Brain Protein Oxidation and Modification for Good or for Bad in Alzheimer’s Disease

Rukhsana Sultana and D. Allan Butterfield

Abstract Alzheimer’s disease (AD) is the most common cause of dementia in the elderly and is characterized by senile plaques, neurofibrillary tangles, synapse loss, and progressive neuronal deficits. There is an abundance of evidence suggesting that oxidative stress is involved in the pathogenesis of Alzheimer’s disease. Several investigations have revealed the presence of oxidation products of proteins, lipids, and DNA in postmortem tissue from AD patients, indices that are indicative of increased oxidative stress. In the present review we discuss the role of protein oxidation in the brain of subjects with AD and MCI.

Keywords Alzheimer’s disease · Protein oxidation · Protein carbonyl · 3-Nitrotyrosine · Mild cognitive impairment

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1 Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly (Evans et al., 1989). AD is characterized by senile plaques, neurofibrillary tangles, synapse loss, and progressive neuronal deficits (Hardy and Selkoe, 2002; McGeer and McGeer, 2003). In addition to the formation of amyloid plaques and NFTs, gliosis, chronic inflammatory reactions, excitotoxic damage, and oxidative stress all appear to contribute to the progression of AD. The proximate cause(s) of the progressive cell death and loss of memory and cognitive functions resulting in profound dementia are still poorly understood. Increased risk factors for AD include stroke, hypertension, diabetes, atherosclerosis, and hypercholesterolemia (Kalaria, 2000; Iadecola, 2003; Casserly and Topol, 2004; Messier and Teutenberg, 2005), and the presence of multiple factors further increases the risk of AD (Luchsinger et al., 2005). These pathologies are associated with chronic inflammation and altered blood vessel responsiveness (Akiyama et al., 2000; Iadecola, 2003; McGeer and McGeer, 2003).

Oxidative stress occurs due to an imbalance in the oxidant and antioxidant levels (Butterfield and Stadtmann, 1997). Oxidants can damage virtually all biological molecules: DNA, RNA, cholesterol, lipids, carbohydrates, proteins, and antioxidants. In AD brain, the levels of antioxidants were found to be decreased with an associated increase in protein oxidation (indexed by protein carbonyls and 3-nitrotyrosine), lipid peroxidation, DNA oxidation, advanced glycation end-products, and reactive oxygen species (ROS) formation, among other indices, strongly suggesting a role for oxidative stress in the pathogenesis of AD (Markesbery, 1997; Smith et al., 1997; Butterfield et al., 2001; Lovell and Markesbery, 2001; Butterfield, 2002; Butterfield and Lauderback, 2002; Castegna et al., 2003; Smith et al., 2004; Sultana et al., 2006a, 2006b). Moreover, the use of vitamin E in cell culture diminishes Aβ(1-42)-induced toxicity, further consistent with a role of oxidative damage in AD pathology (Behl, 1999; Yatin et al., 2000; Butterfield and Lauderback, 2002; Boyd-Kimball et al., 2004; Butterfield and Boyd-Kimball, 2005). In addition, Aβ(1-42) can bind to receptors on neuronal and glial cells, for example, the α7-nicotinic receptor, glutamate, and potassium channels in membrane receptors in adenosine receptor subtypes (Ariste et al., 1993; Etcheberrigaray et al., 1994; Engstrom et al., 1995), decreasing glucose transport across brain endothelial cells (Blanc et al., 1997), and activating the release of chemokines (Fiala et al., 1998) and cytokines (Akama and Van Eldik, 2000). In this review we discuss the role of brain protein oxidation in AD pathology.

2 Role of Aβ(1-42) in Oxidative Stress

Amyloid β-peptide, particularly the 42-mer Aβ(1-42), is thought to be central to the pathogenesis of AD (Selkoe, 2001). In vitro and in vivo studies suggest some et al (1998) role senile oligo...
suggest that Aβ promotes oxidative stress and lipid peroxidation in synaptosomes and neuronal cultures (Butterfield et al., 1994; Keller et al., 1997; Mark et al., 1997; Yatin et al., 1999; Butterfield et al., 2001; Drake et al., 2003; Mohmmad Abdul et al., 2004; Boyd-Kimball et al., 2005a, b; Mattson et al., 1998). Altogether, these studies favor the suggestion that Aβ plays a central role in the pathogenesis of AD as a mediator of oxidative stress. Although senile plaques contain deposited Aβ, the toxic species of Aβ is likely small oligomeric species (Drake et al., 2003; Klein et al., 2004; Ashe, 2005; Walsh et al., 2005).

Aβ peptides (39–43 amino acids) are derived from the amyloid precursor protein (APP) present in both neurons and glial cells in the brain. Although the cellular function of APP has not been completely elucidated, this protein is comprised of a hydrophobic membrane-spanning domain, N-glycosylation sites, and sites for binding Zn^{2+} and Cu^{2+} with high affinity (Hesse et al., 1994). Copper is present at substantial levels in the brain and its release as a result of synaptic activation can reach mM concentrations in the synaptic cleft (Kardos et al., 1989). The interaction between APP and Cu^{2+} may result in the reduction of Cu^{2+} to Cu^{+} and the formation of an intramolecular disulfide bond (Mucke et al., 1994). In the presence of oxidants such as H_2O_2, the APP-Cu interaction may cause APP fragmentation and increase in the production of Aβ. Under normal conditions, Aβs appear to be normal products of APP metabolism and are present in cerebrospinal fluid (CSF) and plasma. Studies by Huang et al. (Huang et al., 1999) reported evidence for a direct interaction between Aβ and Fe^{3+}/Cu^{2+} to create a strong positive formal reduction potential, which can rapidly reduce Fe^{3+} and Cu^{2+} ions and trap molecular oxygen to generate H_2O_2. Transition metals are highly enriched in senile plaques, where they are likely to be bound to amyloid-β (Bush, 2000). Chelation of transition metals efficiently resolves aggregated amyloid-β and senile plaques in vitro (Cherny et al., 2000). The more pathological Aβ(1-42), which has a greater ability to aggregate than Aβ(1-40), has a greater affinity for metals when compared to the latter peptide (Atwood et al., 2000).

The neurotoxic properties of Aβ have also been shown to be associated with methionine at residue 35 of Aβ (Met35) (Butterfield and Boyd-Kimball, 2005). The substitution of methionine by norleucine from Aβ abolishes free radical production, protein oxidation, and toxicity to hippocampal neurons (Butterfield and Boyd-Kimball, 2005). In addition, substitution of a carbon atom for the S atom of methionine completely abrogates Aβ(1-42) neurotoxicity (Yatin et al., 1999; Butterfield and Kanski, 2002), and in vivo studies indicate methionine residue 35 is central for Aβ-induced oxidative damage (Yatin et al., 1999). Studies from our laboratory (Varadarajan et al., 2000) and others (Curtain et al., 2001) showed that Cu^{2+} bound to Aβ(1-42) interacts with Met35 residue to produce free radicals; in the absence of methionine in Aβ(1-42) redox metal ions play no role in the oxidative stress and neurotoxicity induced by the peptide (Varadarajan et al., 2000, 2001). Taken together these results are consonant with the notion that Aβ-induced protein oxidation may in part account for neurodegeneration in AD brain (Butterfield and Boyd-Kimball, 2005).
3 Protein Oxidation in AD

Reactive nitrogen species (RNS) and reactive oxygen species (ROS) can react with proteins leading to oxidatively modified proteins indexed by protein carbonyls and 3-nitrotyrosine (3-NT). Hence, measuring the levels of protein carbonyls and 3-NT reflects the level of protein oxidation in neurons. Increased oxidative stress during aging or in neurodegenerative diseases may lead to accumulation of oxidized proteins. As a consequence, proteins become resistant to proteasomal degradation. On the other hand, oxidative stress is also known to enhance protein turnover accompanied by a specific removal of oxidized proteins. The removal of oxidized proteins has been considered to be impaired in AD due to decreased proteasomal activity (Ding et al., 2006; Halliwell, 2006). Therefore, increased oxidative stress may lead to alterations in both oxidative modification and turnover of proteins and may further trigger immune response. Oxidative damage has been associated with aggregation of proteins, energy dysfunction, calcium dysregulation, mitochondrial malfunction, chronic inflammation, altered antioxidant function, and accumulation of redox-active metals (Butterfield, 2002).

3.1 Protein Carbonyls in AD Brain

Protein carbonyls are formed by backbone fragmentation (Fig. 1), hydrogen atom abstraction at alpha carbons, attack on several amino acid side-chains (Lys, Arg, His, Pro, Thr, etc.), and by the formation of Michael adducts between His, Lys, and Cys residues and reactive alkenals (e.g., hydroxynonenal (HNE)) (Fig. 2). Furthermore, glycation/glycoxidation of Lys amino groups, forming advance glycation end-products (AGEs) (Berlett and Stadtman, 1997; Butterfield and Stadtman, 1997; Dalle-Donne et al., 2003a,b; Stadtman and Levine, 2003), can also lead to protein carbonyl formation. In addition, a number of reactions of protein radicals

![Mechanism of carbonyl formation from peptide backbone scission](image-url)
can give rise to other radicals, which can cause damage to other biomolecules. Protein carbonylation leads to oxidation of side-chain hydroxyls, converting them into ketone or aldehyde derivatives, backbone fragmentation, formation of new reactive species (peroxides, DOPA), release of further radicals and occurrence of chain reactions. Most protein damage is irreparable and could lead to a wide range of downstream functional consequences, such as dimerization or aggregation, unfolding or conformational changes to expose more hydrophobic residues to an aqueous environment, loss of structural or functional activity, alterations in cellular handling/turnover, effects on gene regulation and expression, modulation of cell signaling, induction of apoptosis and necrosis, and so on. These phenomena indicate that protein oxidation has physiological and pathological significance (Butterfield and Stadtman, 1997).

Certain oxidation products of proteins, such as oxidation of Cys to cystine, and Met residues to methionine sulfoxide can be repaired by enzymes such as glutathione reductase and methionine sulfoxide (Gabbita et al., 1999; Moskovitz et al., 2002). Other enzymatic means of removing protein carbonyls involve carbonyl reductase and aldehyde dehydrogenase. The majority of the oxidized proteins are catabolized by proteasomal and lysosomal pathways, but some materials appear to be poorly degraded and accumulate within the cell (Dean et al., 1997; Grune et al., 2003). The accumulation of such damaged material may contribute to a range of human pathologies.
Protein carbonyls are chemically stable compared to the other products of oxidative stress, for example, F2 isoprostanes, which are readily generated during sample storage, processing, and analysis. Hence, protein carbonyls are a general and widely used marker to assess the extent of oxidation of proteins in both in vivo and in vitro conditions (Berlett and Stadtman, 1997; Butterfield and Stadtman, 1997; Dalle-Donne et al., 2003a; Drake et al., 2003; Stadtman and Levine, 2003; Boyd-Kimball et al., 2005b). Several sensitive assays were developed for detection of oxidatively modified proteins (Winterbourn and Buss, 1999; Levine et al., 2000; Dalle-Donne et al., 2003b).

In AD brain and plasma, several proteins have been identified as targets of oxidative stress (Castegna et al., 2002a,b; Castegna et al., 2003; Dalle-Donne et al., 2003a; Butterfield, 2004; Castegna et al., 2004b; Dalle-Donne et al., 2005; Sultana et al., 2006d, 2006a). Immunohistochemical studies have revealed an increase in carbonyl formation in AD brain (Hensley et al., 1995). However, in that study, no oxidatively modified proteins were actually identified. A band of oxidized protein at 78 kDa on one-dimensional oxyblots in AD plasma was observed (Yu et al., 2003). A recent study revealed that several isoforms of fibrinogen α-chain precursor protein and of α-1-antitrypsin exhibited a greater specific oxidation in AD plasma (Choi et al., 2002). Using redox proteomics (Dalle-Donne et al., 2006) our laboratory first identified cytosolic creatine kinase BB isoform, β-actin, glutamine synthase, ubiquitin carboxy-terminal hydrolase L-1, dihydropyrimidinase-related protein 2, alpha-enolase, phosphoglycerate mutase 1 (PGM1), gamma-soluble N-ethylmaleimide-sensitive factor attachment protein (SNAP), peptidyl-prolyl cis/trans isomerase 1 (Pin1), triosephosphate isomerase, ATP synthase alpha chain, and carbonic anhydrase 2 as targets of protein oxidation in AD brain, and further studies showed that the oxidatively modified proteins are generally functionally inactive [see Table 1] (Castegna et al., 2002a,b; Castegna et al., 2003; Butterfield, 2004; Sultana et al., 2006c, 2006a). Thus, identification of carbonylated proteins should be followed by functional assessment of the protein, whether it is enzyme or structural protein. These functional studies may identify metabolic or structural defects caused by oxidative modification.

These data support the notion that protein carbonylation perturbs energy metabolism, pH regulation, cell cycle, and mitochondrial functions, as well as Aβ production (Pastorino et al., 2006).

### 3.2 Protein Nitration in AD

Another marker for protein oxidation is nitration of tyrosine residues, and numerous previous studies support the notion that nitrosative stress also contributes to neurodegeneration in AD (Smith et al., 1997; Tohgi et al., 1999; Castegna et al., 2003; Sultana et al., 2006b). A number of mechanisms for tyrosine nitration of protein have been proposed, and the two widely believed to exist in vivo involve formation of peroxynitrite or mediation via hemeperoxidases (Brennan et al., 2002). These mechanisms involve NO or its by-products that react with ROS (Beckman et al.,
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Table 1 Carbonylated and nitrated proteins in AD brain

<table>
<thead>
<tr>
<th>Protein Functions</th>
<th>Carboxylated Proteins</th>
<th>Nitrated Proteins</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurotransmitter-related proteins</td>
<td>GS</td>
<td>-</td>
<td>Butterfield et al. (1997) Castegna et al. (2002b)</td>
</tr>
<tr>
<td>Proteasome-related proteins</td>
<td>UCHL1</td>
<td>-</td>
<td>Castegna et al. (2002b) Sultana et al. (2005b) Castegna et al. (2003)</td>
</tr>
<tr>
<td>Cholinergic system</td>
<td>HSC 71</td>
<td>Neuropoly-peptide</td>
<td>Castegna et al. (2003)</td>
</tr>
<tr>
<td>PH regulation-protein</td>
<td>CA2</td>
<td>CA2</td>
<td>Sultana et al. (2005b)</td>
</tr>
<tr>
<td>Structural proteins</td>
<td>DRP2</td>
<td>β-actin</td>
<td>Castegna et al. (2002a) Sultana et al. (2005b)</td>
</tr>
<tr>
<td>Cell cycle</td>
<td>Pin</td>
<td></td>
<td>Sultana et al. (2005a, b)</td>
</tr>
<tr>
<td>Synaptic abnormalities and LTP</td>
<td>Gamma-SNAP</td>
<td></td>
<td>Sultana et al. (2005b)</td>
</tr>
<tr>
<td>Mitochondrial abnormalities</td>
<td>-</td>
<td>ATP synthase alpha chain</td>
<td>Sultana et al. (2006b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>VDAC-1</td>
</tr>
</tbody>
</table>

CK, creatine kinase BB; TPI, triose phosphate isomerase; PGM1, phosphoglycerate mutase 1; LDH, lactate dehydrogenase; CA2, carbonic anhydrase 2; GS, glutamine synthase; UCHL1, ubiquitin carboxy-terminal hydrolase L-1; HSC 71, heat shock cognate 71; DRP2, dihydropyrimidinase-related protein 2; Pin1, peptidyl-prolyl cis-trans isomerase; Gamma-SNAP, gamma-Soluble NSF-attachment proteins; VDAC, voltage dependent anion channel protein.

2001). NO reacts with superoxide, a toxic anion produced in the cells, to form peroxynitrite (a potent protein nitrating agent) especially after reacting with carbon dioxide (Radi et al., 1999) (Fig. 3). The level of superoxide is kept low in the cell by superoxide dismutase, an antioxidant enzyme. Increased levels of superoxide may be caused either by the overproduction of NAD(P)H oxidases and NOSs or by processes that produce ROSs, such as the electron transport chain (ETC) in mitochondria or by xanthine oxidase (Beckman, 1996; Xia et al., 1996; Ischiropoulos, 1998).

The AD brain has been reported to show mitochondrial abnormalities (Beal, 1998) which could lead to leakage of $O_2^{-}$ leading to the production of peroxynitrite at diffusion-controlled rates. Peroxynitrite is highly reactive with a very short half-life, and therefore it would react with the proteins, lipids, and carbohydrates near the site of generation and might be involved in the neuronal deterioration observed in AD. The amino acids cysteine, methionine, phenylalanine, and tyrosine are particularly susceptible to nitration. A second mechanism of tyrosine nitration is via hemeproteins. Nitrite, a breakdown product of NO, reacts with hydrogen peroxide to generate nitrogen dioxide. Nitrogen dioxide is also highly unstable and reacts close to the site of its generation as does peroxynitrite.
RNS generated within a physiologically relevant concentration by Cu^{2+}-activated constitutive nitric oxide synthase (NOS) are not toxic; rather RNS so generated are relatively specific in their cellular targets (Lafon-Cazal et al., 1993). NO is generated from three isoenzymes of NOS: neuronal NOS (nNOS), inducible (iNOS), and endothelial NOS (eNOS) (Stuehr, 1999; Alderton et al., 2001). The role of NO in cell physiology is unclear and its role in disease is controversial with both beneficial and detrimental effects. NO may have direct effects such as signaling by interacting with soluble guanylate cyclase leading to vasodilation and causing alterations in gene expression and also may interact with other molecules to generate more reactive species (Ignarro et al., 1987; Beckman, 1996; Ischiropoulos, 1998; Zamora et al., 2002). One such reactivity is to generate potent nitrating species that result in the formation of 3-nitrotyrosine in proteins. The mechanism, regulation, and role of protein tyrosine nitration are controversial (Beckman and Koppenol, 1996; Ischiropoulos, 1998).

Recently, several studies suggested that protein nitration could be a cellular signaling mechanism and is often a reversible and selective process, similar to protein phosphorylation (Aulak et al., 2004; Koeck et al., 2004). In addition, modified proteins are believed to be either degraded or subject to processes that could lead to enzymatic “denitration” (Gow et al., 1996; Kamisaki et al., 1998; Irie et al., 2003). The latter possibility is intriguing because this would allow the process of tyrosine nitration to be reversible and thus enable a more dynamic physiological role. Protein nitration is observed under normal conditions in all tissues. In AD brain levels of nitrated proteins were found to be increased compared to that of control (Smith et al.,
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1997; Castegna et al., 2003; Sultana et al., 2006b). Ubiquitin carboxyl-terminal hydrolase L-1 (UCH-L-1), one of the components of the proteasomal pathway, was identified as an oxidized protein in the inferior parietal and hippocampal regions of AD, further suggesting a role for nitration in protein accumulation (Castegna et al., 2003; Sultana et al., 2006b).

Tyrosine residues are crucial in redox cell signaling and oxidative inflammatory injury, due to the fact that nitration has been shown to alter protein function, including modulation of catalytic activity, cell signaling, and cytoskeletal organization (Schröffer et al., 2003). Addition of nitrite to the protein at the 3-position of tyrosine residues (Fig. 4) sterically hinders the phosphorylation of the tyrosine OH moiety, a prominent functional regulation site of proteins. Thus, 3-NT could, potentially, render a protein dysfunctional. Decreased tyrosine phosphorylation could lead to cell death (Lafon-Cazal et al., 1993; Butterfield and Stadtman, 1997). Nitration of proteins may also lead to irreversible damage to the proteins and also affect the energy status of neurons by inactivating key enzymes (Ischiropoulos, 1998; Aulak et al., 2004; Koeck et al., 2004). This widespread occurrence of oxidative alterations not only decreases or eliminates the normal functions of these macromolecules, but also may activate an inflammatory response (the complement cascade, cytokines, acute phase reactants, and proteases) in the AD brain (Meda et al., 1995; Fiala et al., 1998).

![Fig. 4 Mechanism of 3-NT formation](image)

A number of previous studies showed that dityrosine and 3-NT levels were elevated in the hippocampus, IPL, and neocortical regions of the AD brain and in ventricular cerebrospinal fluid (VF) (Smith et al., 1997; Hensley et al., 1998; Tohgi et al., 1999; Castegna et al., 2003; Sultana et al., 2006b). The increased 3-NT protein adducts in CSF of AD subjects probably reflect increased leakage of mitochondrial electron equivalents and protein nitrating agents, with resultant and increased protein nitration in brain tissue. Furthermore, recent work demonstrates that ONOO\(^-\) can induce α-synuclein oligomerization through covalent 3,3′-dityrosine cross-linking and may facilitate the misfolding and deposition of select proteins through nitrosative and/or oxidative modification. Horiguchi et al. (Horiguchi et al., 2003) demonstrated the presence of nitrated tau in pretangles,
tangles, and tau inclusions in AD brain. The expression of nitration was robust in pretangles of early AD cases compared to those of more advanced cases, suggesting that tau nitration may be an early event in AD.

Using a redox proteomics approach we reported specific nitration of alpha enolase, gamma-enolase, L-lactate dehydrogenase, triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ATP synthase alpha chain, voltage-dependent anion channel protein 1, and carbonic anhydrase 2 in AD brain (Castegna et al., 2003; Sultana et al., 2006b) (Table 1). These data support the notion that nitration of specific proteins perturbs energy metabolism, pH regulation, and mitochondrial functions, which could contribute to the mechanisms for the onset and progression of AD.

The brain depends on glucose as a source of energy, and AD brain has been reported to have altered glucose utilization and consequently altered energy metabolism assessed by PET scanning (Geddes et al., 1996; Messier and Gagnon, 1996; Vanhanen and Soininen, 1998; Rapport, 1999). The identification of CK, ENO1, TPI, GAPDH, PGM1, and α-ATPase as oxidized proteins using redox proteomics suggest that these proteins are involved directly or indirectly in ATP production (Aksenova et al., 2002; Castegna et al., 2002a,b; Castegna et al., 2003; Sultana et al., 2006d, 2006a). Furthermore, the activity of these identified oxidized proteins (e.g., CK, enolase, PGM1, GAPDH, and ATPase activities), were reportedly diminished in AD brain (Hensley et al., 1995; Aksenova et al., 2002; Sultana et al., 2006d). The oxidative modification and consequently the altered enzyme activity would lead to decreased ATP levels that would lead to impaired ion-motive ATPases. These pumps are necessary to maintain potential gradients, operate ion pumps, maintain membrane lipid asymmetry, and so on. Such changes could also lead to exposure of phosphatidylserine to the outer membrane leaflet, a signal for apoptosis (Castegna et al., 2004a; Mohmmad Abdul and Butterfield, 2005). Moreover, a diminution of ATP can also induce hypothermia, causing abnormal tau phosphorylation through differential inhibition of kinases and phosphatases (Planell et al., 2004). Recent studies reported that GAPDH functions as a NO sensor (Hara et al., 2006). Consequently, nitrosative dysfunction of GAPDH conceivably could be involved in the excess nitration observed in AD. Further studies will be necessary to clarify this point.

In AD brain the ubiquitin–proteasome pathway was found to be dysfunctional (Castegna et al., 2002a; Choi et al., 2004; Sultana et al., 2006d), and the identification of UCHL-1 as an oxidized protein with reduced activity could inhibit the process of the degradation of damaged, excess, or altered proteins and may further promote the aggregation of proteins that could lead to synaptic degeneration in AD brain (Castegna et al., 2002a; Choi et al., 2004; Healy et al., 2004; Sultana et al., 2006d). Recent in vitro studies showed that HNE, a lipid peroxidation product, decreased the activity of recombinant UCH-L1 (Okada et al., 1999; Shringarpure et al., 2001; Hyun et al., 2002), suggesting oxidative modification of UCH-L1 inactivates its hydrolase activity. Proteomics identification of UCH-L1 as an oxidatively modified protein in AD (Castegna et al., 2002a; Sultana et al., 2006d)
was recently confirmed by others (Choi et al., 2004). Taken together, these different lines of evidence support a role for dysfunction of the ubiquitin–proteasome pathway in the pathogenesis of AD. Others showed that diminished proteasome function could lead to neurodegeneration (Halliwell, 2002) and oxidative stress (Ding et al., 2003). On the other hand, oxidative stress leads to proteasome dysfunction (Halliwell, 2002, 2006), suggesting a vicious feedforward cycle of oxidative stress, proteasome dysfunction, and neurodegeneration.

Neuropolypeptide h3 (NPH3), a phosphatidylethanolamine-binding protein [PEBP] or cholinergic neurostimulating peptide, may play an important role in regulating choline acetyl transferase (ChAT) and maintaining phospholipid asymmetry, a process that is important to normal mitochondrial and plasma membrane function (Castegna et al., 2004a; Mohmmad Abdul and Butterfield, 2005). Oxidation of this protein could lead to impaired cholinergic properties, mitochondria function, and apoptosis in AD.

β-actin (ACT) and dihydropyrimidinase-related protein 2 (DRP2) were found to be downregulated and oxidatively modified in AD brain (Coleman and Flood, 1987; Lubec et al., 1999; Castegna et al., 2002a,b, 2003). Alterations in the structure of proteins induced due to oxidation could be one of the contributing factors involved in the observed loss of interneuronal connections, neuronal repair, and shortened dendritic lengths in AD brain (Coleman and Flood, 1987), conceivably leading to memory impairment and synapse loss, clearly important for AD.

Another important protein that is found to have reduced expression and is also oxidized and has reduced activity in AD brain is peptidyl-prolyl cis/trans isomerase (Pin1). This protein is colocalized with phosphorylated tau (Holzer et al., 2002; Kurt et al., 2003; Ramakrishnan et al., 2003; Sultana et al., 2006c, d). Pin1 is a chaperone enzyme that recognizes phosphorylated Ser-Pro and phosphorylated Thr-Pro motifs in proteins, and alters the conformation of proteins from cis to trans between a given amino acid and a proline (Schutkowski et al., 1998). One of the target proteins of Pin1 is a protein that removes phosphate moieties from tau (Shen et al., 1998).

Oxidative modification of Pin1 may lead to hyperphosphorylation of tau, and entry into a cell cycle eventually leading to tangle formation and apoptosis (Nagy et al., 1997; Zhou et al., 2000; Smith et al., 2004). In addition to a role of Pin1 in neurofibrillary tangle formation, recent studies suggest that Pin1 plays a role in APP processing, and therefore, in Aβ levels in brain (Pastorino et al., 2006). Thus, Pin1 is involved in two of the major pathological hallmarks of AD. Pin1 is oxidatively modified and dysfunctional in mild cognitive impairment (MCI), a precursor condition to AD (Butterfield, 2006). Further studies are required to understand the role of Pin1 in the disease progression.

Soluble N-ethylmaleimidesensitive factor (NSF) attachment protein (γ-SNAP) is another protein found to be oxidatively modified in AD brain, and this protein is important in vesicular transport for neurotransmitter release, hormone secretion, and mitochondrial integrity. Hence, oxidation may lead to an altered neurotransmission system and impaired learning and memory in AD (Maslia et al., 1994; Scheff and Price, 2003; Sultana et al., 2006d).
The pH of the cell is crucial for the normal functioning of the cells. Carbonic anhydrase 2 (CA2) regulates cellular pH, CO₂, and HCO₃⁻ transport, and maintains H₂O and electrolyte balance (Sly and Hu, 1995) by reversible hydration of CO₂ in normal cells. This protein has been reported to be oxidized in AD brain and also showed a decrease in activity (Meier-Ruge et al., 1984; Poon et al., 2004; Sultana et al., 2006d). Functionally inactive CA2 could induce changes in buffering systems in the brain, which could consequently lead to protein aggregation. Protein aggregation is more pronounced in AD brain, and, because cellular pH could be altered, altered mitochondrial production of ATP could be affected.

The voltage-dependent anion channel (VDAC) is identified as one of the oxidized proteins in AD brain (Sultana et al., 2006a,b). The oxidation of this protein in AD suggests an alteration in the function of the mitochondrial permeability transition pore (MPTP) leading to mitochondrial depolarization and altered signal transduction pathways, which could be crucial in synaptic transmission and plasticity. Moreover, alterations in the MPTP could lead to apoptotic processes. In addition, dysfunction of mitochondria recently has been reported to alter APP metabolism, enhancing the intraneuronal accumulation of amyloid β-peptide and enhancing neuronal vulnerability (Busciglio et al., 2002).

Overall, from the data presented above it is clear that oxidation of specific brain proteins alters the structure and thereby function of the proteins. Such changes could be important in AD pathology.

4 Is Protein Oxidation an Early or Late Event in AD Pathogenesis?

In recent years, the clinical stages preceding AD presenting memory impairment but without overt dementia have attained increased attention in the AD clinical and research fields. Patients with MCI are subjects with memory or other cognitive complaints but who do not fulfill the dementia criteria (Visser et al., 2001). Persons with MCI represent a heterogeneous group of patients with several possible explanations for the cognitive deficits. A high proportion of MCI patients are probably early AD subjects, although other diagnoses are also included in this diagnostic entity. Biochemical markers for AD should reflect the pathogenesis of the disorder.

Both in MCI and AD patients, mean plasma levels of nonenzymatic antioxidants and activity of antioxidant enzymes appeared to be lower than in controls, with no parallel induction of antioxidant enzymes (Keller et al., 2005). In order to explain these results it has been suggested that the increased free radical production in MCI might lead to a rapid consumption of plasma antioxidants without a simultaneous activation of new molecules of antioxidant enzymes. Individuals with MCI, and subsequently with AD, are likely to have an inadequate antioxidant enzymatic activity, unable to counteract the increased production of free radicals during the pathogenesis of the disease.
Subjects with MCI have increased protein oxidation in hippocampus and IPL (Butterfield et al., 2006a) and superior and medial temporal gyri (Keller et al., 2005). Additionally, using redox proteomics we identified three specific proteins, that is, enolase, glutamine synthase, and Pin1 as common targets of protein oxidation between MCI and AD which suggests that protein oxidation of these selected proteins could be important in initial events involved in AD pathogenesis (Fig. 5). Furthermore, several gene mutations associated with AD have been observed in subjects with MCI including mutations in apolipoprotein E, presenilin 1, and the amyloid precursor protein (Traykov et al., 2002; Naemias et al., 2004). Increased levels of lipid peroxidation have been reported in the brain of persons with MCI (Keller et al., 2005; Markesbery et al., 2005; Butterfield et al., 2006b). Thus, increased levels of protein and lipid peroxidation could be implicated as early events in AD pathophysiology and also suggest that pharmacological intervention to prevent protein and lipid peroxidation at the MCI stage or earlier may be a promising therapeutic strategy to delay or prevent progression to AD.

Fig. 5 Comparison between the MCI and AD brain to see the common targets of protein oxidation

Very recent studies reported increased oxidative damage in nuclear and mitochondrial DNA in MCI, as indexed by increased levels of 8-hydroxyguanosine (8-OHdG), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (fapyguanine), 8-hydroxyadenine, 4,6-diamino-5-formamidopyrimidine (fapyadenine), and 5-hydroxy- cytosine (Wang et al., 2006). Due to the crucial role that DNA plays in cells, high levels of oxidation, particularly early in the progression of AD, may result in a decline of normal cell function through altered transcription, changes in protein expression, or cross-linking with proteins. Taken together, these results suggest that oxidative damage is one of the factors involved in the pathogenesis of neurodegeneration in AD and is not simply a late effect of the neurodegenerative process.
5 Conclusions

Protein oxidation may be an early event in AD pathogenesis as supported by the data from the MCI brain. With exceptions related to oxidative signaling or other beneficial processes, excessive oxidation of proteins has been reported to decrease the functionality of most proteins, which suggests that protein oxidation is harmful for cell survival. Further studies are in progress to understand the role of protein oxidation and its abrogation in AD by using in vivo and in vitro models of AD centered around Aβ(1-42) (Sultana et al., 2006a).

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References

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