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Induction of Methionine-Sulfoxide Reductases Protects Neurons from Amyloid β-Protein Insults in Vitro and in Vivo

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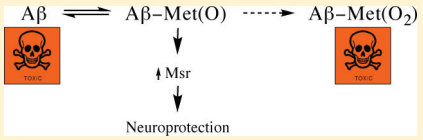
ABSTRACT: Self-assembly of amyloid β-protein (Aβ) into toxic oligomers and fibrillar polymers is believed to cause Alzheimer's disease (AD). In the AD brain, a high percentage of Aβ contains Met-sulfoxide at position 35, though the role this modification plays in AD is not clear. Oxidation of Met^{35} to sulfoxide has been reported to decrease the extent of Aβ assembly and neurotoxicity, whereas surprisingly, oxidation of Met^{35} to sulfone yields a toxicity similar to that of unoxidized Aβ. We hypothesized that the lower toxicity of Aβ-sulfoxide might result not only from structural alteration of the C-terminal region but also from activation of methionine-sulfoxide reductase (Msr), an important component of the cellular antioxidant system. Supporting this hypothesis, we found that the low toxicity of Aβ-sulfoxide correlated with induction of Msr activity. In agreement with these observations, in $MsrA^{-/-}$ mice the difference in toxicity between native Aβ and Aβ-sulfoxide was essentially eliminated. Subsequently, we found that treatment with *N*-acetyl-Met-sulfoxide could induce Msr activity and protect neuronal cells from Aβ toxicity. In addition, we measured Msr activity in a double-transgenic mouse model of AD and found that it was increased significantly relative to that of nontransgenic mice. Immunization with a novel Met-sulfoxide-rich antigen for 6 months led to antibody production, decreased Msr activity, and lowered hippocampal plaque burden. The data suggest an important neuroprotective role for the Msr system in the AD brain, which may lead to development of new therapeutic approaches for AD.



Oxidative stress occurs in biological systems when generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as hydroxyl radicals and peroxynitrite ions, exceeds the system's capacity to eliminate these species.[1] This situation may result from a disturbance in production and/or distribution of antioxidants or from environment-induced elevation of ROS or RNS levels. Oxidative stress is a major deleterious mechanism in Alzheimer's disease (AD),[2] other neurodegenerative diseases,[3] and normal aging.[4] In AD, levels of oxidative damage markers, including lipid peroxidation and nitration, nucleic acid oxidation, and protein carbonylation, are increased in vulnerable brain areas relative to age-matched healthy individuals.[5]

AD is characterized pathologically by extracellular amyloid plaques comprising predominantly fibrillar amyloid β-protein (Aβ) and intracellular neurofibrillary tangles made of hyperphosphorylated tau.[6] Amyloid plaques are surrounded by inflammation, including activated microglia and astrocytes, which contribute to the creation and maintenance of oxidative stress.[6] Though historically amyloid plaques were thought to cause AD,[7] current evidence indicates that the pathological process leading to AD begins with synaptic injury by neurotoxic Aβ oligomers, whereas the formation of plaques and tangles occurs downstream.[8] Oxidative stress is one of the earliest consequences of toxic insults mediated by soluble Aβ oligomers.[9] Mitochondria are particularly sensitive to oxidative stress, and reduced metabolic activity resulting from oxidative damage to vital mitochondrial components has been demonstrated in AD.[10] Consequently, antioxidant therapy has been associated with a reduced risk for AD.[11,12]

Aβ exists predominantly in two major forms comprising 40 (Aβ40) or 42 (Aβ42) amino acid residues. Genetic, physiologic, and biochemical evidence indicates that Aβ42 plays a predominant role in the pathogenesis of AD.[13] A single Met residue in Aβ, Met^{35}, is located in the middle of the hydrophobic C-terminal region [Aβ(29−42)]. Therefore, the dramatic increase in the polarity of the Met side chain that occurs upon oxidation has a profound effect on the hydropathy of the entire region.[14] Met is highly susceptible to oxidation in vivo, particularly under conditions of oxidative stress. The sulfoxide form has been found to comprise 10−50% of Aβ in amyloid plaques of AD brain,[15−18] though it is not clear whether its existence contributes to AD etiology or results from the highly oxidative environment around amyloid plaques where fibrillar Aβ may be trapped for long periods.

In addition to oxidation of Met to Met-sulfoxide [Met(O)], Met can undergo a second oxidation reaction yielding Met-

Received: September 12, 2011
Revised: October 27, 2011
Published: November 7, 2011

sulfone [Met(O_2)]. Met(O_2) has been found in the antioxidant protein DJ-1 in brains from patients with AD or Parkinson's disease (PD)[19] and may exist in Aβ,[20] though its formation requires a high activation energy; consequently, Met(O_2) is not commonly found in vivo. When it does happen, the in vivo oxidation of Met to Met(O_2) is considered irreversible.[21] In contrast, oxidation of Met to Met(O) is reversible, and the reverse reaction is catalyzed in vivo by the methionine-sulfoxide reductase (Msr) system, comprising peptide-methionine (*S*)-*S*-oxide reductase (EC 1.8.4.11, MsrA) and peptide-methionine (*R*)-*S*-oxide reductase (EC 1.8.4.12, MsrB), which reduce the *S* and *R* enantiomers of the sulfoxide group, respectively, providing protection against oxidative stress.[22] Mammalian MsrA is encoded by a single gene[23] and is found in both the cytosol and mitochondria because of the alternative splicing of an N-terminal mitochondrial signal sequence and myristoylation of the cytosolic form.[24] MsrA levels decrease with aging[25] and in AD.[26] Studies in *MsrA*$^{-/-}$ mice have shown increased vulnerability to oxidative stress[27] and oxidative pathology associated with AD[28] and PD.[29] Conversely, overexpression of MsrA in various organisms has been shown to provide enhanced protection against oxidative stress and improve the survival rate.[30−32]

Several laboratories have reported lower toxicity of Aβ-Met(O) relative to WT Aβ.[33] This lower toxicity largely has been attributed to the tendency of Aβ-Met(O) to aggregate with slower kinetics[34] and/or form smaller oligomers relative to WT Aβ,[14] which correlate with structural differences between native and oxidized Aβ in the C-terminal region.[35,36] However, recent examination of the sulfoxide and sulfone forms of Aβ alongside the WT form showed that although Aβ-Met(O) showed reduced toxicity, as expected, the toxicity of Aβ-Met(O_2), which was used as a control, was surprisingly similar to that of WT Aβ in assays of neuronal apoptosis, dendritic spine morphology, and Ca^{2+} homeostasis.[37] These data suggested that the lower activity of Aβ-Met(O) might result not only from an altered structure in the C-terminal region of Aβ or alteration of Aβ oligomerization but also from other mechanisms, possibly Msr activation, which might be unique to the sulfoxide form, despite the similarity in the structure and calculated dipole moment between Met(O) and Met(O_2).[14,38] Consistent with this hypothesis, a recent study has reported elevated MsrA activity and mRNA levels in human neuroblastoma (IMR-32) cells in response to treatment with Aβ42-Met(O), suggesting that the cells sensed the presence of Met(O) in Aβ and upregulated MsrA to provide enhanced cellular protection.[39]

To test the hypothesis that Msr activation contributes to the lower toxicity observed for Aβ-Met(O) relative to Aβ-Met(O_2) and WT Aβ, here, we compared the effect of the WT, sulfoxide, and sulfone forms of Aβ40 and Aβ42 on the viability and Msr activity of rat primary cortical neurons. The findings led us to explore the role of the different Msr isoforms in the cellular response to Aβ by using the same experimental paradigm in primary neurons from WT and *MsrA*$^{-/-}$ mice. In addition, we hypothesized that the Msr system could be used as a target for the development of therapeutic agents against Aβ-induced oxidative stress, and to test this hypothesis, we studied the possibility of inducing a neuroprotective response by activating the Msr system, both in cell culture, using a Met(O) derivative, and in vivo by immunization with a Met(O)-rich antigen.

MATERIALS AND METHODS

Peptide Synthesis. Aβ40, [Met(O)^{35}]Aβ40, [Met(O_2)^{35}]-Aβ40, Aβ42, [Met(O)^{35}]Aβ42, and [Met(O_2)^{35}]Aβ42 were synthesized by incorporating FMOC-Met(O) or FMOC-Met(O_2) (EMD Biosciences, San Diego, CA) in position 35 where appropriate, purified, and characterized in the University of California at Los Angeles (UCLA) Biopolymers Laboratory. Quantitative amino acid analysis and mass spectrometry were used to characterize the expected compositions and molecular weights, respectively, of each peptide. *N*-Acetyl-D,L-Met(O) [Ac-Met(O)] was purchased from Sigma (St. Louis, MO).

Preparation of Peptide Solutions. Purified peptides were stored as lyophilized powders at −20 °C. Before being used, peptides were treated with 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) (TCI America, Portland, OR) to disassemble preformed aggregates and stored as dry films at −20 °C as described previously.[40] Immediately before being used, peptide films were dissolved in 60 mM NaOH at 10% of the desired volume, diluted with cell culture medium followed by sonication for 1 min, and added to the cells at a final concentration of 10 μM unless otherwise stated. The final NaOH concentration was ≤6 mM, and the change in the pH of the medium was negligible.

Animals. All experiments were compliant with the National Research Council Guide for the Care and Use of Laboratory Animals and were approved by the respective Animal Research Councils and the Ethics Committees of UCLA or the University of Kansas. Pregnant (E18) Sprague-Dawley rats and C57Bl/6 mice were purchased from Charles River Laboratories (Wilmington, MA). Double-transgenic (2×Tg) mice overexpressing familial AD-linked mutant forms of amyloid β-protein precursor (APP) and presenilin 1 [B6C3 Tg(APPswe,PSEN1dE9)85Dbo/J] and control, non-Tg mice on the same genetic background were purchased from Jackson Laboratories (Bar Harbor, ME). *MsrA*$^{-/-}$ mice were bred and maintained in house.

Cell Culture. Primary cortical or hippocampal neurons were prepared as described previously.[37] Briefly, pregnant E18 rats or mice were euthanized with CO_2, and the pups were collected immediately. The brains were dissected in chilled Leibovitz's L-15 medium (ATCC, Manassas, VA) in the presence of 1 μg/mL penicillin/streptomycin (Invitrogen, Carlsbad, CA), and the cells were suspended in Dulbecco's modified Eagle's medium (DMEM, obtained from ATCC) containing 10% heat-inactivated fetal bovine serum (ATCC) and penicillin/streptomycin (1 μg/mL) and plated in poly-D-lysine (0.1 mg/mL, Sigma)-coated 96-well COSTAR plates (Corning, Lowell, MA) at a density of 3×10^5 cells/mL. The cultures were maintained for 6 days before being treated with peptides. Twenty-four hours after the cells had been plated, the medium was replaced with fresh medium supplemented with 5 μM cytosine β-D-arabinofuranoside (Sigma) to inhibit the proliferation of glial cells. PC-12 cells were cultured and differentiated with 50 ng/mL nerve growth factor (NGF) 24 h prior to treatment with peptides as described previously.[41]

MTT Reduction Assay. Cells were treated with freshly prepared Aβ analogues for 48 h. Cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell metabolism assay, as described previously.[41] Briefly, following treatment, 15 μL of MTT was added to each well and incubated for 4 h at 37 °C. Then, stop solution was added and kept overnight at 25 °C. The optical

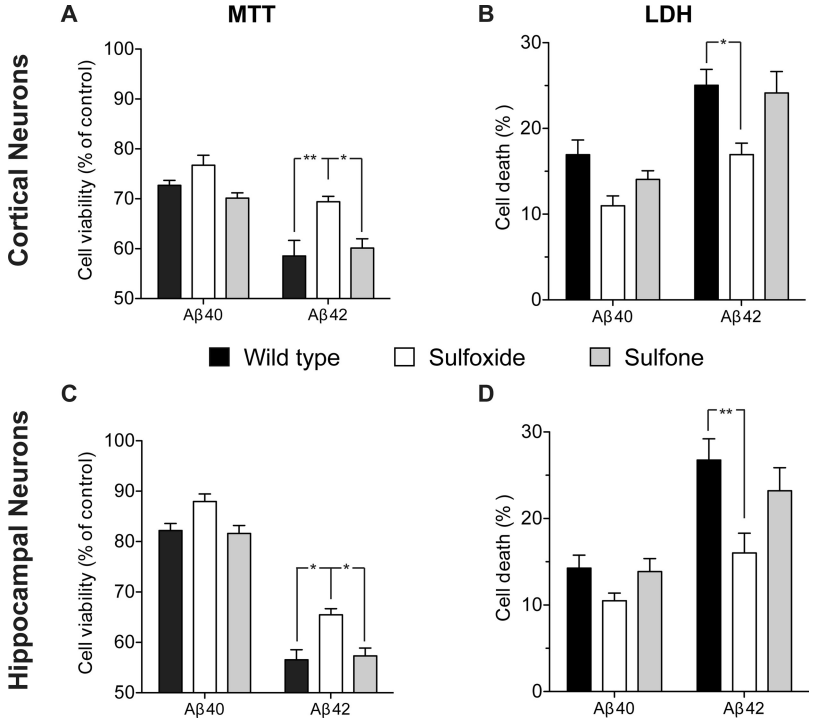


Figure 1. Comparison of neurotoxic effects of native and oxidized Aβ analogues. Rat primary cortical (A and B) or hippocampal (C and D) neurons were cultured for 6 days and then treated with Aβ analogues. Cell viability was measured using the MTT reduction (A and C) or LDH release (B and D) assay following treatment with each Aβ analogue at 10 μM for 48 h as described previously.[37] *p < 0.05; **p < 0.01.

density was measured using a Synergy plate reader (Bio-TEK Instruments, Winooski, VT). The cell viability results of three independent experiments (six wells per data point) were normalized to the medium control group and expressed as the mean ± the standard error of the mean (SEM). Neuroprotection experiments were performed in a similar manner using 10 μM Aβ42 in the absence or presence of Ac-Met(O).

Lactate Dehydrogenase (LDH) Release Assay. Neurons were incubated with Aβ analogues for 48 h, and cell death was assayed by measuring the release of LDH as described previously.[42] Data from six independent experiments (six wells per data point) were normalized to medium control and expressed as means ± SEM.

Measurement of Msr Activity. Total Msr activity was measured in rat or mouse primary cortical neurons, or differentiated PC-12 cells, as described previously.[32] Briefly, the cells were treated with each peptide at 10 μM for 24 h. Similarly, in protection experiments, differentiated PC-12 cells were treated for 24 h with Ac-Met(O) in the absence or presence of 10 μM Aβ42. Following the incubation, the culture medium was removed and the cells were washed with PBS (pH 7.4). Then, the cells were lysed in PBS by a 1 min sonication in an ice−water bath in the presence of a protease inhibitor cocktail (Sigma). Insoluble material was removed by centrifugation at 14000g for 10 min at 4 °C, and supernates were stored at −80 °C until they were used. Protein concentrations were determined using a Bradford protein assay kit (Bio-Rad, Hercules, CA) and used to normalize the volume used for determination of Msr activity. Supernates (100 μg of protein) were incubated with 100 μL of 50 mM Tris-HCl (pH 7.5) containing 20 mM DTT and 200 μM dabsyl-Met(O) for 1 h at 37 °C. Then, the reaction was stopped by addition of an equal volume of acetonitrile, and the mixture was analyzed by a high-performance liquid chromatography (HPLC) system equipped with a C18 column using a gradient starting at 100% 0.14 M sodium acetate (pH 6.0) and increasing the percentage of acetonitrile to 70% over 30 min. The dabsyl-Met(O) and dabsyl-Met peaks were detected at 436 nm. The basal specific activity measured in control cells treated with medium alone was as follows (in picomoles of dabsyl-Met formed per minute per milligram of protein): primary WT rat neurons, 240; primary WT mouse neurons, 150; primary *MsrA*$^{-/-}$ neurons, 80; PC-12 cells, 200. The specific activity in Aβ-treated cells was normalized to the medium-treated cells and expressed as the percentage change in Msr activity (mean ± SEM).

Immunization of 2×Tg Mice. 2×Tg mice[43] were immunized with oxidized *Zea mays* Met-rich protein (DZS18)[44] with complete Freund's adjuvant for the first injection, followed by oxidized DZS18 with incomplete Freund's adjuvant every 2 weeks for 6 months beginning at 3 months of age. 2×Tg mice treated with adjuvant only, unimmunized mice, and non-Tg mice of the same genetic background served as control groups (five mice per group). At the end of the immunization period, the mice were euthanized, their brains were collected, frozen, and sectioned, and brain sections were analyzed for aggregated Aβ deposition using thioflavin S (ThS) staining. Additionally, Msr specific activity in brain was measured using the HPLC assay described above for cultures.

Detection of Serum Immunoglobulins by Western Blotting. Sera were collected from 2×Tg mice immunized with oxidized DZS18 or adjuvant alone or unimmunized mice (five per group). Oxidized, recombinant DZS18 was loaded onto 4 to 20% gradient gels (Pierce, Rockford, IL; 5 μg per lane) and subjected to sodium dodecyl sulfate−polyacrylamide gel electrophoresis. The protein was transferred to nitrocellulose membranes, and each lane was probed with serum from one mouse (1:500 dilution), followed by HRP-conjugated

goat anti-mouse IgG (1:5000 dilution) (Invitrogen, Carlsbad, CA). The bands were visualized using ECL.

ThS Staining. Coronal, 45 μm brain sections were prepared using a Microm HM 360 microtome (Harlow Scientific, Arlington, VA), immersed for 3 min each in 95 and 70% ethanol followed by a 5 min incubation in 1% ThS in deionized water (Sigma) and quick rinses in 80% ethanol and deionized water. The sections then were dehydrated by consecutive 1 min incubations in 70, 95, and 100% ethanol and immersed in xylene for 3 min prior to aqueous mounting in glycerin jelly. ThS fluorescence was imaged and quantified using a fluorescence microscope (GE Healthcare, Waukesha, WI) with a high-content imaging system (GE Healthcare). The area of ThS-positive plaques per equivalent hippocampal area (plaque burden) was measured and quantified using ImageJ.

Data Analysis. Data are expressed as means ± SEM. They were analyzed by one-way analysis of variance (ANOVA) with post hoc Tukey's test.

RESULTS

Effect of WT and Oxidized Aβ on Neuronal Viability. To investigate the neurotoxic effects of native and oxidized Aβ variants on cellular viability and survival, we used two different toxicity assays, MTT reduction and LDH release, in rat primary cortical or hippocampal neurons (Figure 1). We used both assays because methods for determining Aβ toxicity are not consistent across the AD field. Each of these assays addresses a different aspect of cell toxicity. The MTT assay measures mitochondrial activity of viable cells, whereas the LDH assay detects membrane integrity as a direct measurement of cell death.

Compared to the levels in cells treated with medium containing the same concentration of NaOH (used for initial peptide solubilization) but no Aβ, Aβ40 caused decreases of 27 ± 1 and 17 ± 2% in cortical neuron viability and 18 ± 1 and 14 ± 2% in hippocampal neuron viability in the MTT (Figure 1A,C) and LDH (Figure 1B,D) assays, respectively. Aβ40-Met(O) was 5−10% less toxic than WT Aβ40, whereas Aβ40-Met(O_2) had a toxicity similar to that of WT Aβ40. Overall, the differences observed among the Aβ40 analogues were not statistically significant. We also did not find significant differences between the response of cortical (Figure 1A,B) and hippocampal (Figure 1C,D) neurons to Aβ40 analogues.

Under the same conditions, Aβ42 showed 41 ± 3 and 25 ± 2% decreases in cortical neuron viability and 43 ± 2 and 27 ± 3% decreases in hippocampal neuron viability in the MTT (Figure 1A,C) and LDH (Figure 1B,D) assays, respectively. As seen in previously described data,[37] Aβ42-Met(O) was significantly less toxic, causing 31 ± 1 and 17 ± 1% decreases in cortical neuron viability and 34 ± 1 and 16 ± 2% decreases in hippocampal neuron viability, respectively (Figure 1). These levels of toxicity were similar to those of Aβ40 analogues in the LDH assay (Figure 1A,B), whereas in the MTT assay, Aβ42-Met(O) showed toxicity that was intermediate between those induced by Aβ40 and Aβ42 (Figure 1C,D). Aβ42-Met(O_2) exhibited a toxicity similar to that of WT Aβ42 in all cases, causing 40 ± 2 and 24 ± 3% decreases in cortical neuron viability and 43 ± 2 and 23 ± 3% decreases in hippocampal viability in the MTT and LDH assays, respectively (Figure 1). Thus, as reported previously,[37] both assays showed that despite the similar increase in dipole moment upon oxidation of Met[35] to Met(O) or Met(O_2),[14,38] and despite the change in the oligomer size distribution of the sulfoxide and sulfone forms of Aβ42 relative to WT Aβ42,[14] only Aβ42-Met(O) was less toxic to cells than WT Aβ42.

Msr Response to WT and Oxidized Aβ. The toxicity of the six Aβ alloforms correlated with aggregation kinetics[37] but not with oligomer size distribution or polarity of the C-terminus.[14] To test whether the Msr system might be involved, we measured cellular levels of Msr activity following treatment with each Aβ analogue. Because relatively high toxicity levels were observed following a 48 h incubation with Aβ42 or Aβ42-Met(O_2) (Figure 1), we used a 24 h incubation in these experiments.

We found that WT Aβ40 and Aβ42 caused a small (6 ± 6 and 3 ± 5%, respectively), insignificant increase in Msr activity relative to that of untreated cells (Figure 2A), whereas Aβ40-Met(O) and Aβ42-Met(O) increased total Msr activity significantly (25 ± 6 and 27 ± 11%, respectively) relative to that of untreated cells (Figure 2A). In contrast, following treatment with Aβ40-Met(O_2) or Aβ42-Met(O_2), we observed moderate, nonsignificant decreases of 3 ± 4 and 10 ± 4%, respectively, in Msr activity compared to control cells (Figure 2A). Dose−response analysis of the effect of Aβ40-Met(O) or Aβ42-Met(O) on Msr activity showed that in both cases, the measured total Msr activity increased between 1 and 10 μM Aβ and decreased at 30 μM Aβ, likely because of high levels of apoptosis at the highest concentration (Figure 2B). Differences in Msr activity levels caused by Aβ40-Met(O) or Aβ42-Met(O) between panels A and B of Figure 2 reflect experimental variability. These findings support the hypothesis that neurons sense the presence of the sulfoxide group in Aβ and respond by activating the Msr system as self-protection against oxidative stress.

Examination of Aβ Analogues in *MsrA*$^{-/-}$ Neurons. To gain insight into the relative contribution of Msr isozymes to the response to Aβ-Met(O), we measured neurotoxicity and Msr activation in primary cortical neurons from *MsrA*$^{-/-}$ mice and compared the data to those for neurons from WT mice. In these experiments, we used only Aβ42 analogues because the differences observed among the different Aβ forms were similar in trend yet greater in magnitude for Aβ42 than for Aβ40. We predicted that if MsrA were the main isozyme responsible for the lower observed toxicity of Aβ42-Met(O) relative to WT Aβ42 and Aβ42-Met(O_2), the difference among the three Aβ analogues would disappear when they were tested in *MsrA*$^{-/-}$ neurons; i.e., Aβ42-Met(O) would show the same level of toxicity as the other two analogues. In contrast, if MsrB compensated for the absence of MsrA, we expected that each Aβ42 alloform would behave similarly, regardless of whether the neurons were WT or *MsrA*$^{-/-}$.

In WT neurons, Aβ42-Met(O) was significantly less toxic than WT Aβ42 or Aβ42-Met(O_2) in the MTT assay (Figure 3A). In contrast, in neurons from *MsrA*$^{-/-}$ mice, Aβ42, Aβ42-Met(O), and Aβ42-Met(O_2) caused similar decreases in viability (Figure 3A) and the differences among the three alloforms were insignificant. Thus, the absence of MsrA appeared to render the neurons more susceptible to the toxic effect of all three alloforms, suggesting that MsrA was the predominant isozyme protecting the neurons from Aβ42-Met(O) toxicity. Similar results were obtained in the LDH assay. In neurons from WT mice, Aβ42-Met(O) caused significantly less cell death than WT Aβ42 or Aβ42-Met(O_2). In contrast, in *MsrA*$^{-/-}$ neurons, Aβ42, Aβ42-Met(O), or Met(O_2) each induced similar levels of cell death, and the differences were insignificant.

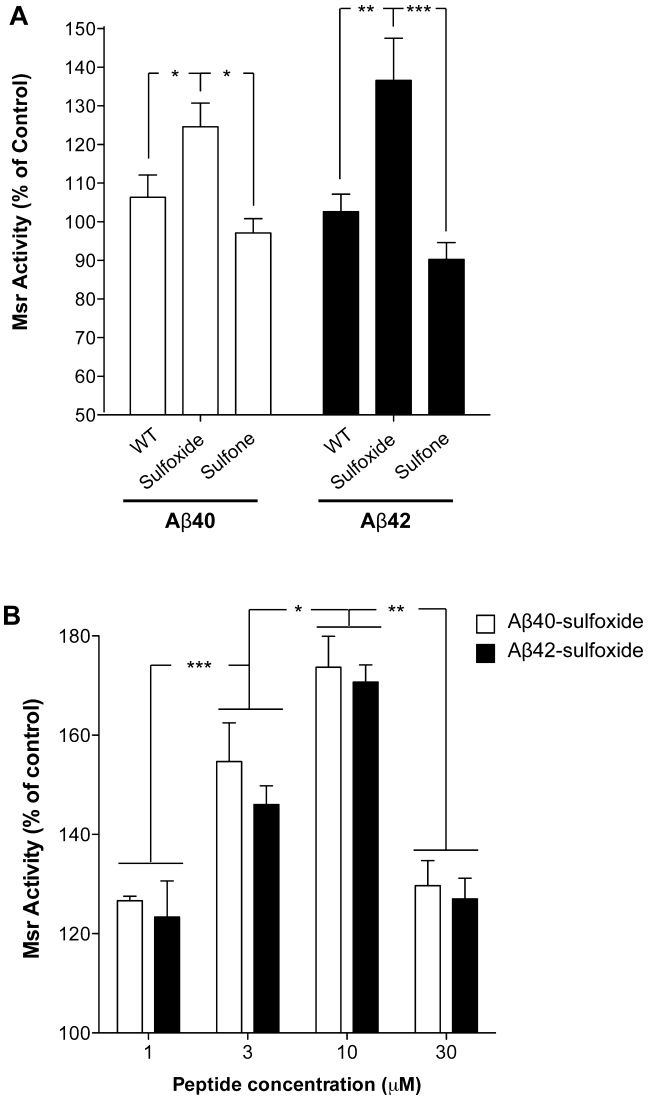


Figure 2. Effect of native and oxidized Aβ on Msr activity. Rat primary cortical neurons were grown for 6 days on poly-D-lysine-coated, 60 mm Petri plates. (A) Cells were incubated in the presence or absence of each Aβ analogue at 10 μM for 24 h at 37 °C. The cells were lysed in PBS (pH 7.4) and centrifuged, and supernatants were used to determine the total specific Msr activity by HPLC using dabsyl-Met(O) as described previously.[60] The results were normalized to untreated cells [240 pmol of dabsyl-Met min^{-1} (mg of protein)$^{-1}$ defined as 100% specific Msr activity]. The data are an average of 10–15 independent experiments. (B) Cells were treated with Aβ40-sulfoxide or Aβ42-sulfoxide at the indicated concentrations, and total specific Msr activity was determined as described above. The data are an average of three independent experiments. *p < 0.05; **p < 0.01; ***p < 0.001.

Measurement of Msr activity showed that the specific Msr activity in *MsrA*$^{-/-}$ neurons [80 pmol of dabsyl-Met min^{-1} (mg of protein)$^{-1}$] was approximately half that of WT neurons [150 pmol of dabsyl-Met min^{-1} (mg of protein)$^{-1}$], consistent with the absence of MsrA. The pattern of response of the *MsrA*$^{-/-}$ neurons to the three Aβ42 analogues was similar to that of WT neurons, though the differences, which reflect MsrB only, did not reach statistical significance. These results suggest that in the absence of MsrA, neurons still sense the presence of the sulfoxide group in Aβ42-Met(O) and respond by increasing MsrB activity, but this response provides little neuroprotection compared to WT neurons.

Induction of a Protective Msr Response by a Met(O) Derivative in Cell Cultures. On the basis of the findings described above, we next asked whether the Msr system could be induced to protect neurons against Aβ42 toxicity. Our first approach was an attempt to protect cultured cells by application of Ac-Met(O). Because of the exploratory nature of these experiments, we used here differentiated PC-12 cells rather than primary neurons.

Evaluation of cell viability using the MTT (Figure 4A) and LDH (Figure 4B) assays showed that 1 mM Ac-Met(O) rescued Aβ42-induced toxicity to the levels of untreated cells. Lower concentrations of Ac-Met(O) showed partial rescue (data not shown). The rescue by 1 mM Ac-Met(O) correlated with a significant, 44 ± 4% increase in Msr activity (Figure 4C). The data suggest that induction of an Msr response by exposure of cells to Ac-Met(O) or derivatives thereof is a viable neuroprotective strategy against Aβ42-induced toxicity.

Induction of a Protective Msr Response by Immunization with a Met(O)-Rich Antigen in Vivo. To test whether induction of the Msr system could be beneficial in vivo, we immunized 2×Tg mice bearing FAD-linked mutant *app* and *psen1* genes[45] with a Met(O)-rich antigen, which recently has been used to create a unique anti-Met(O) antibody.[46] This antigen is an oxidized form of recombinant *Z. mays* methionine-rich protein (DZS18). The anti-Met(O) antibody was developed to recognize Met(O) in any protein. It was shown to detect increased Met(O) levels in plasma from aged WT mice or *MsrA*$^{-/-}$ mice compared with young WT mice, from patients with AD compared with healthy age-matched individuals,[46] and in symptomatic and presymptomatic persons carrying familial AD-linked mutations in *app* or *psen1* compared to noncarriers from the same kindreds.[47]

Here, we used oxidized DZS18 to immunize 2×Tg AD mice. These mice produce high levels of Aβ, particularly Aβ42, in their brain and display Aβ deposition in amyloid plaques as early as 4 months of age with progression up to 12 months of age.[48] We hypothesized that immunization with the Met(O)-rich antigen initially would induce higher Msr activity but over time would produce an immune response that might lead to decreased levels of Met(O) in proteins and a subsequent decrease in Msr activity. Because a large portion of Aβ Met[35] in amyloid plaques is oxidized to sulfoxide,[15] we hypothesized also that immunization with oxidized DZS18 might help clear the amyloid burden in the brains of the mice.

Mice were immunized every 2 weeks for 6 months beginning at 3 months of age. At the end of the immunization period, their serum was analyzed for Msr activity and production of anti-Met(O) antibodies, and brain sections were stained with ThS for visualization of amyloid plaques. As shown in Figure 5, Msr activity in the brain of unimmunized 2×Tg mice was 40 ± 15% higher than in WT mice. Immunization of the 2×Tg mice with oxidized DZS18, but not with adjuvant alone, caused a significant decrease in brain Msr activity of 26 ± 10% (Figure 5A), consistent with our prediction. Analysis of mouse serum showed immunoglobulins reactive toward oxidized DZS18 in immunized mice, but not in mice receiving adjuvant alone or unimmunized mice (Figure 5B). ThS staining showed abundant plaques in unimmunized mice (Figure 5C). Immunization with oxidized DZS18 caused a significant, 28 ± 8% reduction in plaque burden in the hippocampus of the immunized mice relative to the control groups (Figure 5D–F).

dx.doi.org/10.1021/bi201426b | *Biochemistry* 2011, 50, 10687–10697

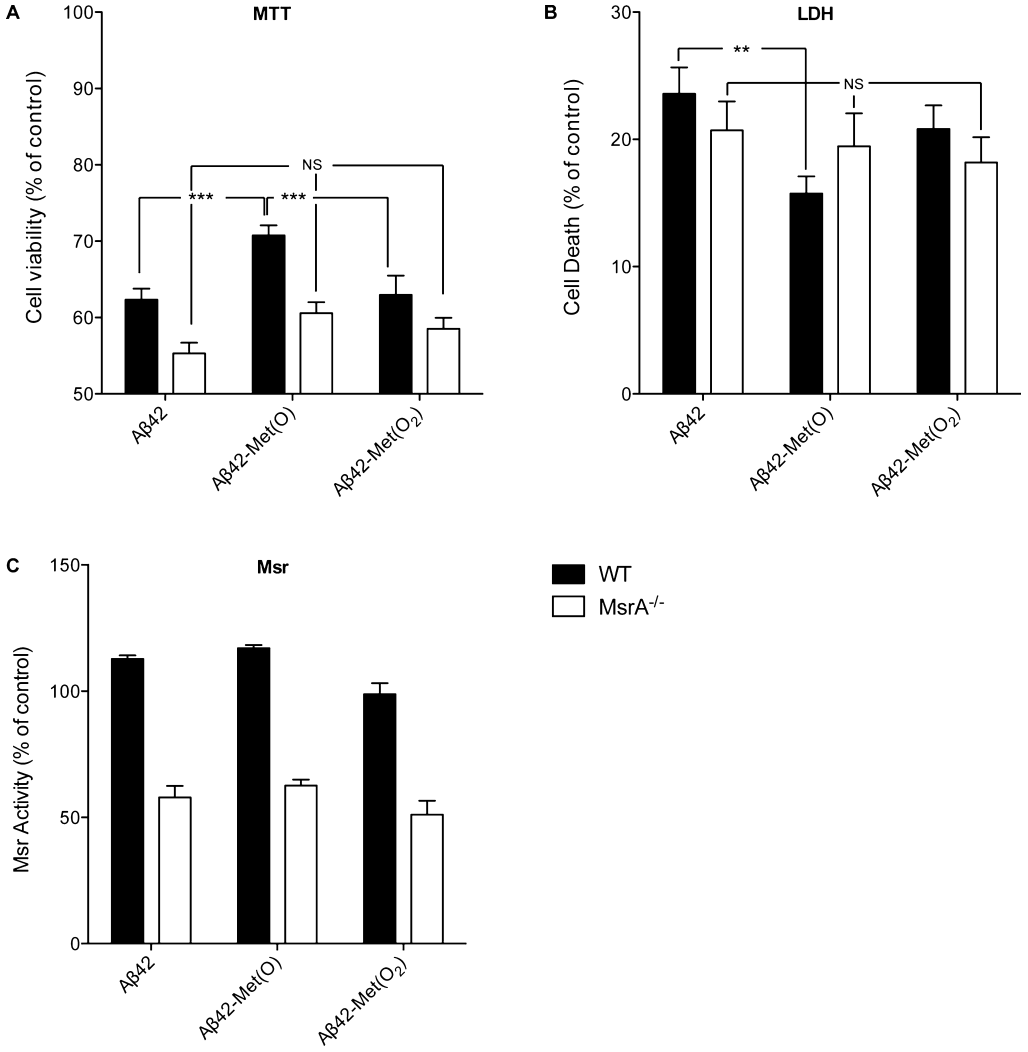


Figure 3. Response of *MsrA*$^{-/-}$ and WT mouse primary cortical neurons to native and oxidized Aβ42. Primary *MsrA*$^{-/-}$ or WT mouse cortical neurons were treated with each Aβ analogue at 10 μM for 48 h. (A) Assessment of cell viability using the MTT assay. (B) Assessment of cell death using the LDH assay. (C) Measurement of specific Msr activity by HPLC using dabsyl-Met(O) as the substrate. The results were normalized to untreated WT cells [150 pmol of dabsyl-Met min^{-1} (mg of protein)$^{-1}$ defined as 100% specific Msr activity]. The data are an average of 5−10 independent experiments. $^{**}p < 0.01$; $^{***}p < 0.001$. NS, nonsignificant.

The data suggest that immunization with a Met(O)-rich, non-Aβ antigen can produce amyloid plaque clearance in the brain of 2×Tg AD mice, presumably by removing the oxidized form of Aβ or other plaque-associated proteins, and that production of an immune response against Met(O) may alleviate in part the oxidative stress that causes increased Msr activity in these mice.

DISCUSSION

Met^{35} is the primary target site for oxidants in Aβ.[33] Formation of methionyl radicals and participation of Met in Fenton chemistry in the presence of transition metal ions leading to production of ROS have been hypothesized to play an important role in Aβ-induced toxicity. Once Met is oxidized to sulfoxide or sulfone, its tendency to participate in further oxidation reactions or form radicals is weakened substantially. Thus, if participation of Met^{35} in these reactions were important for Aβ-induced toxicity, Aβ would be predicted to become less toxic upon oxidation. This hypothesis has been supported by a number of studies comparing the toxicity of WT Aβ42 and Aβ42-Met(O).[33] Surprisingly, however, even though Aβ-Met(O_2) is less likely than Aβ-Met(O) to form radicals or participate in Fenton chemistry, it induces the same levels of neurotoxicity and synaptotoxicity as WT Aβ (Figure 1 and refs 37 and 49). These results have led us to hypothesize that factors other than the effect of oxidation on Aβ conformation and assembly, for example, activation of the Msr system, contributed to the observed differences between the toxicity levels of WT Aβ and Aβ-Met(O).

The observation of a significant increase in Msr activity in response to Aβ-Met(O), but not WT Aβ or Aβ-Met(O_2), in WT rat (Figure 2) and mouse (Figure 3) primary neurons suggests that Msr protects neurons from Aβ-Met(O) toxicity. Supporting our findings, similar observations in IMR-32 cells have recently been described by Misiti et al., who reported also an increase in the level of *MsrA* transcription upon treatment with Aβ-Met(O).[39] Interestingly, although Msr reduces the less toxic Aβ-Met(O) to the more toxic WT Aβ, the overall result is a significantly lower toxicity (Figure 1). This suggests that Msr-mediated reduction of Met(O) to Met in cellular proteins other than Aβ overrides the direct toxicity caused by Aβ itself through

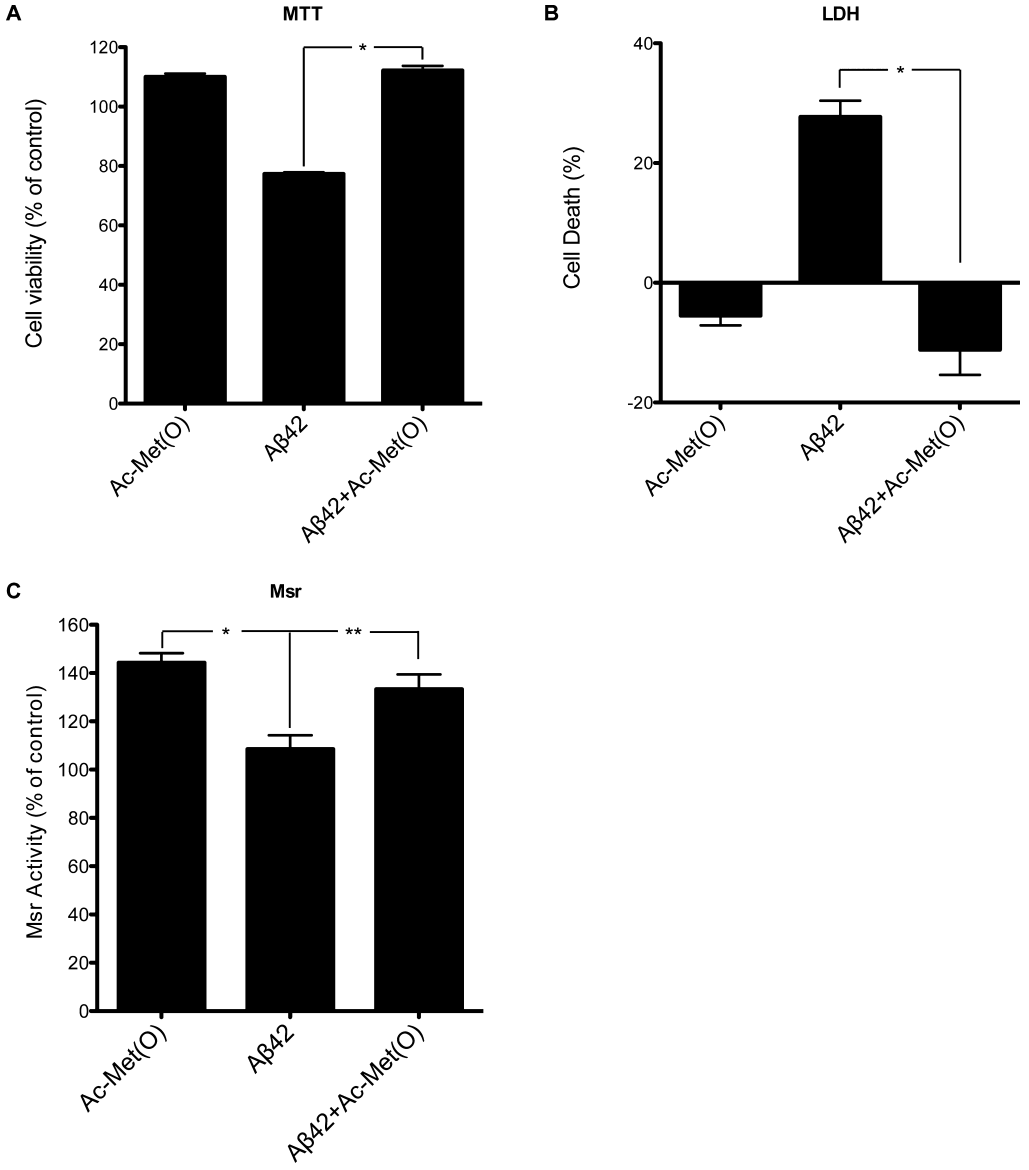


Figure 4. Ac-Met(O) increases Msr activity in differentiated PC-12 cells and protects the cells from Aβ42-induced toxicity. Differentiated PC-12 cells were treated for 24 h with 1 mM Ac-Met(O), 10 μM Aβ42, or 10 μM Aβ42 and 1 mM Ac-Met(O). (A) Assessment of cell viability using the MTT assay. (B) Assessment of cell death using the LDH assay. (C) Measurement of specific Msr activity by HPLC. The results were normalized to untreated cells [200 pmol of dabsyl-Met min^{-1} (mg of protein)$^{-1}$ defined as 100% specific Msr activity]. The data are an average of 10 independent experiments. *$p < 0.05$; **$p < 0.01$.

other mechanisms, emphasizing the role of oxidative damage in the array of toxic mechanisms induced by Aβ.

In both the MTT and LDH assays (Figure 3A,B), the absence of MsrA resulted in elimination of the significant decrease in toxicity induced by Aβ42-Met(O) relative to WT Aβ42 or Aβ42-Met(O_2). These results suggested that the main neuroprotective activity was provided by MsrA rather than MsrB. Indeed, the overall specific Msr activity found in $MsrA^{-/-}$ cells, which must be provided by MsrB, was approximately half that in WT neurons. Previously, ablation of the mouse *MsrA* gene was shown to lower the expression level of MsrB1.[50] Our data suggest that in response to treatment with Aβ42-Met(O), the neurons still upregulate MsrB (Figure 3C); however, this provides only partial protection, and the observed toxicity is higher in the $MsrA^{-/-}$ mice than in WT mice. The putative partial protection provided by MsrB in $MsrA^{-/-}$ cells appeared to be higher in the LDH assay (Figure 3B) than in the MTT assay (Figure 3A). This observation suggests that in mitochondria, where MsrA is the main isoform,[51] the capability of MsrB to mitigate oxidative stress is weaker than in the cytosol. The mitochondrial MsrB isoforms, MsrB2 and MsrB3B, are minor isoforms[52] and presumably have a limited capability to compensate for the absence of MsrA, whereas the cytosolic MsrB1 may offers somewhat higher levels of compensation as reflected in the LDH assay.

Overexpression of MsrA has been shown to be protective against oxidative stress in multiple systems,[22] whereas MsrA ablation enhanced oxidative posttranslational modifications and resulted in the accumulation of damaged proteins, similar to findings in neurodegenerative diseases.[53] These studies and the data presented here suggest that activating the Msr system using sulfoxide-containing compounds may serve as a novel route for the development of therapeutic agents against AD and

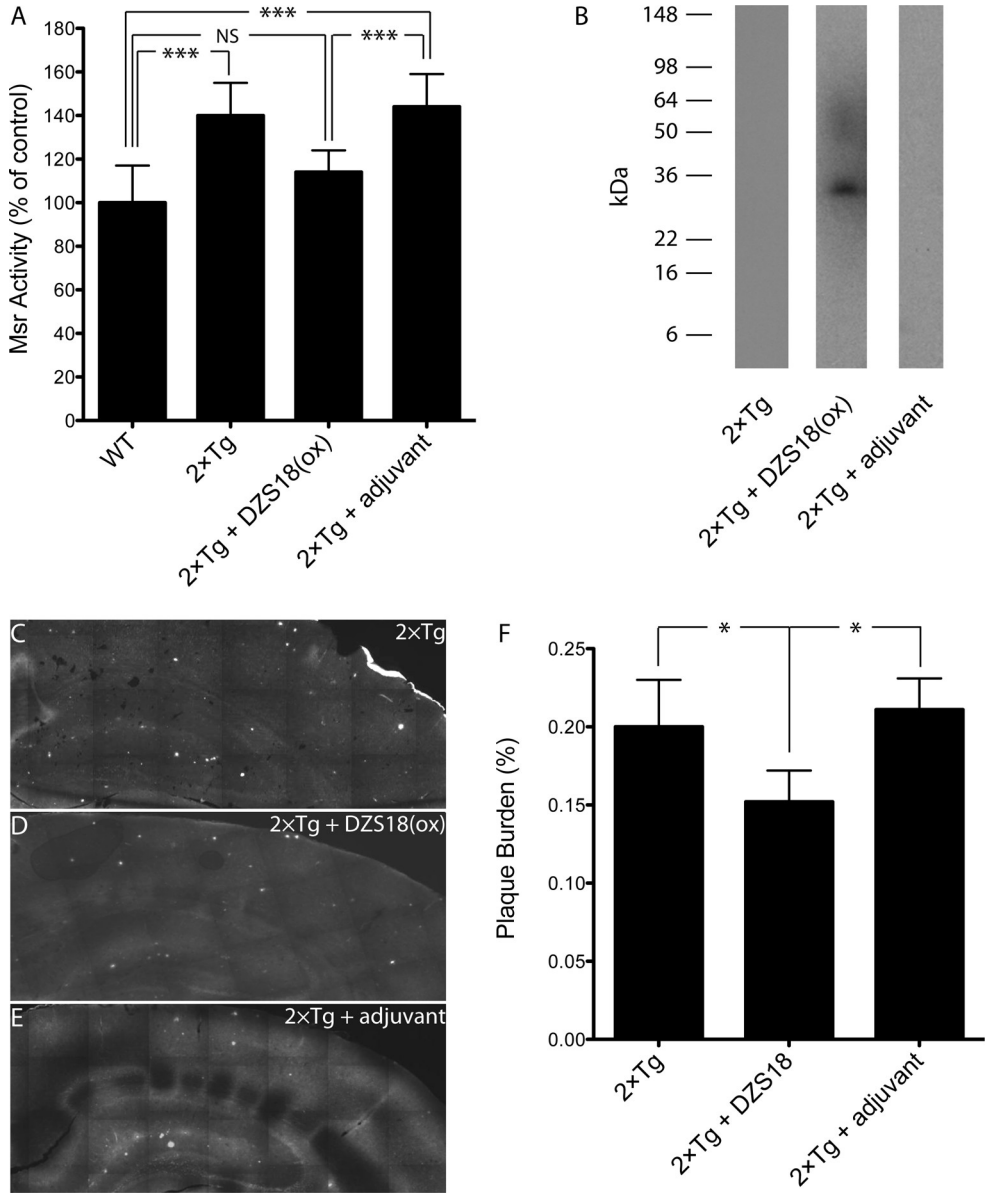


Figure 5. Immunization of 2×Tg mice with oxidized Met-rich protein reduces Msr activity and brain Aβ burden. Mice were immunized for 6 months with oxidized Met-rich protein [DSZ18(ox)] or adjuvant alone. Non-Tg mice served as a negative control and unimmunized mice as a positive control. n = 5 mice per group. (A) Measurement of specific Msr activity in mouse brain. ***$p < 0.001$. NS, nonsignificant. (B) Westren blot analysis for detection of anti-Met(O) antibodies in sera of 2×Tg mice immunized with DZS18(ox), mice immunized with adjuvant alone, or unimmunized mice. The smear between 45 and 60 kDa in the 2×Tg + DZS18(ox) lane is an artifact that was not observed in other blots. (C–E) Fluorescence microscopy images of hippocampal brain slices stained with thioflavin S: (C) unimmunized mice, (D) mice immunized with DZS18(ox), and (E) mice immunized with adjuvant alone. (F) Percent plaque burden quantified by calculation of the total ThS-stained area divided by the total hippocampal area measured and normalized to unimmunized mice using ImageJ (n = 5 mice per condition; *$p < 0.05$).

other neurodegenerative diseases, and for general reduction of aging-related oxidative stress. Here, we used Ac-Met(O) to test this hypothesis and found that this simple amino acid derivative induced elevated Msr activity and protected differentiated PC-12 cells against Aβ42-induced toxicity (Figure 4). These results provide proof of principle for activation of Msr using nontoxic Met(O) derivatives and suggest that exploration of derivatives with higher activity and desirable pharmacokinetic characteristics may yield novel drug candidates for conditions in which oxidative stress is a major deleterious mechanism.

The experiments with Ac-Met(O) did not distinguish between MsrA and MsrB because the compound we used comprised all four diastereomers. MTT experiments using Ac-L-Met(O) or Ac-D-Met(O) showed that both isomers significantly protected differentiated PC-12 cells against Aβ-induced toxicity to a similar extent (data not shown). Experiments using the R or S enantiomer of the sulfoxide group will be pursued in the future.

Immunization has been explored widely as a therapeutic approach for AD, with mixed results.[54] Immunization with Aβ-derived antigens or passive immunization with anti-Aβ antibodies has been shown to reduce Aβ burden in patients with AD and in animal models.[55] However, neuroinflammation, induction of vasogenic edema and/or microhemorrhages, and other adverse effects have raised concerns regarding the safety of this approach, and multiple attempts to develop safer

dx.doi.org/10.1021/bi201426b | *Biochemistry* 2011, 50, 10687–10697

immunization strategies have been made.[56] One such strategy is to use surrogate antigens based on Aβ-unrelated protein sequences that may promote Aβ clearance without causing the problems mentioned above.[57] Here we used a similar approach with a unique antigen, oxidized maize Met-rich protein, which has no sequence similarity with Aβ, and observed a significant reduction in plaque burden in the 2×Tg mice (Figure 5C−F). These data suggest that anti-Met(O) antibodies, similar to the one reported previously,[58] were produced in the mice (Figure 5B) and contributed to the observed clearance of deposited Aβ via binding to Aβ-Met(O). This offers an advantage relative to antibodies that recognize Aβ itself because only an aberrant form of Aβ, the one containing Met(O), is targeted.

The immunization likely reduced the overall level of brain oxidative stress using several mechanisms. First, the inflammatory processes surrounding the plaques were relieved by reducing the number of amyloid plaques. A cellular immune response (e.g., activation of microglia) also might have participated in plaque clearance, though exploration of this aspect of the immune response was beyond the scope of this work. Second, the antibodies might have promoted clearance of other oxidized [Met(O)-containing] proteins, resulting in lower levels of overall cellular oxidative damage. Third, because oxidative damage is known to upregulate Aβ production,[59] the decrease in the level of oxidative stress signals might have lowered Aβ production and further facilitated reduction of the Aβ burden.

Msr activity has been shown to decline in post-mortem AD brain[26] yet to increase in cell culture in response to Aβ. Our study presents for the first time evidence showing that Msr levels are elevated in a mouse model of AD at an age in which abundant plaque deposition is observed (Figure 5). These findings could reflect simply a difference between mice and humans. However, if mouse models of AD represent relatively early stages of the disease, our findings suggest that in early AD, Msr activation is one way by which the brain attempts to mitigate oxidative stress, yet this attempt fails in late stages. Testing this hypothesis in human studies will validate the Msr system as a new therapeutic target and may lead to the development of novel treatments for AD that would utilize natural brain defense mechanisms. Such treatments may have a broad impact because oxidative stress is a common deleterious mechanism in many degenerative diseases and in normal aging.

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Funding

The work was supported by National Institute on Aging Grants AG027818 to G.B. and AG027363 to J.M. and by Alzheimer's Association Grant IIRG-07-5833 to G.B.

ACKNOWLEDGMENTS

We thank Dr. David Teplow for the use of his microplate reader.

ABBREVIATIONS

AD, Alzheimer's disease; Aβ, amyloid β-protein; APP, amyloid β-protein precursor; ANOVA, analysis of variance; DMEM, Dulbecco's modified Eagle's medium; DTT, dithiothreitol; FAD, familial AD; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; LDH, lactate dehydrogenase; Msr, methionine-sulfoxide reductase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ThS, thioflavin S.

REFERENCES

(1) Floyd, R. A., and Hensley, K. (2002) Oxidative stress in brain aging. Implications for therapeutics of neurodegenerative diseases. *Neurobiol. Aging 23*, 795−807.

(2) Sultana, R., and Butterfield, D. A. (2010) Role of oxidative stress in the progression of Alzheimer's disease. *J. Alzheimer's Dis. 19*, 341−353.

(3) Halliwell, B. (2001) Role of free radicals in the neurodegenerative diseases: Therapeutic implications for antioxidant treatment. *Drugs Aging 18*, 685−716.

(4) Terman, A., and Brunk, U. T. (2006) Oxidative stress, accumulation of biological 'garbage', and aging. *Antioxid. Redox Signaling 8*, 197−204.

(5) Nunomura, A., Perry, G., Pappolla, M. A., Wade, R., Hirai, K., Chiba, S., and Smith, M. A. (1999) RNA oxidation is a prominent feature of vulnerable neurons in Alzheimer's disease. *J. Neurosci. 19*, 1959−1964.

(6) Selkoe, D. J. (2001) Alzheimer's disease: Genes, proteins, and therapy. *Physiol. Rev. 81*, 741−766.

(7) Hardy, J. A., and Higgins, G. A. (1992) Alzheimer's disease: The amyloid cascade hypothesis. *Science 256*, 184−185.

(8) Roychaudhuri, R., Yang, M., Hoshi, M. M., and Teplow, D. B. (2009) Amyloid β-protein assembly and Alzheimer disease. *J. Biol. Chem. 284*, 4749−4753.

(9) Nunomura, A., Perry, G., Aliev, G., Hirai, K., Takeda, A., Balraj, E. K., Jones, P. K., Ghanbari, H., Wataya, T., Shimohama, S., Chiba, S., Atwood, C. S., Petersen, R. B., and Smith, M. A. (2001) Oxidative damage is the earliest event in Alzheimer disease. *J. Neuropathol. Exp. Neurol. 60*, 759−767.

(10) Hirai, K., Aliev, G., Nunomura, A., Fujioka, H., Russell, R. L., Atwood, C. S., Johnson, A. B., Kress, Y., Vinters, H. V., Tabaton, M., Shimohama, S., Cash, A. D., Siedlak, S. L., Harris, P. L. R., Jones, P. K., Petersen, R. B., Perry, G., and Smith, M. A. (2001) Mitochondrial abnormalities in Alzheimer's disease. *J. Neurosci. 21*, 3017−3023.

(11) Pratico, D. (2008) Evidence of oxidative stress in Alzheimer's disease brain and antioxidant therapy: Lights and shadows. *Ann. N.Y. Acad. Sci. 1147*, 70−78.

(12) Dumont, M., Lin, M. T., and Beal, M. F. (2010) Mitochondria and antioxidant targeted therapeutic strategies for Alzheimer's disease. *J. Alzheimer's Dis. 20* (Suppl. 2), S633−S643.

(13) Younkin, S. G. (1995) Evidence that Aβ42 is the real culprit in Alzheimer's disease. *Ann. Neurol. 37*, 287−288.

(14) Bitan, G., Tarus, B., Vollers, S. S., Lashuel, H. A., Condron, M. M., Straub, J. E., and Teplow, D. B. (2003) A molecular switch in amyloid assembly: Met^{35} and amyloid β-protein oligomerization. *J. Am. Chem. Soc. 125*, 15359−15365.

(15) Näslund, J., Schierhorn, A., Hellman, U., Lannfelt, L., Roses, A. D., Tjernberg, L. O., Silberring, J., Gandy, S. E., Winblad, B., Greengard, P., et al. (1994) Relative abundance of Alzheimer Aβ amyloid peptide variants in Alzheimer disease and normal aging. *Proc. Natl. Acad. Sci. U.S.A. 91*, 8378−8382.

(16) Kuo, Y. M., Kokjohn, T. A., Beach, T. G., Sue, L. I., Brune, D., Lopez, J. C., Kalback, W. M., Abramowski, D., Sturchler-Pierrat, C., Staufenbiel, M., and Roher, A. E. (2001) Comparative analysis of amyloid-β chemical structure and amyloid plaque morphology of transgenic mouse and Alzheimer's disease brains. *J. Biol. Chem. 276*, 12991−12998.

(17) Dong, J., Atwood, C. S., Anderson, V. E., Siedlak, S. L., Smith, M. A., Perry, G., and Carey, P. R. (2003) Metal binding and oxidation

of amyloid-β within isolated senile plaque cores: Raman microscopic evidence. *Biochemistry 42*, 2768−2773.
(18) Boutte, A. M., Woltjer, R. L., Zimmerman, L. J., Stamer, S. L., Montine, K. S., Manno, M. V., Cimino, P. J., Liebler, D. C., and Montine, T. J. (2006) Selectively increased oxidative modifications mapped to detergent-insoluble forms of Aβ and β-III tubulin in Alzheimer's disease. *FASEB J. 20*, 1473−1483.
(19) Choi, J., Sullards, M. C., Olzmann, J. A., Rees, H. D., Weintraub, S. T., Bostwick, D. E., Gearing, M., Levey, A. I., Chin, L. S., and Li, L. (2006) Oxidative damage of DJ-1 is linked to sporadic Parkinson and Alzheimer diseases. *J. Biol. Chem. 281*, 10816−10824.
(20) Ali, F. E., Separovic, F., Barrow, C. J., Cherny, R. A., Fraser, F., Bush, A. I., Masters, C. L., and Barnham, K. J. (2005) Methionine regulates copper/hydrogen peroxide oxidation products of Aβ. *J. Pept. Sci. 11*, 353−360.
(21) Ejiri, S. I., Weissbach, H., and Brot, N. (1979) Reduction of methionine sulfoxide to methionine by *Escherichia coli*. *J. Bacteriol. 139*, 161−164.
(22) Oien, D. B., and Moskovitz, J. (2008) Substrates of the methionine sulfoxide reductase system and their physiological relevance. *Curr. Top. Dev. Biol. 80*, 93−133.
(23) Moskovitz, J., Weissbach, H., and Brot, N. (1996) Cloning the expression of a mammalian gene involved in the reduction of methionine sulfoxide residues in proteins. *Proc. Natl. Acad. Sci. U.S.A. 93*, 2095−2099.
(24) Kim, G., Cole, N. B., Lim, J. C., Zhao, H., and Levine, R. L. (2010) Dual sites of protein initiation control the localization and myristoylation of methionine sulfoxide reductase A. *J. Biol. Chem. 285*, 18085−18094.
(25) Stadtman, E. R., Moskovitz, J., Berlett, B. S., and Levine, R. L. (2002) Cyclic oxidation and reduction of protein methionine residues is an important antioxidant mechanism. *Mol. Cell. Biochem. 234*, 3−9.
(26) Gabbita, S. P., Aksenov, M. Y., Lovell, M. A., and Markesbery, W. R. (1999) Decrease in peptide methionine sulfoxide reductase in Alzheimer's disease brain. *J. Neurochem. 73*, 1660−1666.
(27) Moskovitz, J., Bar-Noy, S., Williams, W. M., Requena, J., Berlett, B. S., and Stadtman, E. R. (2001) Methionine sulfoxide reductase (MsrA) is a regulator of antioxidant defense and lifespan in mammals. *Proc. Natl. Acad. Sci. U.S.A. 98*, 12920−12925.
(28) Pal, R., Oien, D. B., Ersen, F. Y., and Moskovitz, J. (2007) Elevated levels of brain-pathologies associated with neurodegenerative diseases in the methionine sulfoxide reductase A knockout mouse. *Exp. Brain Res. 180*, 765−774.
(29) Oien, D. B., Osterhaus, G. L., Latif, S. A., Pinkston, J. W., Fulks, J., Johnson, M., Fowler, S. C., and Moskovitz, J. (2008) MsrA knockout mouse exhibits abnormal behavior and brain dopamine levels. *Free Radical Biol. Med. 45*, 193−200.
(30) Moskovitz, J., Rahman, M. A., Strassman, J., Yancey, S. O., Kushner, S. R., Brot, N., and Weissbach, H. (1995) *Escherichia coli* peptide methionine sulfoxide reductase gene: Regulation of expression and role in protecting against oxidative damage. *J. Bacteriol. 177*, 502−507.
(31) Chung, H., Kim, A. K., Jung, S. A., Kim, S. W., Yu, K., and Lee, J. H. (2010) The *Drosophila* homolog of methionine sulfoxide reductase A extends lifespan and increases nuclear localization of FOXO. *FEBS Lett. 584*, 3609−3614.
(32) Moskovitz, J., Flescher, E., Berlett, B. S., Azare, J., Poston, J. M., and Stadtman, E. R. (1998) Overexpression of peptide-methionine sulfoxide reductase in *Saccharomyces cerevisiae* and human T cells provides them with high resistance to oxidative stress. *Proc. Natl. Acad. Sci. U.S.A. 95*, 14071−14075.
(33) Butterfield, D. A., and Boyd-Kimball, D. (2005) The critical role of methionine 35 in Alzheimer's amyloid β-peptide (1−42)-induced oxidative stress and neurotoxicity. *Biochim. Biophys. Acta 1703*, 149−156.
(34) Hou, L., Kang, I., Marchant, R. E., and Zagorski, M. G. (2002) Methionine 35 oxidation reduces fibril assembly of the amyloid β-(1−42) peptide of Alzheimer's disease. *J. Biol. Chem. 277*, 40173−40176.
(35) Riek, R., Guntert, P., Döbeli, H., Wipf, B., and Wüthrich, K. (2001) NMR studies in aqueous solution fail to identify significant conformational differences between the monomeric forms of two Alzheimer peptides with widely different plaque-competence, Aβ(1−40)^{ox} and Aβ(1−42)^{ox}. *Eur. J. Biochem. 268*, 5930−5936.
(36) Yan, Y., McCallum, S. A., and Wang, C. (2008) M35 oxidation induces Aβ40-like structural and dynamical changes in Aβ42. *J. Am. Chem. Soc. 130*, 5394−5395.
(37) Maiti, P., Piacentini, R., Ripoli, C., Grassi, C., and Bitan, G. (2010) Surprising toxicity and assembly behavior of amyloid β-protein oxidized to sulfone. *Biochem. J. 443*, 323−332.
(38) Triguero, L., Singh, R., and Prabhakar, R. (2008) Comparative molecular dynamics studies of wild-type and oxidized forms of full-length Alzheimer amyloid β-peptides Aβ(1−40) and Aβ(1−42). *J. Phys. Chem. B 112*, 7123−7131.
(39) Misiti, F., Clementi, M. E., and Giardina, B. (2010) Oxidation of methionine 35 reduces toxicity of the amyloid β-peptide(1−42) in neuroblastoma cells (IMR-32) via enzyme methionine sulfoxide reductase A expression and function. *Neurochem. Int. 56*, 597−602.
(40) Rahimi, F., Maiti, P., and Bitan, G. (2009) Photo-induced cross-linking of unmodified proteins (PICUP) applied to amyloidogenic peptides. *J. Visual Exp.*, 1071 DOI: doi: 10.3791/1071.
(41) Fradinger, E. A., Monien, B. H., Urbanc, B., Lomakin, A., Tan, M., Li, H., Spring, S. M., Condron, M. M., Cruz, L., Xie, C. W., Benedek, G. B., and Bitan, G. (2008) C-terminal peptides coassemble into Aβ42 oligomers and protect neurons against Aβ42-induced neurotoxicity. *Proc. Natl. Acad. Sci. U.S.A. 105*, 14175−14180.
(42) Maiti, P., Lomakin, A., Benedek, G. B., and Bitan, G. (2010) Despite its role in assembly, methionine 35 is not necessary for amyloid β-protein toxicity. *J. Neurochem. 113*, 1252−1262.
(43) Jankowsky, J. L., Slunt, H. H., Gonzales, V., Jenkins, N. A., Copeland, N. G., and Borchelt, D. R. (2004) APP processing and amyloid deposition in mice haplo-insufficient for presenilin 1. *Neurobiol. Aging 25*, 885−892.
(44) Andersson, M., Ostman, A., Westermark, B., and Heldin, C. H. (1994) Characterization of the retention motif in the C-terminal part of the long splice form of platelet-derived growth factor A-chain. *J. Biol. Chem. 269*, 926−930.
(45) Jankowsky, J. L., Fadale, D. J., Anderson, J., Xu, G. M., Gonzales, V., Jenkins, N. A., Copeland, N. G., Lee, M. K., Younkin, L. H., Wagner, S. L., Younkin, S. G., and Borchelt, D. R. (2004) Mutant presenilins specifically elevate the levels of the 42 residue β-amyloid peptide in vivo: Evidence for augmentation of a 42-specific γ secretase. *Hum. Mol. Genet. 13*, 159−170.
(46) Andersson, A., Bohman, S., Borg, L. A., Paulsson, J. F., Schultz, S. W., Westermark, G. T., and Westermark, P. (2008) Amyloid deposition in transplanted human pancreatic islets: A conceivable cause of their long-term failure. *Exp. Diabetes Res. 2008*, 562985.
(47) Ringman, J. M., Fithian, A. T., Gylys, K., Cummings, J. L., Coppola, G., Elashoff, D., Pratico, D., Moskovitz, J., and Bitan, G. (2011) Plasma methionine sulfoxide in persons with familial Alzheimer's disease mutations, submitted for publication.
(48) Garcia-Alloza, M., Robbins, E. M., Zhang-Nunes, S. X., Purcell, S. M., Betensky, R. A., Raju, S., Prada, C., Greenberg, S. M., Bacskai, B. J., and Frosch, M. P. (2006) Characterization of amyloid deposition in the APPswe/PS1dE9 mouse model of Alzheimer disease. *Neurobiol. Dis. 24*, 516−524.
(49) Ripoli, C., Piacentini, R., Riccardi, E., Leone, L., Bitan, G., and Grassi, C. (2011) Synaptotoxic effects of different amyloid β-protein analogues in rodent hippocampal neurons and brain slices, submitted for publication.
(50) Moskovitz, J., and Stadtman, E. R. (2003) Selenium-deficient diet enhances protein oxidation and affects methionine sulfoxide reductase (MsrB) protein level in certain mouse tissues. *Proc. Natl. Acad. Sci. U.S.A. 100*, 7486−7490.
(51) Hansel, A., Kuschel, L., Hehl, S., Lemke, C., Agricola, H. J., Hoshi, T., and Heinemann, S. H. (2002) Mitochondrial targeting of the human peptide methionine sulfoxide reductase (MSRA), an

enzyme involved in the repair of oxidized proteins. *FASEB J. 16*, 911−913.
(52) Marchetti, M. A., Pizarro, G. O., Sagher, D., Deamicis, C., Brot, N., Hejtmancik, J. F., Weissbach, H., and Kantorow, M. (2005) Methionine sulfoxide reductases B1, B2, and B3 are present in the human lens and confer oxidative stress resistance to lens cells. *Invest. Ophthalmol. Visual Sci. 46*, 2107−2112.
(53) Oien, D. B., Shinogle, H. E., Moore, D. S., and Moskovitz, J. (2009) Clearance and phosphorylation of α-synuclein are inhibited in methionine sulfoxide reductase a null yeast cells. *J. Mol. Neurosci. 39*, 323−332.
(54) Schenk, D. B., Seubert, P., Grundman, M., and Black, R. (2005) Aβ immunotherapy: Lessons learned for potential treatment of Alzheimer's disease. *Neurodegener. Dis. 2*, 255−260.
(55) Schenk, D., Barbour, R., Dunn, W., Gordon, G., Grajeda, H., Guido, T., Hu, K., Huang, J., Johnson-Wood, K., Khan, K., Kholodenko, D., Lee, M., Liao, Z., Lieberburg, I., Motter, R., Mutter, L., Soriano, F., Shopp, G., Vasquez, N., Vandevert, C., Walker, S., Wogulis, M., Yednock, T., Games, D., and Seubert, P. (1999) Immunization with amyloid-β attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature 400*, 173−177.
(56) Nicoll, J. A., Wilkinson, D., Holmes, C., Steart, P., Markham, H., and Weller, R. O. (2003) Neuropathology of human Alzheimer disease after immunization with amyloid-β peptide: A case report. *Nat. Med. 9*, 448−452.
(57) Goni, F., Prelli, F., Ji, Y., Scholtzova, H., Yang, J., Sun, Y., Liang, F. X., Kascsak, R., Mehta, P., and Wisniewski, T. (2010) Immunomodulation targeting abnormal protein conformation reduces pathology in a mouse model of Alzheimer's disease. *PLoS One 5*, e13391.
(58) Oien, D. B., Canello, T., Gabizon, R., Gasset, M., Lundquist, B. L., Burns, J. M., and Moskovitz, J. (2009) Detection of oxidized methionine in selected proteins, cellular extracts and blood serums by novel anti-methionine sulfoxide antibodies. *Arch. Biochem. Biophys. 485*, 35−40.
(59) Bayer, T. A., Schafer, S., Breyhan, H., Wirths, O., Treiber, C., and Multhaup, G. (2006) A vicious circle: Role of oxidative stress, intraneuronal Aβ and Cu in Alzheimer's disease. *Clin. Neuropathol. 25*, 163−171.
(60) Moskovitz, J., Berlett, B. S., Poston, J. M., and Stadtman, E. R. (1997) The yeast peptide-methionine sulfoxide reductase functions as an antioxidant in vivo. *Proc. Natl. Acad. Sci. U.S.A. 94*, 9585−9589.