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Amyloid β-peptide (1–42)-induced Oxidative Stress and Neurotoxicity: Implications for Neurodegeneration in Alzheimer's Disease Brain. A Review*

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Oxidative stress, manifested by protein oxidation, lipid peroxidation, DNA oxidation and 3-nitrotyrosine formation, among other indices, is observed in Alzheimer's disease (AD) brain. Amyloid β-peptide (1–42) [Aβ(1–42)] may be central to the pathogenesis of AD. Our laboratory and others have implicated Aβ(1–42)-induced free radical oxidative stress in the neurodegeneration observed in AD brain. This paper reviews some of these studies from our laboratory.

Recently, we showed both *in-vitro* and *in-vivo* that methionine residue 35 (Met-35) of A**β**(1–42) was critical to its oxidative stress and neurotoxic properties. Because the C-terminal region of A**β**(1–42) is helical, and invoking the $i + 4$ rule of helices, we hypothesized that the carboxyl oxygen of lle-31, known to be within a van der Waals distance of the S atom of Met-35, would interact with the latter. This interaction could alter the susceptibility for oxidation of Met-35, i.e. free radical formation. Consistent with this hypothesis, substitution of lle-31 by the helix-breaking amino acid, proline, completely abrogated the oxidative stress and neurotoxic properties of A**β**(1–42). Removal of the Met-35 residue from the lipid bilayer by substitution of the negatively charged Asp for Gly-37 abrogated oxidative stress and neurotoxic properties of A**β**(1–42).

The free radical scavenger vitamin E prevented A**β**(1–42)-induced ROS formation, protein oxidation, lipid peroxidation, and neurotoxicity in hippocampal neurons, consistent with our model for A**β**-associated free radical oxidative stress induced neurodegeneration in AD.

ApoE, allele 4, is a risk factor for AD. Synaptosomes from apoE *knock-out* mice are more vulnerable to A**β**-induced oxidative stress (protein oxidation, lipid peroxidation, and ROS generation) than are those from wild-type mice. We also studied synaptosomes from allele-specific human apoE *knock-in* mice. Brain membranes from human apoE4 mice have greater vulnerability to A**β**(1–42)-induced oxidative stress than brain membranes from apoE2 or E3, assessed by the same indices, consistent with the notion of a coupling of the oxidative environment in AD brain and increased risk of developing this disorder.

Using immunoprecipitation of proteins from AD and control brain obtained no longer than 4 h PMI, selective oxidized proteins were identified in the AD brain. Creatine kinase (CK) and **β**-actin have increased carbonyl groups, an index of protein oxidation, and Glt-1, the principal glutamate transporter, has increased binding of the lipid peroxidation product, 4-hydroxy-2-nonenal (HNE). A**β** inhibits CK and causes lipid peroxidation, leading to HNE formation. Implications of these findings relate to decreased energy utilization, altered assembly of cytoskeletal proteins, and increased excitotoxicity to neurons by glutamate, all reported for AD. Other oxidatively modified proteins have been identified in AD brain by proteomics analysis, and these oxidatively-modified proteins may be related to increased excitotoxicity (glutamine synthetase), aberrant proteasomal degradation of damaged or aggregated proteins (ubiquitin C-terminal hydrolase L-1), altered energy production (**α**-enolase), and diminished growth cone elongation and directionality (dihydropyrimindase-related protein 2). Taken together, these studies outlined above suggest that Met-35 is key to the oxidative stress and neurotoxic properties of A**β**(1–42) and may help explain the apoE allele dependence on risk for AD, some of the functional and structural alterations in AD brain, and strongly support a causative role of A**β**(1–42)-induced oxidative stress and neurodegeneration in AD.

Keywords: Oxidative stress; Amyloid β-peptide; Protein oxidation; Lipid peroxidation; Methionine; Alzheimer's disease

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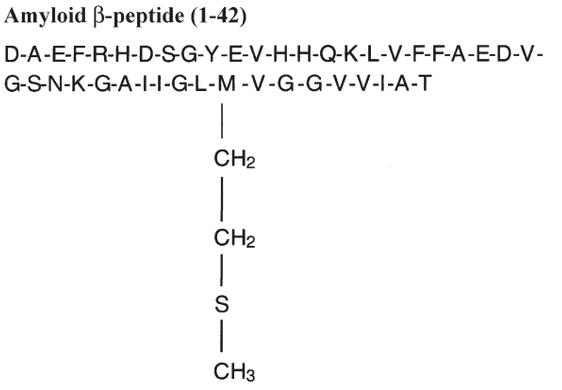

FIGURE 1 Sequence of Aβ(1–42) with the side chain of methionine residue 35 indicated.

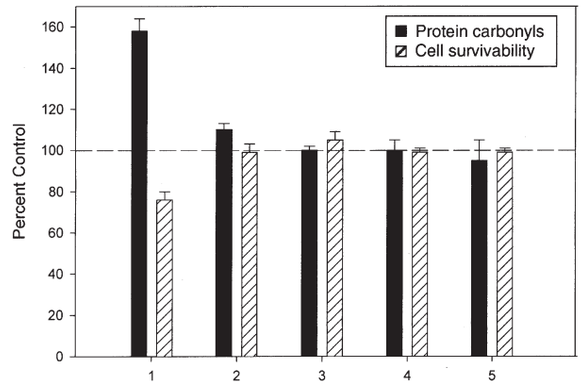

FIGURE 2 Protein oxidation (black columns) and cell survivability (hatched columns) after treatment of cultured hippocampal neurons with Aβ(1–42) or modified peptide. (1) Aβ(1–42); (2) Aβ(1–42) plus vitamin E; (3) Aβ(1–42)M35Norleucine; (4) Aβ(1–42)l31P; and (5) Aβ(1–42)G37D. Statistically significant increases ($p < 0.01$) in protein oxidation and decreased cell survivability were found with native Aβ(1–42) but not with the other modified peptides or with use of the antioxidant vitamin E.

INTRODUCTION

Alzheimer's disease (AD) brain is under intense oxidative stress, manifested by increased protein oxidation, lipid peroxidation, free radical formation, DNA/RNA oxidation, nitrotyrosine levels, and advanced glycation end products (recently reviewed in Refs. [1,2]). Further, based mostly on genetic grounds, the 42-amino acid peptide, amyloid β-peptide (1–42) [Aβ(1–42), Fig. 1], may be central to the pathogenesis of the disease.[3] Our laboratory combined these two concepts into a comprehensive model for neurodegeneration in AD brain based on the free radical oxidative stress associated with the peptide.[1,2,4–6] This brief review outlines some of the evidence to support this model.

Aβ(1–42) INDUCES OXIDATIVE STRESS AND NEUROTOXICITY

Aβ(1–42) Causes Protein Oxidation in and Death to Hippocampal Neurons

Addition of Aβ(1–42) to neurons leads to increased protein carbonyls and decreased cell survival compared to controls[5,7–11] (Fig. 2). Vitamin E inhibits both these effects,[11] expected for a free radical process (Fig. 2). Protein oxidation is increased in AD brain in regions rich in Aβ(1–42).[12,13]

Aβ(1–42) Causes Lipid Peroxidation in Brain Membranes

Following our initial finding that a short fragment of Aβ(1–42), the 11-mer Aβ(25–35), caused lipid peroxidation in brain membranes,[14] many laboratories have reported Aβ-induced lipid peroxidation.[15–23] More recently, Aβ(1–42) addition to neuronal cultures or synaptosomal membranes was shown to lead to the formation of 4-hydroxy-2-nonenal (HNE) or isoprostanes, both products of lipid peroxidation.[17,19,22] Vitamin E[23] and numerous other antioxidants inhibited Aβ-induced lipid peroxidation (reviewed in Refs. [1,2,4]. Lipid peroxidation is increased in AD brain as assessed by increased levels of thiobarbituric acid reactive substances (TBARS), isoprostanes and neuroprostanes, HNE, and acrolein (reviewed in Refs. [1,2,4,24].

Aβ(1–42) Induces Reactive Oxygen Species (ROS) Formation

ROS, assessed by increased fluorescence of dichlorofluoroscein previously loaded into neurons, were elevated following treatment with Aβ(1–42), and vitamin E was effective in limiting this ROS formation.[11] The latter result is, once again, what one would expect for a free radical process. The inhibition of Aβ(1–42)-induce ROS formation in neurons is not due to the inhibition of fibrils by the peptide, as fibrils that appear to be the same as those of native peptide are found in the presence of vitamin E.[11]

THE SINGLE METHIONINE OF Aβ(1–42) AT RESIDUE 35 IS IMPORTANT FOR THE OXIDATIVE STRESS AND NEUROTOXIC PROPERTIES OF THE PEPTIDE

Substitution for Met of Aβ(1–42) Inhibits the Oxidative Stress and Neurotoxic Properties of the Peptide

In contrast to native Aβ(1–42), substitution of the S atom of the single methionine residue at position 35 by a methylene group [CH_2, making norleucine the amino acid at residue 35] completely abolished

the oxidative and neurotoxic properties of the peptide when added to cultured neurons.[7] Further, if in place of methionine, the oxidized form of this amino acid [methionine sulfoxide] is substituted into Aβ(1–42), again no protein oxidation nor cell death occurred.[10] That is, if the S atom of methionine is already oxidized, then addition of this modified Aβ(1–42) to cultured neurons is not toxic nor oxidative. Likewise, *in vivo* studies of Aβ(1–42)-induced oxidative stress showed that transgenic *C. elegans*, in which human Aβ(1–42) is produced, had increased protein oxidation, but if the codon for methionine in Aβ(1–42) were substituted by the codon for a different amino acid no increased protein oxidation was found *in vivo*.[7]

In both the case of oxidized methionine and of the norleucine derivative of Aβ(1–42) apparently normal-looking fibrils are formed, consistent with the notion that fibrils, *per se*, are not necessary toxic.[10] Rather, our results are consistent with growing evidence that suggests small aggregates of Aβ(1–42) are the toxic species of this peptide.[25–29] In marked contrast to large fibrillar structures, such small aggregates, being hydrophobic, could insert into the neuronal lipid bilayer to induce lipid peroxidation with subsequent HNE formation.

The reactive alkenals HNE and acrolein, increased in AD CNS,[30,31] can react by Michael addition with adjacent transmembrane proteins,[32,33] covalently modifying their structure and disrupting their function. For example, increased HNE binding to the glial glutamate transporter GLT-1 [EAAT2] is observed in AD brain,[22] probably explaining the loss of function of this transporter.[34] Further, addition of Aβ(1–42) to synapstomal preparations leads to increased HNE binding to GLT-1,[22] suggesting that this is one cause of the oxidative modification of this transporter in AD brain.[4] Coupled to the loss of activity of glutamine synthetase (GS) in AD brain[12] and loss of activity induced by Aβ,[27,35,36] then two means of removing potentially excitotoxic glutamate from the external portion of neurons are dysfunctional, leading to increased possibility that glutamate-stimulated excitotoxic mechanisms could cause neurodegeneration in AD brain.[37] Incubation of neurons with Aβ(1–42) leads to formation of HNE,[17,22] and several transmembrane proteins are functionally and structurally modified by Aβ, HNE or acrolein.[4,8,9,17,18,38–41]

Disruption of the Alpha-helical Structure of the C-terminus of Aβ(1–42) Abolishes the Oxidative Stress and Neurotoxic Properties of the Peptide

NMR studies of monomeric Aβ(1–42) or Aβ(1–40) suggest that the C-terminal region of the peptide encompassing residues 28–42 has helical secondary structure.[42,43] Like any helix, a prediction is that every residue interacts with the residue four units away. Indeed, NMR studies showed that the peptide carbonyl of lle-31 was within a van der Waals distance of the S atom of methionine residue 35 (Met-35) of Aβ(1–42).[42,43] This interaction could increase the susceptibility for oxidation of the S atom of Met-35 in Aβ(1–42) leading to the presumed reactive species, the sulfuramyl free radical.[10] To test this idea, we substituted the helical-breaking amino acid proline for lle-31, reasoning that breaking the helical interactions of residue-31 with the Met-35 S atom would preclude this "priming potential for oxidation" present in the native peptide. Addition of Aβ(1–42)l31P to hippocampal neurons, in sharp contrast to the native peptide, led to no oxidative stress nor neurotoxicity[44] (Fig. 2), consistent with the notion that the secondary structure of Aβ(1–42) coupled to the chemistry of thioethers like methionine is important in the oxidative stress and neurotoxic properties of the peptide.

Removal of Methionine in Aβ(1–42) from its Presumed Lipid Bilayer-resident Location Leads to a Non-oxidative and Non-neurotoxic Peptide

As noted above, we believe that small aggregates of Aβ(1–42) insert into the lipid bilayer to induce lipid peroxidation in a free radical-dependent mechanism that involves the Met-35 residue of the peptide. This would suggest that the Met-35 residue is inserted into the hydrophobic region of the neuronal membrane bilayer, where the unsaturated sites on the phospholipid acyl chains are located. Hydrogen atoms on lipid carbon atoms adjacent to these sites are the most vulnerable to free radical attack, leading to a chain reaction of radical processes and subsequent membrane damage.[45] Molecular modeling and physical studies of Aβ suggest that Met is indeed located in the lipid bilayer of neurons.[46] If lipid peroxidation, induced by free radical processes involving Met-35, is an early event in the oxidative stress and neurotoxic properties of Aβ(1–42), then removal of the methionine residue from the bilayer is predicted to abrogate these properties. To test this idea, we substituted aspartic acid for glycine-37, reasoning that the negative charge on residue 37 of Aβ(1–42) would drag the methionine residue out of the bilayer and away from C–H bonds on carbon atoms adjacent to the unsaturated lipid sites that are vulnerable to free radical attack. All the chemistry of thioethers (such as methionine) and the structural aspects of monomeric Aβ(1–42) presumably would still be present, but the targets for Aβ(1–42)-induced free radical attack on the lipids would not be available to the methionine. Consistent with this prediction, Aβ(1–42)G37D is no longer oxidative nor neurotoxic, in marked contrast to the native

peptide[47] (Fig. 2). This result supports the notion that lipid peroxidation is an early event in the neurotoxic properties of Aβ(1–42) and is consistent with the observation that vitamin E, a hydrophobic chain-breaking antioxidant, is able to protect neurons against the oxidative stress and neurotoxicity associated with Aβ(1–42).[11]

ALLELE 4 OF APOLIPOPROTEIN E MAY BE A RISK FACTOR FOR AD, IN PART, DUE TO ITS INABILITY TO HANDLE THE OXIDATIVE STRESS ASSOCIATED WITH Aβ(1–42)

Apolipoprotein E, a lipid and cholesterol carrier and potential chaperone protein,[48] has three principal alleles, apoE2, apoE3 and apoE4. Many studies confirm that inheritance of the apoE4 allele confers a significant risk of developing AD (reviewed in Ref. [49]. In composition, the only differences at the protein level in the three alleles of apoE are the number of cysteine residues in the protein: apoE2 has two cysteines, apoE3 has an arginine substituted for one of the cysteines, while apoE4 has both cysteines substituted by arginine residues.

Given the importance of Aβ to the pathogenesis of AD, we performed a series of studies on the interaction of this peptide with brain membranes from well-defined apoE mouse populations.[50–52] Synaptosomes from mice in which the gene for apoE was deleted (*knock-out*) had increased basal oxidative stress[50] and were more vulnerable to Aβ(1–40)-induced oxidative stress, including lipid and protein oxidation.[51] We extended these findings that suggested that endogenous apoE could serve an antioxidant role to examine the allele-specific effects of human apoE on Aβ(1–42)-induced oxidative stress. Mice were generated in which the exons for mouse apoE were substituted by exons for human apoE2, apoE3 or apoE4 (*knock-in*). That is, the mouse apoE promoter was still present; consequently, the normal amount of apoE was produced in the mouse and at the correct location, however, the apoE produced was human and specifically either apoE2, apoE3 or apoE4 as desired. Cortical synaptosomes from each knock-in mouse were subjected to Aβ(1–42) addition and oxidative stress parameters measured.[52] No matter if ROS, lipid peroxidation, or protein oxidation markers were examined, synaptosomes from apoE4 mice were most vulnerable to the oxidative stress associated with Aβ(1–42) relative to those from human apoE2 or apoE3 knock-in mice.[52] One interpretation of these results is that the increased risk of developing AD upon inheritance of the apoE4 allele may be due in part to the inability of this protein, relative to apoE2 or apoE3, to handle the oxidative stress associated with Aβ(1–42) that is produced in excess in the AD brain. Continued studies are in progress to explore this idea.

IDENTIFICATION OF SPECIFICALLY OXIDIZED PROTEINS IN AD BRAIN

Protein oxidation occurs in AD brain in regions where Aβ(1–42) is present, but not in the cerebellum that is largely spared the pathology of AD.[12,13] But which specific proteins are oxidized? An answer to this question may provide insight into potential mechanisms for neurodegeneration and synapse loss in AD brain. To begin to answer this question of the identity of specific, oxidatively modified proteins, we initially utilized selective immunochemical precipitation of oxidized proteins, detected by reaction with their increased protein carbonyl functionality, to prove their identify. In this way, we identified creatine kinase (CK, BB isoform) and β-actin as specifically oxidized proteins in AD brain.[53,54] However, this method is laborious, requires prior knowledge (or a good guess) of the identity of the oxidized protein in order to use the correct antibody, and necessitates the availability of specific antibodies to the proteins thought to be oxidized. To circumvent these difficulties, we have used proteomics for the first time to identify selectively oxidized proteins in AD brain.[55,56]

In this proteomics method, AD or control brain proteins, separated by 2-dimensional gel electrophoresis, are treated with 2,4-dinitrophenylhydrazine to form the hydrazone and an antibody to this protein-bound Schiff base added. Analysis and comparison of the images of control and AD brain samples (never more than 4 h post mortem interval) allows one to select spots on the 2-dimensional protein map that represent proteins that are more oxidized in AD brain. These spots are isolated and digested by trypsin. The digests are subjected to Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) from which the m/z ratios of peptides generated by trypsin digestion are determined. The information obtained is submitted to a protein database (or more than one database if necessary), from which the identity of the protein is obtained. Confirmation of the proteins identified by proteomics can be made using immunochemical methods if desired. Using this approach, we identified CK (BB isoform), GS, ubiquitin C-terminal hydrolase L-1 (UCH), dihydropyrimidinase related protein 2 (CRMP-2), and α-enolase as specifically oxidized proteins in AD inferior parietal lobule[55,56] (Table I). Continued proteomics analysis will lead to further identity of oxidatively modified proteins in AD brain.

TABLE I Identity of oxidatively modified proteins in AD brain

Protein	Method used	References
Creatine kinase (BB isoform)	Proteomics; Immunochemistry	[53–55]
β-Actin	Immunochemistry	[54]
Glutamine synthetase	Proteomics (confirmed by immunochemistry)	[55]
Ubiquitin C-terminal hydrolase L-1	Proteomics	[55]
Dihydropyrimidnase related protein-2	Proteomics	[56]
α-Enolase	Proteomics	[56]

Each of these specifically oxidatively-modified proteins identified by proteomics potentially can, in plausible mechanisms, be associated with neurodegeneration in AD brain. For example, CK is involved in ATP production and its activity is decreased in AD brain.[12] Under oxidative stress, the synaptic regions of the neurons require ATP to maintain membrane integrity, ion gradients, and other aspects of the cell. Yet, CK is unable to produce high-energy phosphate bonds efficiently due to its oxidative modification, thereby decreasing the energy availability in AD brain. Others have shown that energy utilization is compromised in AD brain,[57] and we showed that CK is inhibited by Aβ in a way that is inhibited by vitamin E.[58] Likewise, if activity of α-enolase is decreased as a result of its oxidative modification, decreased energy utilization would result. As mentioned above, GS activity is decreased in AD brain,[12] and Aβ inhibits GS activity and alters its structure.[27,35,36] Also mentioned above, decreased activity of GS in AD,[12] coupled to the oxidative modification of GLT-1 in AD brain and by Aβ (1–42)[22] and decreased activity of GLT-1 in AD brain,[34] suggest that excitotoxic mechanisms of glutamate may lead to neuronal death. The proteomics results provide the first direct evidence that GS is oxidatively modified in AD brain. Ubiquitin is added to proteins that have been damaged or aggregated as a signal for the 26S proteasome to degrade these proteins. Yet, there is a fixed pool of ubiquitin, so UCH is necessary to remove the protein-bound ubiquitin for recycling to other damaged or aggregated proteins for subsequent proteasomal degradation.[59] If UCH function is compromised in AD brain as a result of its oxidative modification, then accumulation of damaged or aggregated proteins is predicted, some of which can damage the neuron, perhaps even leading to neuronal death. In AD, of course, one observes the presence of abnormally aggregated proteins, though we do not yet know the reason for this accumulation. Still, it is plausible that diminished UCH activity could be involved in this observation. Conceivably, this could also be involved in the compromised function of the proteasome suggested for AD.[59–62] UCH lacking its catalytic residue in animal models leads to Aβ deposition and neurodegeneration.[63] Finally, CRMP-2 functions in the elongation and directionality of the growth cone of neurons.[64] Should oxidative modification of collapsin lead to decreased activity, then decreased neurite extension and decreased neuronal networks would be predicted. This is precisely what one observes upon addition of Aβ(1–42) to neuronal cultures.[11] Such shortening of neurite processes would diminish neuronal contact and compromise interneuronal communication. One could speculate that decreased information (memory) processing could result, which, of course, is a hallmark of AD. Much more research is needed to flesh out this highly speculative notion.

CONCLUSIONS

Taken together, the results summarized in this brief review suggest that the single methionine of Aβ (1–42) at residue 35 is key to the oxidative stress and neurotoxic properties of this peptide. Further, the apoE allele dependence on increased risk of developing AD conceivably could be explained in part due to the decreased ability of apoE4 to handle the oxidative stress of Aβ(1–42) that accumulates in AD brain. Lastly, the emerging techniques of proteomics, applied for the first time to identify oxidatively modified proteins in AD brain, have provided plausible mechanisms for neurodegeneration in AD brain based on compromised function of these specifically oxidatively modified proteins. All these studies strongly suggest a causative role for Aβ(1–42)-induced oxidative stress in the neurodegeneration and synapse loss observed in AD brain. Studies to continue these lines of investigation are in progress.

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References

[1] Butterfield, D.A., Drake, J., Pocernich, C. and Castegna, A. (2001) "Evidence of oxidative damage in Alzheimer's disease brain: central role of amyloid β-peptide", *Trends Mol. Med.* **7**, 548–554.

[2] Butterfield, D.A. and Lauderback, C.M. (2002) "Lipid peroxidation and protein oxidation in Alzheimer's disease brain: potential causes and consequences involving amyloid β-peptide-associated free radical oxidative stress", *Free Radic. Biol. Med.* **32**, 1050–1060.

[3] Selkoe, D.J. (2001) "Clearing the brain's amyloid cobwebs", *Neuron* **25**, 177–180.

[4] Butterfield, D.A., Castegna, A., Lauderback, C.M. and Drake, J. (2002) "Review: evidence that amyloid beta-peptide-induced lipid peroxidation and its sequelae in Alzheimer's disease brain contributes to neuronal death", *Neurobiol. Aging*, In press.

[5] Varadarajan, S., Yatin, S., Aksenova, M. and Butterfield, D.A. (2000) "Review: Alzheimer's amyloid β-peptide-associated free radical oxidative stress and neurotoxicity", *J. Struct. Biol.* **130**, 184–208.

[6] Butterfield, D.A. (1997) "β-Amyloid-associated free radical oxidative stress and neurotoxicity: implications for Alzheimer's disease", *Chem. Res. Toxicol.* **10**, 495–506.

[7] Yatin, S.M., Link, C.D. and Butterfield, D.A. (1999) "*In-vitro* and *in-vivo* oxidative stress associated with Alzheimer's amyloid β-peptide (1–42)", *Neurobiol. Aging* **20**, 325–330.

[8] Yatin, S.M., Yatin, M., Aulick, T., Ain, K.B. and Butterfield, D.A. (1999) "Alzheimer's amyloid β-peptide generated free radicals increase rat embryonic neuronal polyamine uptake and ODC activity: protective effect of vitamin E", *Neurosci. Lett.* **263**, 17–20.

[9] Yatin, S.M., Yatin, M., Varadarajan, S., Ain, K.B. and Butterfield, D.A. (2001) "Role of spermine and amyloid β-peptide (1–42) associated free radical oxidative stress induced neurotoxicity", *J. Neurosci. Res.* **63**, 395–401.

[10] Varadarajan, S., Kanski, J., Aksenova, M., Lauderback, C.M. and Butterfield, D.A. (2001) "Different mechanisms of oxidative stress and neurotoxicity for Alzheimer's Aβ(1–42) and Aβ(25–35)", *J. Am. Chem. Soc.* **123**, 5625–5631.

[11] Yatin, S.M., Varadarajan, S., Aksenova, M. and Butterfield, D.A. (2000) "Vitamin E prevents Alzheimer's amyloid β-peptide (1–42)-induced protein oxidation and reactive species formation", *J. Alzheimer Dis.* **2**, 123–131.

[12] Hensley, K., Hall, N., Subramaniam, R., Cole, P., Harris, M., Aksenov, M., Aksenova, M., Gabbita, S.P., Wu, J.F., Carney, J.M., Lovell, M.A., Markesbery, W.R. and Butterfield, D.A. (1995) "Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation", *J. Neurochem.* **65**, 2146–2156.

[13] Smith, C.D., Carney, J.M., Starke-Reed, P.E., Oliver, C.N., Stadtman, E.R., Floyd, R.A. and Markesbery, W.R. (1991) "Excess brain protein oxidation and enzyme dysfunction in normal aging and Alzheimer's disease", *Proc. Natl Acad. Sci. USA* **88**, 10540–10543.

[14] Butterfield, D.A., Hensley, K., Harris, M., Mattson, M. and Carney, J. (1994) "β-Amyloid peptide free radical fragments initiate synaptosomal lipoperoxidation in a sequence-specific fashion", *Biochem. Biophys. Res. Commun.* **200**, 710–715.

[15] Avdulov, N.A., Chochina, S.V., Igbavboa, U., O'Hare, E.O., Schroeder, F., Cleary, J.P. and Wood, G.W.G. (1997) "Amyloid beta-peptides increase annular and bulk fluidity and induce lipid peroxidation in brain synaptic plasma membranes", *J. Neurochem.* **68**, 2086–2091.

[16] Bruce-Keller, A.J., Begley, J.G., Fu, W., Butterfield, D.A., Bredesen, D.E., Hutchins, J.B., Hensley, K. and Mattson, M.P. (1998) "Bcl-2 protects isolated plasma and mitochondrial membranes against lipid peroxidation induced by hydrogen peroxide and amyloid β-peptide", *J. Neurochem.* **70**, 31–39.

[17] Mark, R.J., Lovell, M.A., Markesbery, W.R., Uchida, K. and Mattson, M.P. (1997) "A role for 4-hydroxynonenal, an aldehydic product of lipid peroxidation in disruption of ion homeostasis and neuronal death induced by amyloid β-peptide", *J. Neurochem.* **68**, 255–264.

[18] Mark, R.J., Pang, Z., Geddes, J.W., Uchida, K. and Mattson, M.P. (1997) "Amyloid β-peptide impairs glucose transport in hippocampal and cortical neurons: involvement of membrane lipid peroxidation", *J. Neurosci.* **17**, 1046–1054.

[19] Mark, R.J., Fuson, K.S. and May, P.C. (1999) "Characterization of 8-epiprostaglandin F2alpha as a marker of amyloid β-peptide-induced oxidative damage", *J. Neurochem.* **72**, 1146–1153.

[20] Daniels, W.M., van Rensburg, S.J., van Zyl, J.M. and Taljaard, J.J. (1998) "Melatonin prevents beta-amyloid-induced lipid peroxidation", *J. Pineal Res.* **24**, 78–82.

[21] Gridley, K.E., Green, P.S. and Simpkins, J.W. (1997) "Low concentrations of estradiol reduce beta-amyloid (25–35)-induced toxicity, lipid peroxidation, and glucose utilization in human SK-N-SH neuroblastoma cells", *Brain Res.* **778**, 158–165.

[22] Lauderback, C.M., Hackett, J.M., Huang, F.F., Keller, J.N., Szweda, L.I., Markesbery, W.R. and Butterfield, D.A. (2001) "The glial glutatmate transporter Glt-1 is oxidatively modified by 4-hydroxy-2-nonenal in Alzheimer's disease brain: role of Aβ(1–42)", *J. Neurochem.* **78**, 413–416.

[23] Koppal, T., Subramaniam, R., Drake, J., Prasad, M.R., Dhillon, H. and Butterfield, D.A. (1998) "Vitamin E protects against amyloid peptide (25–35)-induced changes in neocortical synaptosomal membrane lipid structure and composition", *Brain Res.* **786**, 270–273.

[24] Markesbery, W.R. (1997) "Oxidative stress hypothesis in Alzheimer's disease", *Free Radic. Biol. Med.* **23**, 134–147.

[25] Oda, T., Wals, P., Osterburg, H.H., Johnson, S.A., Pasinetti, G.M., Morgan, T.E., Rozovsky, I., Stine, W.B., Snyder, S.W. and Holzman, T.F. (1995) "Clusterin (apoJ) alters the aggregation of amyloid beta-peptide (A beta 1–42) and forms slowly sedimenting A beta complexes that cause oxidative stress", *Exp. Neurol.* **136**, 22–31.

[26] Klein, W.L. (2002) "ADDLs and protofibrils—the missing links?", *Neurobiol. Aging* **23**, 231–235.

[27] Aksenov, M.Y., Aksenova, M.V., Butterfield, D.A., Hensley, K., Vigo-Pelfrey, C. and Carney, J.M. (1996) "Glutamine synthetase-induced neurotoxicity accompanied by abrogation of fibril formation and amyloid β-peptide fragmentation", *J. Neurochem.* **66**, 2050–2056.

[28] Walsh, D.M., Hartley, D.M., Kusumoto, Y., Fezoui, Y., Condron, M.M., Lomakin, A., Benedek, G.B., Selkoe, D.J. and Teplow, D.B. (1999) "Amyloid beta-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates", *J. Biol. Chem.* **274**, 25945–25952.

[29] Walsh, D.M., Klyubin, I., Fadeeva, J.V., cullen, W.K., Anwyl, R., Wolfe, M.S., Rowan, M.J. and Selkoe, D.J. (2002) "Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation *in vivo*", *Nature* **416**, 535–539.

[30] Markesbery, W.R. and Lovell, M.A. (1998) "4-Hydroxynonenal, a product of lipid peroxidation, is increased in the brain in Alzheimer's disease", *Neurobiol. Aging* **19**, 33–36.

[31] Lovell, M.A., Xie, C. and Markesbery, W.R. (2001) "Acrolein is increased in Alzheimer's disease brain and is toxic to primary hippocampal cultures", *Neurobiol. Aging* **22**, 187–194.

[32] Esterbauer, H., Schaur, R.J. and Zollner, H. (1991) "Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde, and related aldehydes", *Free Radic. Biol. Med.* **11**, 81–128.

[33] Butterfield, D.A. and Stadtman, E.R. (1997) "Protein oxidation processes in aging brain", *Adv. Cell Aging Gerontol.* **2**, 161–191.

[34] Masliah, E., Alford, M., Deteresa, R., Mallory, M. and Hensen, L. (1996) "Deficient glutamate transport is associated with neurodegeneration in Alzheimer's disease", *Ann. Neurol.* **40**, 759–766.

[35] Butterfield, D.A., Hensley, K., Cole, P., Subramanaim, R., Aksenov, M., Aksenova, M., Bummer, P.M., Haley, B.E. and Carney, J.M. (1997) "Oxidatively-induced structural alteration of glutamine synthetase assessed by analysis of spin label incorporation kinetics: relevance to Alzheimer's disease", *J. Neurochem.* **68**, 2151–2157.

[36] Aksenov, M.Y., Aksenova, M.V., Carney, J.M. and Butterfield, D.A. (1997) "Oxidative modification of glutamine synthetase by amyloid β-peptide", *Free Radic. Res.* **27**, 267–281.
[37] Butterfield, D.A. and Pocernich, C.B. (2002) "The glutamatergic system and Alzheimer's disease: therapeutic implications", *CNS Drugs*, In press.
[38] Mark, R.J., Hensley, K., Butterfield, D.A. and Mattson, M.P. (1995) "Amyloid beta-peptide impairs ion-motive ATPase activities: evidence for a role in loss of Ca^{2+} homeostasis and cell death", *J. Neurosci.* **15**, 6239–6249.
[39] Keller, J.N., Mark, R.J., Bruce, A.J., Blanc, E., Rothstein, J.D., Uchida, K., Waeg, G. and Mattson, M.P. (1997) "4-Hydroxynonenal, an aldehydic product of lipid peroxidation, impairs glutamate transport and mitochondrial function in synaptosomes", *Neuroscience* **80**, 685–696.
[40] Subramaniam, R., Roediger, F., Jordan, B., Mattson, M.P., Keller, J.N., Waeg, G. and Butterfield, D.A. (1997) "The lipid peroxidation product, 4-hydroxy-2-*trans*-nonenal, alters the conformation of cortical synaptosomal membrane proteins", *J. Neurochem.* **69**, 1161–1169.
[41] Pocernich, C.B., Cardin, A., Racine, C., Lauderback, C.M. and Butterfield, D.A. (2001) "Glutathione elevation and its protective role in acrolein-induced protein damage in synaptosomal membranes: relevance to brain lipid peroxidation in neurodegenerative disease", *Neurochem. Int.* **39**, 141–149.
[42] Shao, H., Jao, S.-C., Ma, K. and Zagorski, M.G. (1999) "Solution structures of micelle-bound amyloid β(1–40) and β(1–42) peptides of Alzheimer's disease", *J. Mol. Biol.* **285**, 755–773.
[43] Coles, M., Bicknell, W., Watson, A.A., Fairlie, D.P. and Craik, D.J. (1998) "Solution structure of amyloid β-peptide (1–40) in a water-micelle environment. Is the membrane-spanning domain where we think it is?", *Biochemistry* **37**, 11064–11077.
[44] Kanski, J., Aksenova, M., Schoneich, C. and Butterfield, D.A. (2002) "Substitution of isoleucine-31 by helical-breaking proline abolishes stress and neurotoxic properties of Alzheimer's amyloid β-peptide (1–42)", *Free Radic. Biol. Med.* **32**, 1205–1211.
[45] Halliwell, B. and Gutteridge (1989) Free Radicals in Biology and Medicine (Clarendon, Oxford).
[46] Curtain, C.C., Ali, F., Volitakis, I., Cherny, R.A., Norton, R.S., Beyreuther, K., Barrow, C.J., Masters, C.L., Bush, A.I. and Barnham, J.J. (2001) "Alzheimer's disease amyloid binds copper an zinc to generate an allosterically ordered membrane-penetrating structure containing superoxide dismutase-like subunits", *J. Biol. Chem.* **276**, 20466–20473.
[47] Kanski, J., Aksenova, M. and Butterfield, D.A. (2002) "The hydrophobic environment of Met35 of Alzheimer's Aβ(1–42) is important for the neurotoxic and oxidative properties of the peptide", *Neurotoxicity Res.* **4**, 219–223.
[48] Mahley, R. (1988) "Apolipoprotein E: cholesterol transport protein with expanding role in cell biology", *Science* **240**, 622–630.
[49] Beffert, U., Cohn, J.S., Petit-Turcottee, C., Tremblay, M., Aumont, N., Ramassamy, C., Davignon, J. and Poirier, J. (1999) "Apolipoprotein E and beta-amyloid levels in the hippocampus and frontal cortex of Alzheimer's disease subjects are disease-related and apolipoprotein E genotype dependent", *Brain Res.* **843**, 87–94.
[50] Keller, J.N., Lauderback, C.M., Butterfield, D.A., Kindy, M.S. and Markesbery, W.R. (2000) "Potential role for apolipoprotein E in maintaining synaptic homeostasis", *J. Neurochem.* **74**, 1579–1586.
[51] Lauderback, C.M., Hackett, J.M., Keller, J.N., Varadarajan, S., Szweda, L., Kindy, M., Markesbery, W.R. and Butterfield, D.A. (2001) "Vulnerability of synaptosomes from apoE knock-out mice to structural and oxidative modifications induced by Aβ(1–40)", *Biochemistry* **40**, 2548–2554.
[52] Lauderback, C.M., Kanski, J., Hackett, J.M., Maeda, N., Kindy, M.S. and Butterfield, D.A. (2002) "Apolipoprotein E modulates Alzheimer's Aβ(1–42)-induced oxidative damage to synaptosomes in an allele-specific manner", *Brain Res.* **924**, 90–97.
[53] Aksenov, M.Y., Aksenova, M.V., Butterfield, D.A. and Markesbery, W.R. (2000) "Oxidative modification of creatine kinase BB in Alzheimer's disease brain", *J. Neurochem.* **74**, 2520–2527.
[54] Aksenov, M.Y., Aksenova, M.V., Butterfield, D.A., Geddes, J.W., Markesbery, W.R. (2001) "Protein oxidation in the Alzheimer's disease brain: analysis of protein carbonyls by immunochemistry and two-dimensional western blotting", *Neuroscience* **103**, 373–383.
[55] Castegna, A., Aksenov, M., Aksenova, M., Thongboonkerd, V., Klein, J.B., Pierce, W.M., Booze, R., Markesbery, W.R. and Butterfield, D.A. (2002) "Proteomic identification of oxidatively modified proteins in Alzheimer's disease brain: Part I. Creatine kinase BB, glutamine synthetase, and ubiquitin C-terminal hydrolase L-1", *Free Radic. Biol. Med.* **33**, 562–571.
[56] Castegna, A., Aksenov, M., Thongboonkerd, V., Klein, J.B., Pierce, W.M., Booze, R., Markesbery, W.R. and Butterfield, D.A. (2002) "Proteomic identification of oxidatively modified proteins in Alzheimer's disease brain: Part II. Dihydropyrimidinase-related protein 2, α-enolase, and heat shock cognate 71", *J. Neurochem.* **82**, 1524–1532.
[57] Blass, J.P. (2001) "Brain metabolism and brain disease: is metabolic deficiency the proximate cause of Alzheimer dementia?", *J. Neurosci. Res.* **66**, 851–856.
[58] Yatin, S.M., Aksenov, M. and Butterfield, D.A. (1991) "The antioxidant vitamin E modulated amyloid β-peptide-induced creatine kinase activity inhibition and increased protein oxidation: implications for the free radical hypothesis of Alzheimer's disease", *Neurochem. Res.* **24**, 427–435.
[59] Keller, J.N., Gee, J. and Ding, Q. (2002) "The proteasome in brain aging", *Aging Res. Rev.* **1**, 279–293.
[60] Shringarpure, R. and Davies, K.J. (2002) "Protein turnover by the proteosome in aging and disease", *Free Radic. Biol. Med.* **32**, 1084–1089.
[61] Lee, M., Hyun, D.H., Marshall, K.A., Ellerby, L.M., Bredesen, D.E., Jenner, P. and Halliwell, B. (2001) "Effect of overexpression of BCL-2 on cellular oxidative damage, nitric oxide production, antioxidant defenses, and the proteasome", *Free Radic. Biol. Med.* **31**, 1550–1559.
[62] Keller, J., Hanni, K.B. and Markesbery, W.R. (2000) "Impaired proteasome function in Alzheimer's disease", *J. Neurochem.* **75**, 436–439.
[63] Kurihaka, L.K., Kikuchi, T., Wada, K. and Tilghman, S.M. (2001) "Loss of *Uch L-1* and *Uch L-3* leads to neurodegenaration, posterior paralysis, and dysphagia", *Human Mol. Genet.* **10**, 1963–1970.
[64] Gu, Y. and Ihara, Y. (2000) "Evidence that collapsin response mediator protein-2 is involved in the dynamics of microtubules", *J. Biol. Chem.* **275**, 17917–17920.