among older people. Currently there are more than 4 million Americans suffering from the disease and the number is assumed to increase to nearly 15 million over the coming several decades. The exact mechanism of AD is largely unknown, but mutation of presenilin-1 (PS-1), presenilin-2 (PS-2) [1], and amyloid precursor protein (APP) [2] genes has been observed in inherited AD [3]. In addition, there is also an association between AD and apolipoprotein E (APOE) gene [4,5], endothelial nitric oxide synthase −3 gene [6], and alpha-2-macroglobulin [7].

Pathologically, AD is characterized by the presence of extracellular amyloid plaques containing aggregated amyloid β-peptide (Aβ), intracellular neurofibrillary tangles (NFT), and loss of synaptic connections within selective brain regions. The presence of detectable entorhinal NFTs is considered to be the histological correlate of MCI (a possible prodrome to dementia) also referred to as incipient AD [8]. NFT consists of aggregates of hyperphosphorylated microtubule associated protein tau that forms paired helical filaments and related straight filaments [9]. Aβ is considered to play a causal role in the development and progression of AD [10]. Aβ40 and Aβ42 peptides are generated from its precursor, Aβ peptide precursor protein (APP), by the action of β- and γ-secretases. Aβ is present in soluble form, aggregated form, oligomeric form, protofibrils (PF), and fibrils. Indeed, oligomers, PF, and amyloid-derived diffusible

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* Corresponding author. Tel.: +1 859 257 3184; fax: +1 859 257 5876.
E-mail address: dabcns@uky.edu (D.A. Butterfield).

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ligands (ADDLs) are believed to generate the potent toxicity of Aβ [11–16]. However, most researchers now agree that fibril Aβ is not the primary toxic species of this peptide [11–16].

Several mechanisms have been proposed to explain AD pathogenesis that include: amyloid cascade, excitoxicity, oxidative stress, and inflammation. There is accumulating evidence that suggests a role of oxidative stress in the pathophysiology of AD. Oxidative stress in AD brain is manifested by increased protein oxidation, lipid peroxidation, DNA oxidation, advanced glycation end products, and ROS formation, among other indices [17–22]. Further, the use of vitamin E in cell culture diminishes Aβ(1–42)-induced toxicity, further supporting a role of oxidative damage in AD pathology [17,23,24]. Aβ-induced lipid peroxidation leads to increased formation of 4-hydroxy-2-nonenal (HNE) in neurons and is also observed in AD brain and CSF [25–28].

Using immunuprecipitation techniques followed by immunochemical detection Lauderback et al., showed the HNE modification of the glutamate transporter (GLT-1), a transporter involved in regulating the levels of glutamate within the cell, in AD brain [26]. This oxidative modification could lead to loss of function of the transporter eventually leading to neuronal death [29]. Aβ(1–42) added to rodent brain preparations containing GLT-1 also led to HNE modification of this transporter, demonstrating a direct linkage between Aβ-mediated oxidative stress and potential mechanism of neurodegeneration [26]. One of the mechanisms for removal of HNE from neurons is by conjugation to GSH, catalyzed by the action of glutathione-*S*-transferase (GST) followed by the action of the multidrug resistant protein-1 (MRP-1) to efflux out this conjugate from the cell [30]. However, in AD brain GST and MRP-1 were reported to have excess bound HNE and showed a reduced activity, supporting both the idea that oxidative modification leads to loss of functionality and the accumulation of HNE in AD brain [31].

There are several serious limitations to the use of immunoprecipitation to identify proteins; for example, the necessary availability of the antibody for the protein of interest, knowledge about the proteins, and the time- consuming and laborious nature of the process. Moreover, sometimes a posttranslational modification can change the structure of proteins, thereby preventing the formation of the appropriate antigen-antibody complex. Redox proteomics, that couples the 2D-gel electrophoresis separation of proteins with mass spectrometric techniques, is a facile approach to identify oxidatively modified brain proteins [32]. Proteomics has enabled us to identify a large number of oxidatively modified proteins in AD brain and models thereof.

2. Principles of redox proteomics

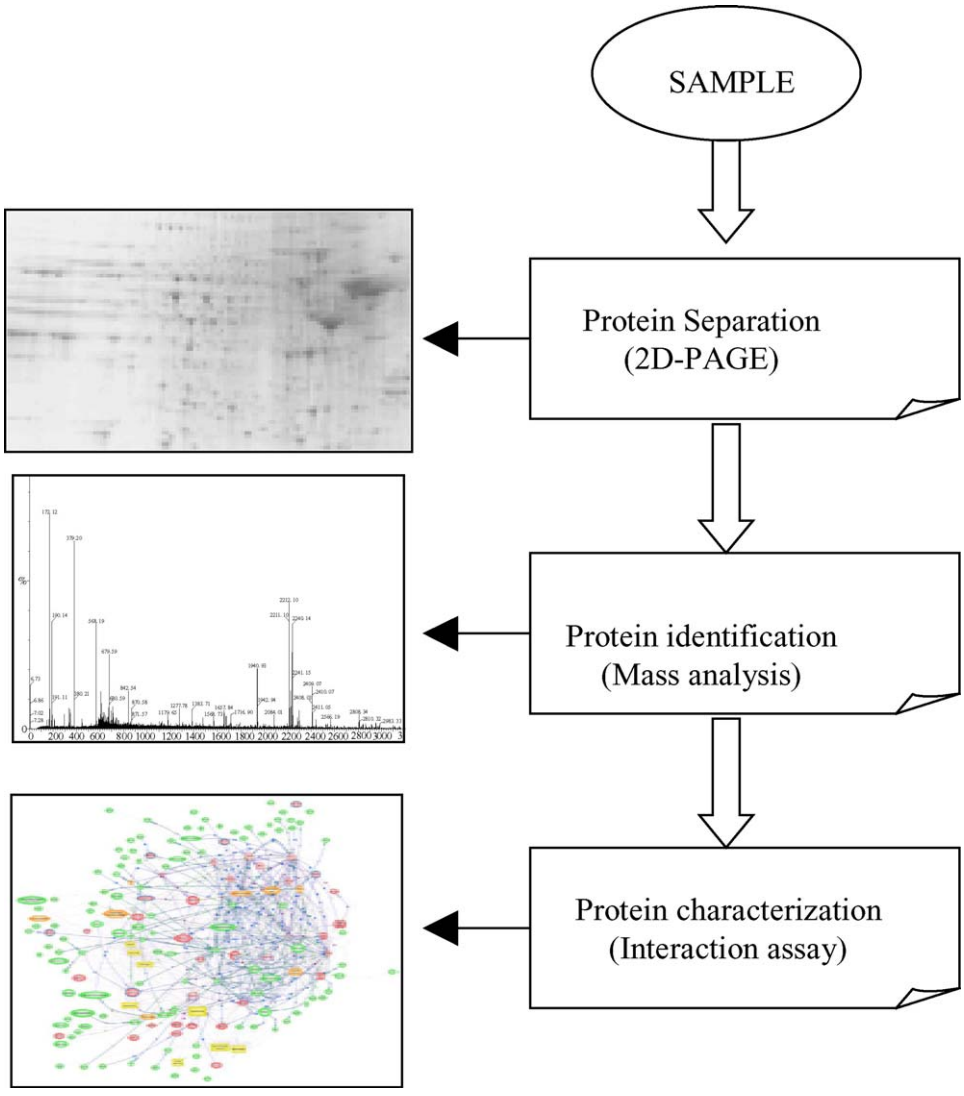
Proteome analysis is “the analysis of the entire PROTEin complement expressed by a genOME”. The analysis involves the systematic separation, identification, and quantification of many proteins simultaneously from a single sample (Scheme 1). Proteomic analysis encompasses the qualitative, quantitative and functional characterization of the entire protein profile of a given cell, tissue and/or organism.

In our laboratory, two-dimensional electrophoresis is used in this analysis that allows the separation of proteins based on two physicochemical properties, i.e., isoelectricfocusing (IEF) followed by the second dimension separation of proteins based on their relative mobility (M_r) [33]. The protein maps so obtained allow comparison of different sets of samples in terms of profiling of isoforms, splice variants, mutants, and post-translationally modified species, and the definition of protein–protein interactions, etc., using a computer-assisted program. Each spot on the resulting two-dimensional gel corresponds to a single protein species in the sample [34]. Thousands of different proteins can thus be separated, and information such as the protein p*I*, the apparent molecular weight, and the amount of each protein is obtained. The serious limitation of 2D is the solubilization of the membrane proteins [35], since ionic detergents are not compatible with IEF. The second limitation is the inability to detect low-abundance proteins, and the third limitation of 2D-PAGE is the insensitivity to proteins of high trypsin content. Chaotropic agents, such a urea and thiourea, coupled with nonionic or zwitterionic detergents can be used to solubilize proteins and avoid protein precipitation during the IEF and the SDS gel [35,37]. The use of immobilized pH IEF strips improved the reproducibility between the samples and also eliminates the typical cathodic drift associated with previously used tube gels [36]. Further, the use of narrow range IEF strips enables the investigator to separate proteins over a wide range of pH with a unit pH difference of one. However, the normally employed IEF strip pH range, i.e., 3–10, limits the identification of highly basic proteins. The identification of low-abundance proteins in a given sample is a limitation, as noted above, one that is important when a protein of this group is involved in the pathogenesis of a disease.

2D-PAGE represents undoubtedly one of the most-used techniques for protein separation, but non-SDS-PAGE methods are also employed. 2D-HPLC achieves separation of a protein mixture by eluting the sample through a series of columns with a coupled MS analysis [38,39]. Alternatively, an expression profiling can also be obtained by labeling a mixture of two samples with different isotopes that bind to specific amino acid side chains. The resulting isotopic labeling is further analyzed by a mass spectrometer. This technique is referred as isotopically coded affinity tags (ICAT) [40].

The redox proteomics studies performed in our laboratory aimed to identify post-translational modification of AD brain proteins essentially resulting as a consequence of oxidative damage. It has been demonstrated that covalent modifications of proteins are induced by reactive oxygen intermediates and by-products of oxidative stress [41–43]. Among general types of protein modifications, we focused our attention on protein carbonyls, lipid peroxidation adducts (HNE-adducts) and nitration of tyrosine residues (3-NT).

Our proteomic analysis to identify specifically oxidized proteins in AD brain and animal models of the disease, coupled 2D-PAGE with immunochemical detection of protein carbonyl derivatized by 2,4-dinitrophenyhydrazine (DNPH), nitrated proteins indexed by 3-nitrotyrosine (3-NT), and HNE-bound proteins followed by MS analysis (Fig. 1). A 2D western blot map is



Scheme 1. Schematic diagram of 2D-PAGE-based proteomics identification of proteins.

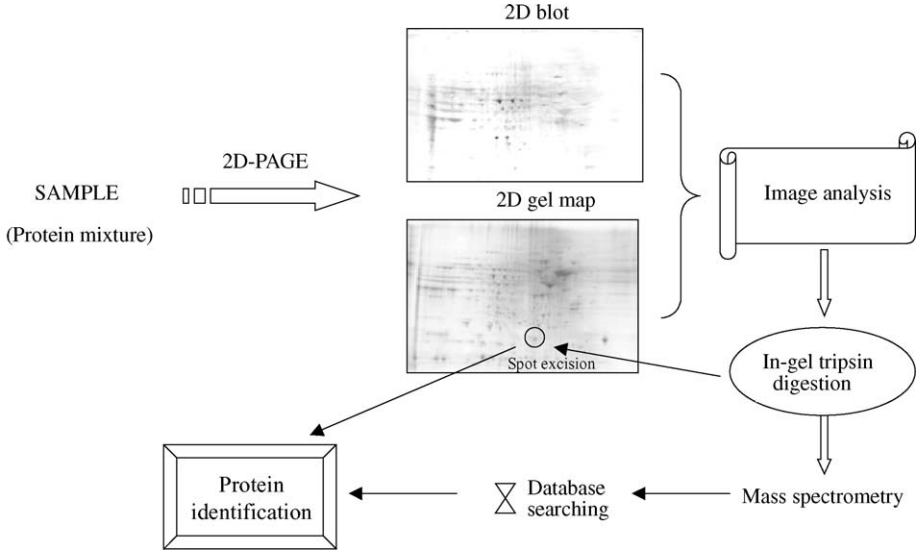


Fig. 1. Protocol for the identification of oxidized proteins by redox proteomics used in our laboratory.

achieved by using specific antibodies, e.g., anti-DNP, anti-3-NT or anti-HNE, that react with those proteins containing reactive carbonyl groups/3-NT/HNE in AD and control brain. 2D gel images, used to obtain the protein expression profile, and the 2D western blots are analyzed by image software (PD Quest, BioRad). This sophisticated software offers powerful comparative analysis and is specifically designed to analyze many gels or blots at once that were performed under identical experimental conditions. Powerful automatching algorithms quickly and accurately match gels or blots and sophisticated statistical analysis tools identify experimentally significant spots. The principles of measuring intensity values by 2D analysis software are similar to those of densitometric measurements. After completion of spot matching, the normalized intensity of each protein spot from individual gels (or membranes) is compared between groups using statistical analysis.

Statistical significance is assessed by a two-tailed Student's *t* test. *P* values <0.05 are considered significant for comparison between control (age-matched control brain) and experimental data (AD brain). Only the proteins in AD brain that were significantly different from aged-matched control brain assessed by the Student's *t*-test were selected for identification. Similar statistical analyses are usually used for proteomics data analysis, since sophisticated statistical analyses used for microarray data are not applicable to redox proteomics data [44–46]. The selected protein spots are excised and digested in-gel; the resulting peptides are then extracted and submitted for mass spectrometry analysis.

2.1. Mass spectrometry and data base searching

The protein of interest is cleaved enzymatically or chemically and analyzed by mass spectrometry. MALDI can tolerate moderate salt and buffer concentrations in the protein sample mixture and almost produces singly charged ions compared to other mass techniques like ESI. The identification of a protein from its peptide sequence derived from the mass spectrum has been facilitated by the development of proteomics databases (Table 1). The first major protein database is Swiss-Prot, which allows protein identification by using computer algorithms [47], freely accessible online. These search engines provide a theoretical protease digestion of the proteins contained in the database by comparing the resulting peptide masses to the experimental masses obtained from the in-gel digested proteins. Several factors have to be considered to obtain correct protein identification, such as the protein size and the probability of a single peptide to occur in the whole database. Any hit with a score higher that the one obtained from the search is considered statistically significant and has an excellent chance to be the protein cut from a given spot. In addition, the molecular weight and the p*I* of the protein is calculated based on the position in the 2D map to avoid any false identification. Immunochemical methods are also performed to validate protein identification [48,49].

Table 1
Mass spectrometry search engines for engines for peptide mass fingerprinting

Search engine	URL
Mascot	http://www.matrixscience.com
MOWSE	http://www.hgmp.mrc.ac.uk/Bioinformatics/Webapp/mowse
Profound	http://prowl.rockefeller.edu/profound_bin/WebProFound.exe
MS-fit	http://prospector.ucsf.edu/ucsfhtml4.0/msfit.htm
Peptident	http://ca.expasy.org/tools/peptident.html

3. Redox proteomics studies in Alzheimer's disease

Several proteomic approaches have been used to study degenerative diseases, including Alzheimer's disease, and many protein classes from signalling, metabolic, cytoskeleton, chaperone, antioxidant, and proteasome have been shown to be tentatively involved in pathological mechanisms [50]. While some protein polymorphisms are linked to disease states, most are not. Yet protein isoforms do have in many cases a direct or indirect effect on the activities of the proteins concerned. For example, it is estimated that each human protein exists, on the average, in 10–15 different post-translationally modified forms.

Much of the information processing in healthy and diseased human cells can be studied only at the protein level, and there is increasing evidence linking minor changes in expression of some modifications with specific diseases. There are many potential applications of proteomics in neuroscience: comparative protein expression profiling, mapping of protein–protein interaction, post-translational protein modification profiling, determination of neuroproteome and many others.

Our laboratory has used a redox proteomics approach to study post-translational modifications of proteins in AD brains, with particular regard to oxidative modifications. It has been widely accepted that oxidative stress plays a central role in the pathogenesis of AD [17,51,52]. Reactive oxygen and nitrogen species may cause various types of chemical modifications of proteins. Such modifications if irreversible are often associated with permanent loss of function and may lead to the elimination of accumulation of the damaged proteins [53]. Since oxidized forms of proteins are thermodynamically unstable, the partially unfolded proteins are assumed more susceptible to irregular proteolysis. To understand how oxidative stress contributes to neurodegenerative processes and to evaluate the therapeutic effect of antioxidant drugs, it would be desirable to have a comprehensive profiling of the types of oxidative modification that occur to cellular proteins.

Our redox proteomics approach has made possible the identification of specifically oxidized proteins in AD brain, providing for the first time evidence on specific proteins in which oxidative stress may play a crucial role in AD-related neurodegeneration. The information obtained has significant potential for providing insight into AD pathogenesis, detecting disease markers, and identifying potential targets for drug therapy in AD. Application of redox proteomics to study cellular events, especially related to disease dysfunction, may provide an efficient tool to understand the main mechanisms involved in disease pathogenesis and progression. Protein oxidation levels were reported to be increased in AD brain as indexed by increased protein carbonyls and 3-nitrotyrosine levels [19,22,54,55]. Redox

proteomics offers a tool to identify such oxidized proteins and represents a significant step in understanding how oxidized proteins affect cellular metabolism that ultimately results in synapse loss and neuronal cell death. Using redox proteomics, we identified the proteins that were significantly oxidized in AD inferior parietal lobule (IPL) and hippocampus [19,46,56–58]. The proteins we identified play roles in different cellular functions, and here we discuss their potential link in AD pathology. Oxidatively modified brain proteins identified by our redox proteomic approach are: creatine kinase (CK), α-enolase, triosephosphate isomerase (TPI), phosphoglycerate mutase 1 (PGM1), gamma-soluble *N*-ethylmaleimide-sensitive factor attachment protein (SNAP), glutamine synthase (GS), glutamate transporter (EAAT2), ubiquitin carboxy hydrolase L-1 (UCHL1), heat shock proteins (HSPs), Neuropolypeptide h3, dihydropyrimidinase-related protein 2 (DRP2), beta-actin, peptidyl prolyl cis, trans isomerase (PIN1), and carbonic anhydrase II (CA2) (Table 2).

3.1. Energy dysfunction

CK, TPI, PGM1 and α-enolase are enzymes involved in ATP production. The impairment of energy metabolism has been shown to selectively contribute to neurodegenerative processes. The brain depends upon continuous glucose metabolism for normal function. Although the brain represents only 2% of the body mass, it accounts for 20% of the body's glucose consumption. Even minimal interruption of brain glucose metabolism impairs brain function. For example, reducing the oxygen or glucose to the brain diminishes memory [59]. Many cellular functions rely on ATP consumption, especially in those cellular compartments where a high rate of ATP is fundamental to maintain signaling pathways, such as the synapse. Consistent with this notion, it has been demonstrated that once oxidized CK, PGM1 and enolase showed a loss of enzymatic activity ultimately leading to a decrease in ATP production [46,60,61]. Considering that glucose metabolism is the primary source of energy in the brain, oxidation, and consequent activity decrease of target metabolic enzymes, play roles in the known energy dysfunction in AD brain and contributes directly or indirectly to metabolic abnormalities associated with a decrease of cellular ATP content.

3.2. Excitotoxicity

It has been previously shown that the glutamate transporter EAAT2 is oxidatively modified by HNE in AD brain [26], and that Aβ(1–42) also leads to HNE binding to EAAT2 [26]. EAAT2 and GS regulate the extraneuronal levels of glutamate and neurotransmission. Based on the assumption that oxidative modifications affect protein function, we demonstrated that the enzymatic activity of GS decreased in AD brain [62]. Impaired function of EAAT2 and GS may result in excess glutamate on the outside of neurons, followed by an influx of Ca^{2+} and activation of NMDA and AMPA receptors that are ultimately responsible and cause neuronal excitotoxic death [63,64].

3.3. Proteosomal dysfunction

UCHL1, as part of the ubiquitin–proteasome system necessary for protein degradation, has been implicated in the death of neurons in neurodegenerative diseases, such as Parkinson's, Alzheimer's, and Huntington's [65–67]. The ubiquitin-proteasome system acts as the cell's quality control system by disposing of damaged, misshapen, and excess proteins. Although the exact function of UCHL1 protein is not fully understood, it appears to have two enzyme activities. One activity, called hydrolase, removes and recycles ubiquitin molecules from degraded proteins [68]. This recycling step is important to sustain the degradation process. The other enzyme activity, known as ligase, links together ubiquitin molecules for use in tagging proteins for disposal [68]. In the human disease states, UCHL1 levels are low [65] or its function is compromised [69]. UCHL1 has found to be oxidized in AD brain [46]. The oxidative modifications may lead to decreased hydrolase activity, which may disrupt the ubiquitin–proteasome system. Instead

Table 2
Redox proteomics identification of oxidatively modified proteins in AD and models thereof

	Human AD brain	SAMP8	Synaptosomes plus Aβ(1–42)	In vivo Aβ(1–42) model	Neuronal cell culture plus Aβ(1–42)
Energy-related enzymes	CK, Enolase, TPI, PGM1	CK, Enolase, LDH	PGM1, GAPDH Pyruvate dehydrogenase	PGM1, pyruvate dehydrogenase, GAPDH	GAPDH
Neurotrasmitter-related proteins	EATT2, GS	–	–	–	
Proteasome-related proteins	UCHL1 HSPs	–	HSP 60	HSP 60	
Cholinergic system	Neuropoly-peptide h3	–	–	–	
pH regulation-protein	CA2 II	–	–	–	
Structural proteins	DRP2 β-actin	DRP2 α-spectrin	β-Synuclein	β-Synuclein	
Signal transduction			14-3-3 Zeta	14-3-3 Zeta	14-3-3 Zeta
Cell cycle	PIN1	–	–	–	
Antioxidant	–	–	GST	–	

of being degraded, unwanted proteins may accumulate to toxic levels that impair or kill nerve cells in the brain. In addition, a recent in vitro study showed that HNE, a lipid peroxidation product, decreased hydrolase activity of recombinant UCHL1 [70]. Inhibition of the proteasomal pathway for degrading abnormal proteins leads to protein aggregation, increased oxidative damage and increased protein nitration [71]. The deleterious effects of proteasome dysfunction on neuronal survival are pronounced.

3.4. Lipid abnormalities and cholinergic alterations

Neuropolypeptide h3, a phosphatidyloethanolamine binding protein [PEBP] or cholinergic neurostimulating peptide, has been identified as a specifically oxidized protein in AD brain [19]. Phospholipid asymmetry is essential for membrane structure and integrity [72], and it is reasonable to argue that loss of activity of PEBP as a consequence of its oxidative modification might compromise phospholipid asymmetry. Therefore, phosphatidylserine would become exposed to the outer bilayer of the membrane and initiate an apoptosis cascade leading to neuronal death. Neuropolypeptide h3 is also a cholinergic neurostimulating peptide by upregulating the activity of choline acetyltransferase. This latter enzyme has found to be HNE-modified in synaptosomes exposed to Aβ(1–42) [17], consistent with previous studies that reported a decrease activity of the enzyme in AD brain [73].

3.5. Neuritic abnormalities

One of the characteristic hallmarks of AD pathology is memory loss, potentially associated with decreased interneuronal connections and to a shortened dendritic length [74]. DRP2 performs important functions in neuronal repair and in axonal outgrowth [75,76]. Decreased expression of DRP2 was observed in AD brain, adult Down's syndrome [77], schizophrenia and affective disorders [78]. DRP2 was shown to be oxidatively modified in AD brain [56], and its decreased function could be responsible of shortened dendritic length and synapse loss. β-Actin, a specifically oxidatively modified protein in AD brain [19] and whose expression is altered in AD, is involved in cytoskeleton network integrity. In adult brain, actin is concentrated in dendritic spines where it can produce rapid change in their shape that might be involved in memory function [79]. Hence, decreased function of both DRP2 and actin, as a consequence of oxidative modifications, are consistent with the memory impairment and synapse loss observed in AD.

3.6. Tau hyperphosphorylation

Tau is a microtubule-associated protein that is involved in microtubule assembly and stabilization. In recent years, tau-protein has attracted considerable interest as tau pathology is central in AD as well as to a number of disorders presenting with dementia and/or motor syndromes, where it is found in a hyperphosphorylated state assembled into filamentous nerve cell inclusions [80–82]. Neurofibrillary degeneration involves hyperphosphorylation of tau, which critically impairs its binding capacity to microtubule and, therefore, is believed to disrupt the axonal cytoskeleton. Recently, Lu et al. [83] described the ability of peptidyl-prolyl cis-trans isomerase Pin1 (PPIases) to recover microtubule-binding affinity and microtubule stabilization of phosphorylated tau. Pin1 is a chaperone enzyme that binds to mitotic serine or threonine phosphoproteins and catalyzes the conversion of the cis to trans and vice versa of proteins between given amino acids and a proline [84]. Pin1 is essential for cell growth and is required for proper progression through the cell cycle in dividing cells [85]. Recent studies showed that Pin1 is colocalized with phosphorylated tau in AD and other tauopathies [86]. We showed that Pin1 is oxidatively modified in AD brain [57]. This post-translational modification could be responsible of altered binding capacity of Pin1 to many of its target proteins, thus impairing the downstream cell cycle machinery. The oxidation of Pin1 may play a role in the accumulation of phosphorylated tau proteins observed in AD, but the involvement of Pin1 in the disease progression requires further investigation.

3.7. Synaptic abnormalities and LTP

Morphologic studies of the neuropathology in Alzheimer's disease (AD) have demonstrated significant loss of synaptic connectivity in many regions of the neocortex and hippocampus [87]. The strongest correlation with cognitive decline in AD is with the synaptic density. Soluble NSF-attachment proteins (SNAPs) are highly conserved proteins that participate in intracellular membrane fusion and vesicular trafficking. In mammals, there are three different isoforms of SNAPs, alpha-, beta- and gamma-SNAP. Alpha- and gamma-SNAP are ubiquitously expressed, whereas beta-SNAP is the brain-specific isoform. This family of proteins is involved in neurotransmitter release, hormone secretion and mitochondrial organization and play important role in vesicular transport in the secretory pathway. The oxidation of SNAP could lead to impaired learning and memory processes and altered neurotransmission system in AD brain.

3.8. pH maintenance

Carbonic anhydrase II (CA2) is a Zn^{2+} metallo-enzyme that catalyzes reversible hydration of carbon dioxide (CO_2) to bicarbonate (HCO_3^-). CA2 regulates CO_2 and HCO_3^- transport, cellular pH and also regulates H_2O and electrolyte balance [88]. The finding that CA2 is oxidatively modified in AD brain supports previously studies that showed a decrease enzyme activity of CA2 in AD brain [61]. Oxidative modification of CA2 may lead to loss of the buffering system in brain, mitochondrial alterations and eventually impaired synthesis of glucose and lipids. pH homeostasis, both intracellular and extracellular, is fundamental for regulation of catalytic activity of enzymes by maintaining their proper conformation. Also, altered pH could affect protein aggregation, which is clearly pronounced in AD brain. Consequently, altered neuronal pH conceivably may contribute to the progression of AD.

4. Redox proteomics in Aβ(1–42) in vitro, ex vivo and in vivo models

4.1. Neuronal cells model

The use of animal models of AD has been of principal importance for providing insight into the neurochemical and cellular changes associated with this disorder and also for drug discovery. Several knock-in and knock-out models were constructed to study the role of the genetic mutations of AD, such as APP, PS-1, PS-1/APP, tau, and Tau/APP/PS-1 animal models, which represent one or more pathological markers of AD [89–91]. In addition, these animal models also allow study of the consequences of oxidation of proteins on cellular activities, as exemplified by the gracile axonal dystrophy (GAD) mouse model that has a defective UCHL-1 protein, an oxidized protein in AD brain [46,92]. Redox proteomics of GAD mouse brain showed oxidization of a number of specific proteins [92].

Aβ(1–42) aggregates are the pathological hallmark of AD, and oligomers of Aβ(1–42) are believed to be involved in the pathogenesis of AD [17]. A number of in vivo and in vitro studies showed Aβ(1–42) as a mediator of oxidative stress [17,18]. In primary neuronal cell culture Aβ(1–42) treatment showed an increased protein oxidation, lipid peroxidation and ROS, each of which can be blocked by use of vitamin E and γ-glutamylcysteine ethyl ester (precursor to glutathione) [24,93,94]. Using redox proteomics we identified 14-3-3 zeta and glyceraldehyde 3-phosphate dehydrogenase as oxidatively modified proteins following exposure to Aβ(1–42) [94]. Further, the elevation of cytosolic GSH ameliorated Aβ(1–42) induced oxidation [94].

4.2. Synaptosomes model

Synaptosomes are sealed presynaptic nerve terminals that can respire, take up oxygen and glucose, extrude Na^+, accumulate K^+, maintain anormal membrane potential and, on depolarization, release transmitter in a Ca^{2+}-dependent manner [95,96]. Therefore, synaptosomes were used to determine the changes occurring at the synapse. Synapse loss is thought to be an early pathological event in AD [97] and could be a key event in early cognitive decline [98–100]. In AD, Aβ accumulation was observed at the synapse that may increase oxidative stress leading to synaptic degeneration and impaired normal synaptic functions [101–103]. The loss of synaptic connections in the hippocampal dentate gyrus disrupts the circuitry between the hippocampus and the entorhinal cortex leading to the memory deficits associated with AD [99]. A recent redox proteomics study showed that incubation of synaptosomes with 10 μM Aβ(1–42) partially replicates what is observed in AD brain. Aβ-treated synaptosomes showed specific oxidation of β-actin, glial fibrillary acidic protein, and dihydropyrimidinase related protein-2 [103]. Further, a trend towards increase oxidation was observed for H^+-transporting two-sector ATPase, syntaxin binding protein 1, glutamate dehydrogenase, β-actin, and elongation factor Tu [103]. The oxidation of these proteins could lead to loss of a variety of cellular functions including energy metabolism, cytoskeletal integrity, and neuronal communication that are pathological hallmark of AD. These results further suggest a role of oxidative stress in the degeneration of neurons.

4.3. Intracerebral injection of Aβ(1–42) model

Intracerebral injection of Aβ(1–42) into the nucleus basalis magnocellarius of rat brain replicates cholinergic loss observed in AD [104]. In AD brain basal forebrain cholinergic neurons undergo degeneration that is associated with cognitive deficits [105,106]. A region-specific redox proteomics showed extensive protein oxidation in hippocampus compared to cortex and nucleus basalis in this model [107]. The oxidized proteins identified in this model, includes proteins involved in cellular structure (β-synuclein), signal transduction (14-3-3 zeta), energy metabolism [phosphoglycerate mutase 1 (PGM1), pyruvate dehydrogenase, and glyceraldehyde 3-phosphate dehydrogenase], and stress responses (HSP60) [107], and the oxidation of these proteins could impair all these cellular functions.

4.4. Senescence-accelerated mice prone 8 (SAMP8)

The SAMP8 strain of mouse shows accelerated senescence and develops cognitive deficits. SAMP8 mice have increased amounts of Aβ and Aβ-like-protein immunoreactive granular structures in brain similar to those moieties observed in AD [108,109]. SAMP8 mice also show an increased oxidative stress [62] and deposit Aβ 4–12 months [110]. This Aβ level observed in the SAMP8 mouse brain is much closer to the estimated 50% increase in Aβ observed in AD brain [111] than is observed in APP transgenic mouse models. Hence, SAMP8 mouse is an excellent model for studying Aβ toxicity in vivo.

Previously, oxidized proteins in brain aging were identified using a combination of MudPIT techiniques with a hydrazine biotin-streptavidin isolation of carbonylated proteins in aged mice [112]. Recently, we identified alpha enolase, gamma enolase, lactate dehydrogenase 2, alpha spectrin, creatine kinase and DRP-2 as oxidized proteins in SAMP8 mice brain. Treatment of SAMP8 mice with lipoic acid [48] or with antisence oligonucleotide to APP [113] led to decreased oxidative stress [113,114] and loss of specifically oxidized proteins [48,115]. Such treatment also improved learning and memory in these animals, suggesting that Aβ contributes to these defects in SAMP8 mice and, by extension, in AD. Among these proteins alpha enolase, gamma enolase, LDH, and CK are the common oxidized proteins observed in human AD brain and SAMP8 mice by proteomics. Further, the expression of alpha spectrin and DRP-2 was reported to be altered in AD brain.

5. Conclusions

Our redox proteomics approach to identify oxidized proteins in AD brain provides insight into the mechanisms involved in the pathogenesis and progression of AD. The information obtained by proteomics analysis offers the possibility of establishing new hypotheses for the mechanisms of neurodegeneration found in AD in which particular proteins play a key role. Moreover,

our current findings demonstrate how proteomics could be of help in developing therapeutic strategies to prevent or delay neurodegeneration observed in AD. Despite current limitations of this technique, proteomics still represents a promising tool to gain insight the molecular basis of the disease, and further studies to achieve fast, reliable and sensitive results are in progress. With the improvement of proteomics tools, other kinds of protein post-translational modifications can be investigated. Our laboratory is actively employing proteomic technology to study a wide variety of neurodegenerative disorders and models thereof.

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