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Oxidative Modification of Glutamine Synthetase by Amyloid Beta Peptide

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β-Amyloid peptide (Aβ), the main constituent of senile plaques and diffuse amyloid deposits in Alzheimer's diseased brain, was shown to initiate the development of oxidative stress in neuronal cell cultures. Toxic lots of Aβ form free radical species in aqueous solution. It was proposed that Aβ-derived free radicals can directly damage cell proteins via oxidative modification. Recently we reported that synthetic Aβ can interact with glutamine synthetase (GS) and induce inactivation of this enzyme. In the present study we present the evidence that toxic Aβ(25–35) induces the oxidation of pure GS *in vitro*. It was found that inactivation of GS by Aβ, as well as the oxidation of GS by metal-catalyzed oxidation system, is accompanied by an increase of protein carbonyl content. As it was reported previously by our laboratory, radicalization of Aβ is not iron or peroxide-dependent. Our present observations consistently show that toxic Aβ does not need iron or peroxide to oxidize GS. However, treatment of GS with the peptide, iron and peroxide together significantly stimulates the protein carbonyl formation. Here we report also that Aβ(25–35) induces carbonyl formation in BSA. Our results demonstrate that β-peptide, as well as other free radical generators, induces carbonyl formation when brought into contact with different proteins.

Keywords: Amyloid peptide, glutamine synthetase, inactivation, protein carbonyls

INTRODUCTION

Amyloid β-peptide (Aβ) is a 39–43-amino acid oligopeptide that is the major component of amyloid deposits in the human brain during normal aging and during the development of Alzheimer's disease (AD).[1,2,3] It was demonstrated that purified peptide components of senile plaques are neurotoxic *in vivo*.[4,5] Different Aβ peptides were synthesized and made commercially available. After several years of investigation it is now accepted that synthetic analogues of Aβ are neurotoxic to cultured neuronal cells.[6–10] The investigation of cytotoxic properties of different fragments of the Aβ sequence localized the toxicity of beta amyloid peptide to its highly hydrophobic portion spanning the residues 25–35.[11] Aβ cytotoxicity usually requires "pre-aging" of the β-peptide in solution for several hours to days before the application to the neuronal cell culture. Only Aβ(25–35) was found to be cytotoxic immediately after dissolving. It was subsequently demonstrated that beta peptides

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[either Aβ(25–35) or Aβ(1–40)] generate ROS in oxygenated solution.[12–14] Aβ(25–35) was shown to incorporate into the hydrocarbon core of model lipid membranes[15] and promote lipid peroxidation *in vitro*.[16] When added to neuronal cell culture, Aβ associates with the plasma membrane and induces Ca^{+2} influx[17] and inactivation of membrane-associated enzymes and cytosolic enzymes.[10,18,19] Short-term treatment of hippocampal cell cultures with amyloid β-peptide was shown to cause the increase of intracellular levels of reactive oxygen species and increase the level of protein oxidation.[10,19] Thus, there is a significant body of evidence which suggests that beta amyloid peptide is a potential prooxidant (for review see 20). It was proposed that oxidative damage of proteins by Aβ-derived radicals may contribute to the mechanism of Aβ-toxicity.[12] However, it is not clear if toxic Aβ can directly oxidize cell proteins, or the excess protein oxidation is a consequence of intracellular ROS production stimulated by Aβ.

Oxidative damage of proteins results in chemical modification of a variety of amino acid residues. Protein carbonyls formed by oxidation of arginine, lysine, threonine or proline residues are often employed as a marker of protein oxidation.[21,22] Thus, the formation of protein carbonyls in proteins able to interact with Aβ *in vitro* will provide direct evidence for the ability of Aβ-generated radicals to induce protein oxidation.

Recently we reported that both Aβ(1–40) and its hydrophobic fragment Aβ(25–35) induce inactivation of sheep brain glutamine synthetase (GS) *in vitro*.[13] In human brain GS (glutamate-ammonia ligase; EC 6.3.1.2) is mainly expressed in astrocytes.[23] Its activity and expression are sensitive to oxidative stress and change significantly in AD.[24–28] Glutamine synthetase either of mammalian or bacterial origin has been well-studied for oxidative modification by ROS-generating systems.[29,30] In the current study we report protein carbonyl formation in pure GS as a result of Aβ(25–35)-induced inactivation of the enzyme.

MATERIALS AND METHODS

Chemicals

Aβ(25–35) was purchased from Bachem Chemicals (Torrance, CA), RBI (Natick, MA), QCB (Hopkinton, MA). Aβ(1–40), Aβ(1–28), Aβ(11–28), Aβ(1–11), Aβ(1–16) were purchased from Bachem Chemicals (Torrance, CA). Aβ(35–25) and scrambled Aβ(25–35) were generous gifts from Athena Neurosciences (San Francisco, CA). All peptides were stored in the dry state at 4°C. Purified sheep brain GS, bovine serum albumin (BSA) and protein standards for electrophoresis were purchased from Sigma (St. Louis, MO). Sulfo-phenyl-*tert*-butyl nitrone (sulfo-PBN) was provided by Centaur Pharmaceuticals, Inc (Sunnyvale, CA).

Glutamine Synthetase Activity Assay

GS activity was determined by the method of Rowe *et al.*[31] as modified by Miller *et al.*[32] and corrected for nonspecific glutaminase activity by comparison in the presence and absence of ADP and arsenate. The specific GS activity is given in units per mg of protein (1 unit = 1 μmol of γ-glutamyl hydroxamate/ 1 min), or as % of control. The results are represented as mean values ± SEM.

Coincubation of β-Amyloid Peptides with GS and Oxidation of GS by Fenton Reagent (Fe^{2+}/H_2O_2)

For coincubation with ovine GS Aβ peptides were solubilized in double-deionized water and immediately mixed with the enzyme and buffer. Coincubation of ovine GS with different Aβ peptides was performed as previously described[33] at 37°C for 1–24 h. The protective effect of sulfo-phenyl-*tert*-butyl nitrone (sulfo-PBN) was estimated with 10 mM final concentration of sulfo-PBN added to the reaction mixture. Protein carbonyl formation during GS/Aβ(25–35) interaction and/or during oxidation of the enzyme by 50 μM $FeSO_4$/1 mM H_2O_2 was studied in 100 mM potassium phosphate buffer, pH 7.2 for 1 hour at 37°C. Oxidation reactions were stopped by addition of deferoxamine mesylate (Sigma) as described elsewhere.[12] The GS concentration was adjusted to 0.14 mg/ml. The concentration of Aβ peptides was 1 mg/ml. The ability of Aβ(25–35) to induce the oxidative modification of protein was also checked with BSA under the same experimental conditions.

Electrophoresis and Western Blot Analysis

To determine the level of protein oxidation an Oxidized Protein Detection Kit (Oxyblot, ONCOR Cat# S7150-Kit) was used. This kit is based on immunochemical detection of protein carbonyl groups derivatized with 2,4-dinitrophenylhydrazine (DNPH).[21,22] The samples were treated with DNPH and derivatization-control solution according to the protocol supplied with the kit before the electrophoresis. After derivatization and neutralization with 2M Tris/30% glycerol (neutralization solution, Oxyblot Kit) plus 19% 2-mercaptoethanol, samples were loaded onto the gel. The oxidized BSA with known concentration of carbonyls (20 nmol of carbonyls/mg of protein) was treated with DNPH and loaded as a standard (1 pmol of protein carbonyl per lane) with each set of the samples. For the standard preparation the BSA (Standard for Gel Filtration Chromatography, Cat# A3581, Sigma) was dissolved in deionized water at 2 mg/ml and oxidized by Fe^{2+}/H_2O_2 (100 μM/1 mM subsequently) for 2 hours at 37°C. The reaction was stopped with deferoxamine mesylate and the small molecular weight substances were removed from the protein by passage through a Sephadex G-25 desalting column. The concentration of carbonyl groups per mg of protein was determined by calorimetric carbonyl assay.[22] SDS-PAGE (12%) was carried out in minislabs (0.75 × 60 × 70 mm) according to method of Laemmli.[34] Gels were stained with Coomasie brilliant blue or transferred on nitrocellulose for futher immunoblotting analysis. Western blotting was performed according the procedure adapted from Glenney.[35] The transfer of proteins after SDS-PAGE on nitrocellulose was completed in two hours. Transfer buffer was Tris-Glycine pH 8.5 with 20% methanol. After transfer, membranes were blocked in 3% BSA (in PBS with sodium azide 0.01% and Tween-20 0.2%) for 1 hour at room temperature. Rabbit anti-DNP antibody from ONCOR oxyblot Kit (1:150 working dilution) was used as a primary antibody. Secondary antibodies (anti-Rabbit IgG conjugated with alkaline phosphatase, Sigma) were diluted in blocking solution 1:15000 and incubated with a membrane for 1 hour at 37°C. Membranes were washed after every step in washing buffer (PBS with 0.01% sodium azide and 0.2% Tween 20) for 10 minutes at room temperature. Washed membranes were developed using BCIP-NBT solution (SigmaFast tablets, Sigma).

Imaging Analysis

Western blots were digitized and quantified by computer assisted imaging using MCID/M4 software supplied by Imaging Research Inc. (Ontario, Canada).

Statistical Analysis

Statistical comparisons were made using ANOVA followed by Dunnett's test for multiple comparisons.

RESULTS

The ability of Aβ-peptides to interact with glutamine synthetase was reported in several recently published papers.[33,36,37] Figure 1 shows the time course of the Aβ(1–40)-mediated inactivation of GS. When co-incubated with Aβ(1–40), GS activity usually starts to decrease after 6–12 hrs of

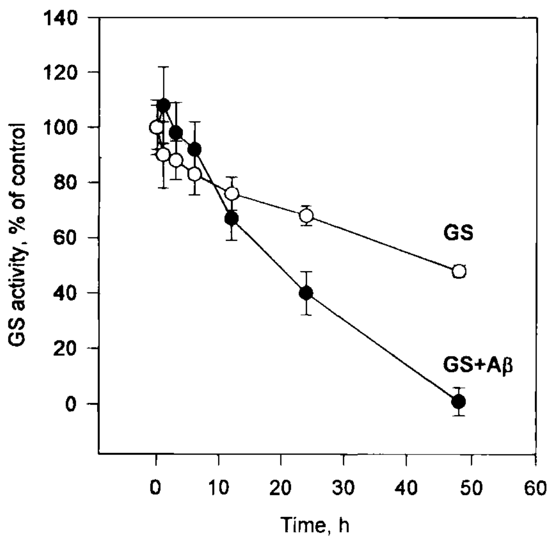

FIGURE 1 Time course of GS inactivation during co-incubation with Aβ(1–40). Results are presented as an average of 3 independent experiments. For each experiment GS activity measurement was performed in duplicate. Error bars indicate standard error of the mean (±SEM) GS activity (expressed as percent of control). GS activity at the beginning of the experiment was 180 ± 6.7 U/mg for GS control. After 48 hrs of incubation GS activity in control samples was 86 ± 4.5 U/mg.

incubation, which coincides with the occurence of EPR-detectable species in Aβ-peptide solutions.[10,12] The data in Figure 2 demonstrate that the 25–35 fragment of Aβ-sequence is essential for the peptide enzyme toxicity. None of the synthetic peptides derived from the hydrophilic part of Aβ was able to induce GS inactivation even after 24 hours of co-incubation with the enzyme. The results presented in Table I show the effect of different lots of Aβ(25–35) on the activity of GS. Different lots of Aβ(25–35) caused from 21% to 72% decrease of GS activity after 1 hour of co-incubation (Table I). It should be noted that several lots of Aβ(25–35) did not induce the inactivation of GS. Lots of Aβ(25–35) unable to inactivate GS were nontoxic to hippocampal cell cultures (Table I) and produced a weak 4-line spectrum or no EPR signal at all with the spin-trapping agent PBN.[13] Scrambled Aβ(25–35) and reversed Aβ(35–25) were not able to inactivate GS (Table I), and none of these peptides pro-

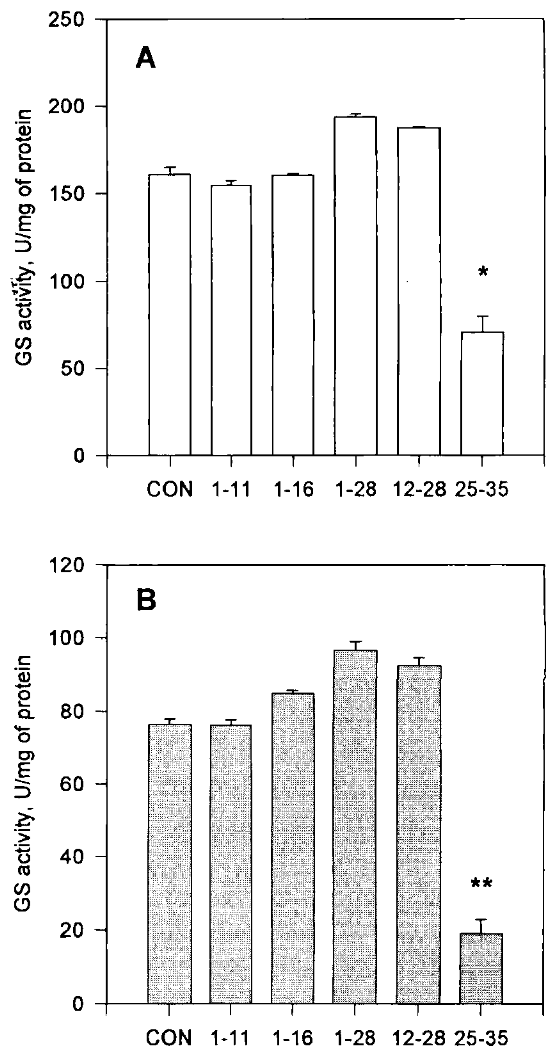

FIGURE 2 GS activity after co-incubation of the enzyme with different Aβ peptides: A- 1 hour incubation, B- 24 hour incubation. This experiment was repeated twice. For each experiment GS activity measurement was performed in triplicate. Error bars represent standard error of the mean (± SEM). *$p < 0.01$ vs. control and **$p < 0.001$ vs. control, ANOVA followed by Dunnett's test.

duced PBN-detected 3-line spectra, in contrast to toxic Aβ(25–35) (data not shown).

The protective effect of sulfo-PBN (a more water-soluble analog of PBN) on the GS activity in cell-free brain extracts treated with Aβ(25–35) (Fig. 3) is consistent with the notion that the free radicals are involved in the process of the enzyme inactivation.

TABLE I Inactivation of GS enzyme upon treatment with Aβ

	Source	Lot and batch number	Number of measurements	GS activity (% of control)
		WJ744**^	15	43.8 ± 2.1
Aβ(25–35)(toxic)*	Bachem	ZJ744^	15	20.7 ± 3.4
		ZK600**	12	49 ± 3.3
		ZL6502	12	38.3 ± 2.5
		ZL650^	12	31 ± 3.1
		BOO961	9	60.3 ± 5.7
		WL650**	9	38.7 ± 3.6
	QCB	01014008**^	9	72 ± 1.2
		Average for different lots of Aβ(25–35):		44.2 ± 5.7
Aβ(25–35) (non-toxic)*	Bachem	ZL744#221	6	137.5 ± 2.3
		ZL744#210	6	116 ± 2.6
		ZL650DNPE#276**	6	127.5 ± 2.6
	RBI	DKL-195A**^	9	147.7 ± 2.7
	Athena	2292	3	100 ± 2.1
		2256	3	102 ± 1.8
		Average for different lots of Aβ(25–35):		121.8 ± 7.9
Aβ(35–25) (reversed)	Bachem	ZL817	6	94 ± 2.6
	Athena	a gift	9	97 ± 2.3
Aβ(25–35) (scrambled)	Athena	a gift	6	94 ± 2.0

* Lots of Aβ(25–35) were considered "toxic" or "non-toxic" if they were able or unable to cause significant decrease of GS activity in vitro. **Indicated lots of Aβ(25–35) were simultaneously checked for the ability to inactivate GS and for the cytotoxicity to hippocampal cell cultures. The ability of the particular batch of Aβ(25–35) to inactivate GS always correlated with its ability to produce free radicals and induce the cell damage.
^ The loss of GS protein (CBB staining and/or Anti-GS immunostaining) was estimated when the enzyme was coincubated with indicated lots of Aβ(25–35).

An increase of carbonyl content in pure GS treated by Aβ would provide direct evidence for Aβ-associated free radicals to cause oxidative modification of the protein. The immunochemical technique for protein carbonyl determination provides the possibility to assess the carbonyl formation in the samples with relatively low protein concentration and when the volume of the sample available for analysis is restricted. Aβ(25–35) was chosen for these experiments because the significant inactivation of GS with this Aβ peptide could be observed within 1–2 hr. In addition, this fragment of Aβ sequence does not contain amino acids residues, which may be transformed to carbonyl derivatives as a result of self-oxidation of the peptide.

A small amount of fragmentation and cross-linking was detected after metal-catalyzed oxidation of bacterial GS.[38] It was observed previously that the inactivation of GS caused by Aβ (either Aβ(25–35) or Aβ(1–40)) is accompanied by loss of a significant amount of GS protein.[33] Thus, the inactivation of GS by Aβ or oxidation of GS by iron/peroxide might lead to the decrease of 43 kDa GS protein content and might induce the formation of additional protein bands as a result of GS fragmentation or cross-linking. To control the changes of the GS protein during treatment with toxic Aβ or during treatment with iron/peroxide, Western blot analysis for GS immunoreactivity was performed together with Western blot analysis for protein carbonyl formation.

The GS protein carbonyl formation in the GS + Aβ(25–35) samples, in the GS + Fe^{2+}/H_2O_2 samples, in GS + Aβ(25–35)/Fe^{2+}/H_2O_2, and in con-

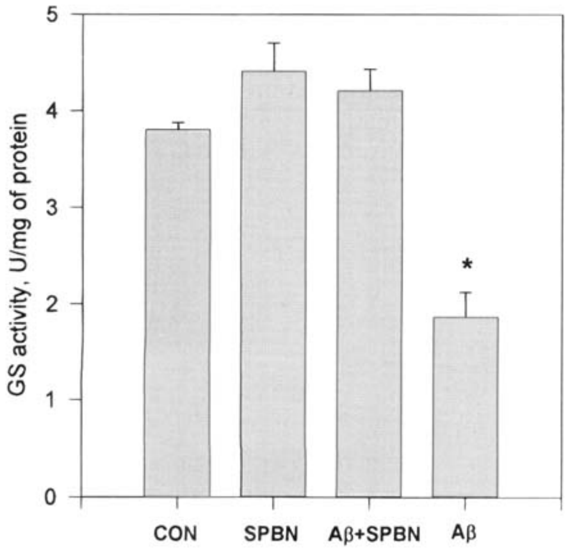

FIGURE 3 Sulfo-PBN protects GS from the "toxic" Aβ(25–35). 10 mM Sulfo-PBN was co-incubated for 1 hour with the GS-Aβ mixture. This experiment was repeated twice. For each experiment GS activity measurement was performed in triplicate. Error bars represent standard error of the mean (± SEM). *$p < 0.01$ vs. control, ANOVA followed by Dunnett's test.

trol GS was estimated by scanning and digitizing of Anti-DNP/Anti-GS positive 43 kDa band on Western blots. The treatment of sheep brain GS with "toxic" Aβ(25–35), as well as the oxidation of GS by iron/peroxide, led to an increase of the carbonyl content (Fig. 4A, Fig. 5). "Non-toxic" Aβ(25–35) did not promote the oxidation of the enzyme (Fig. 4B). The significant stimulation of carbonyl formation was observed upon treatment of GS with "toxic" Aβ(25–35) mixed with Fenton reagent (Fig. 6).

The quantitative data from Western blots were normalized to the immunoreactive GS content (Anti-DNP stain density per Anti-GS stain density) and presented as % of control (Fig 7A). The protein carbonyl content in GS treated with "toxic" Aβ(25–35) was found more than twice that of control (264 ± 36%). The oxidation of GS by Fe^{2+}/H_2O_2 increased the carbonyl content 1.4 times compared to control (147 ± 10%). The addition of Aβ(25–35) and Fe^{2+}/H_2O_2 together to pure GS led to a 10-fold increase in carbonyl content (998/±30%). The dramatic increase of the carbonyl formation in GS co-incubated with Aβ(25–35)/ Fe^{2+}/H_2O_2 was not accompanied by the same dramatic loss of the enzyme activity. The GS activity in samples treated with Aβ(25–35), Fe^{2+}/H_2O_2, and Aβ(25–35)/Fe^{2+}/H_2O_2 was 72 ± 1.2%, 77 ± 0.3%, and 66 ± 0.5% of control subsequently (Fig. 7B).

To check the ability of Aβ(25–35) to induce oxidation of other proteins we co-incubated BSA with the β-peptide. It was observed that BSA treated with Aβ(25–35) contains more reactive carbonyl groups than control (Fig. 8). No increase of protein carbonyls was observed in BSA incubated with "non-toxic" lot of Aβ(25–35) (data not shown). Thus, β-peptide, as well as other free radical generators, induces carbonyl formation when brought into contact with different proteins.

DISCUSSION

The results presented here demonstrate that interaction of toxic Aβ with proteins mimics one of the most important characteristics of enzymic and nonenzymic metal ion-catalyzed oxidation (MCO) systems: it causes the conversion of some amino acid residues to carbonyl derivatives. This is the first demonstration that Aβ is able to produce oxidative damage in proteins in a simple *in vitro* cell-free system containing only amyloid beta peptide, the protein of interest, and water or appropriate buffer. When the prooxidant abilities of β-peptides were studied in complex systems like cell cultures or even cell-free membraneous preparations or tissue extracts, it always could be argued that the excess protein oxidation produced by Aβ was due to stimulation of ROS-generating enzymes and/or due to the increased lipid peroxidation, but not due to the direct interaction of peptidyl radicals with proteins. In contrast to MCO systems, Aβ does not need iron or peroxide to be added to the sample to produce free radical species[12] and/or to induce the GS

A

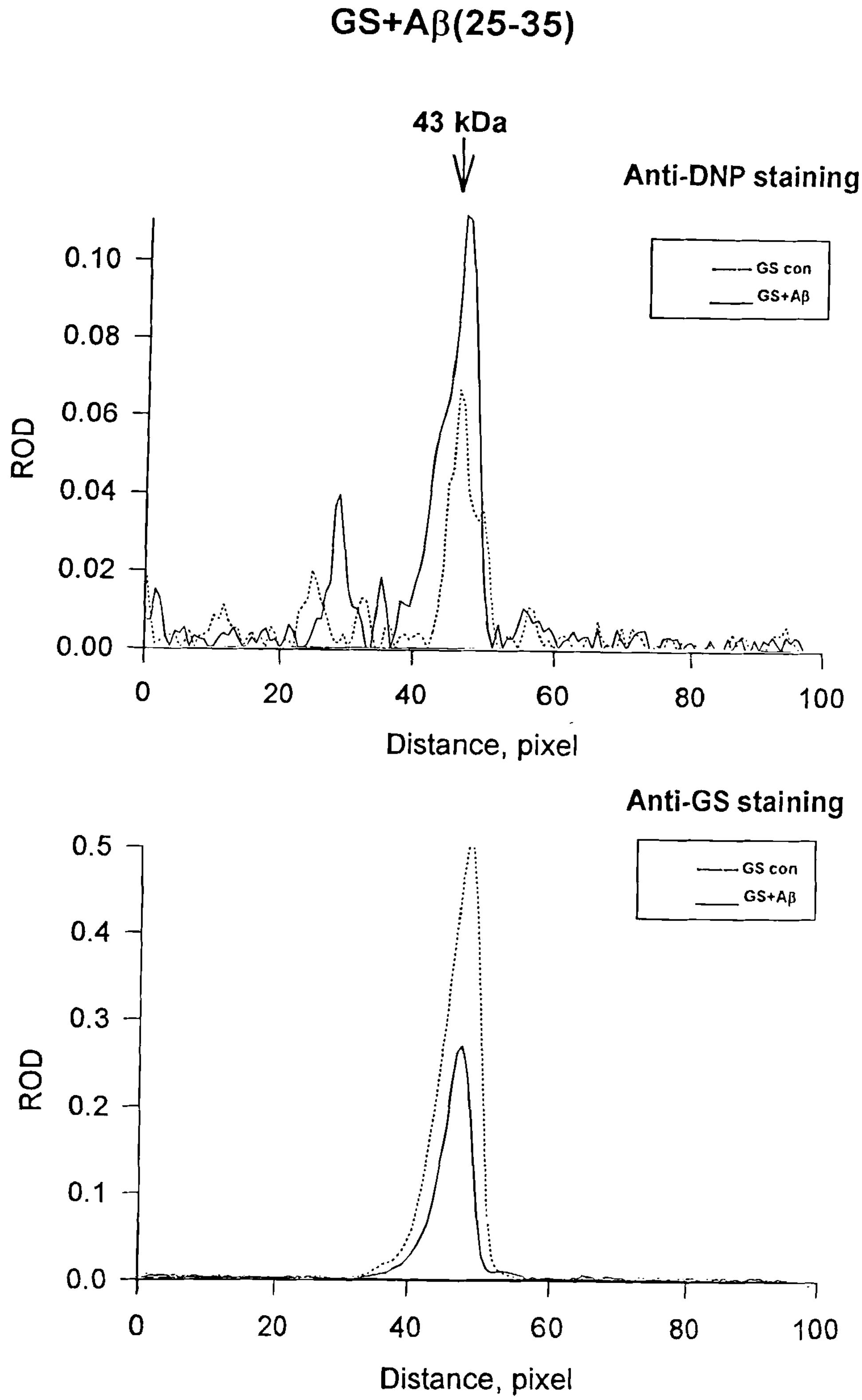

FIGURE 4 A, B Typical scan of anti-DNP- and anti-GS- stained Western blots of GS co-incubated with "toxic" Aβ(25–35) (**A**) or "non-toxic" Aβ(25–35) (**B**). For each trial the Western analysis was repeated 3 times and the number of trials was 3 for "toxic" and 2 for "non-toxic" Aβ(25–35).

B

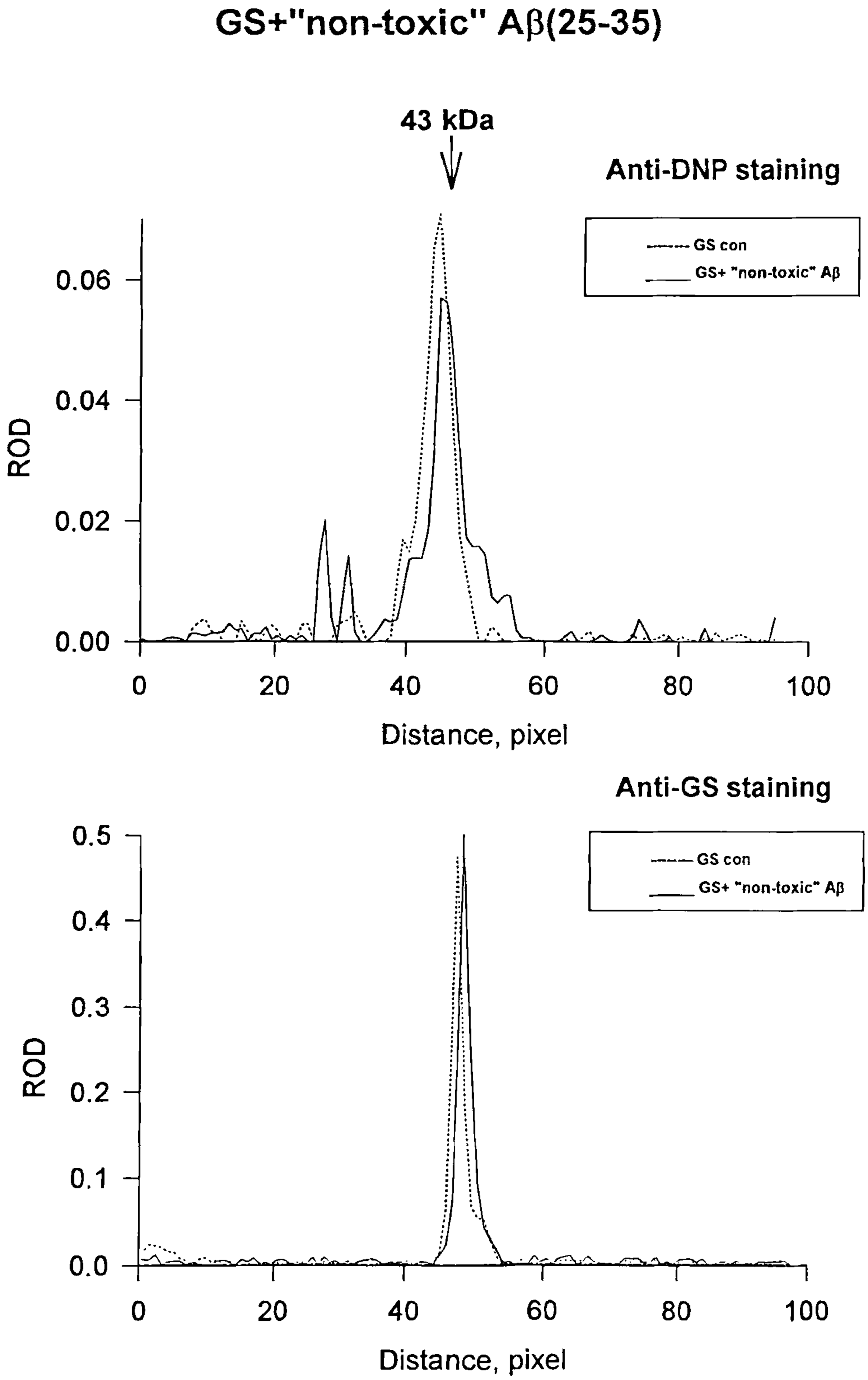

FIGURE 4 (*Continued*)

$GS+/Fe^{2+}/H_2O_2$

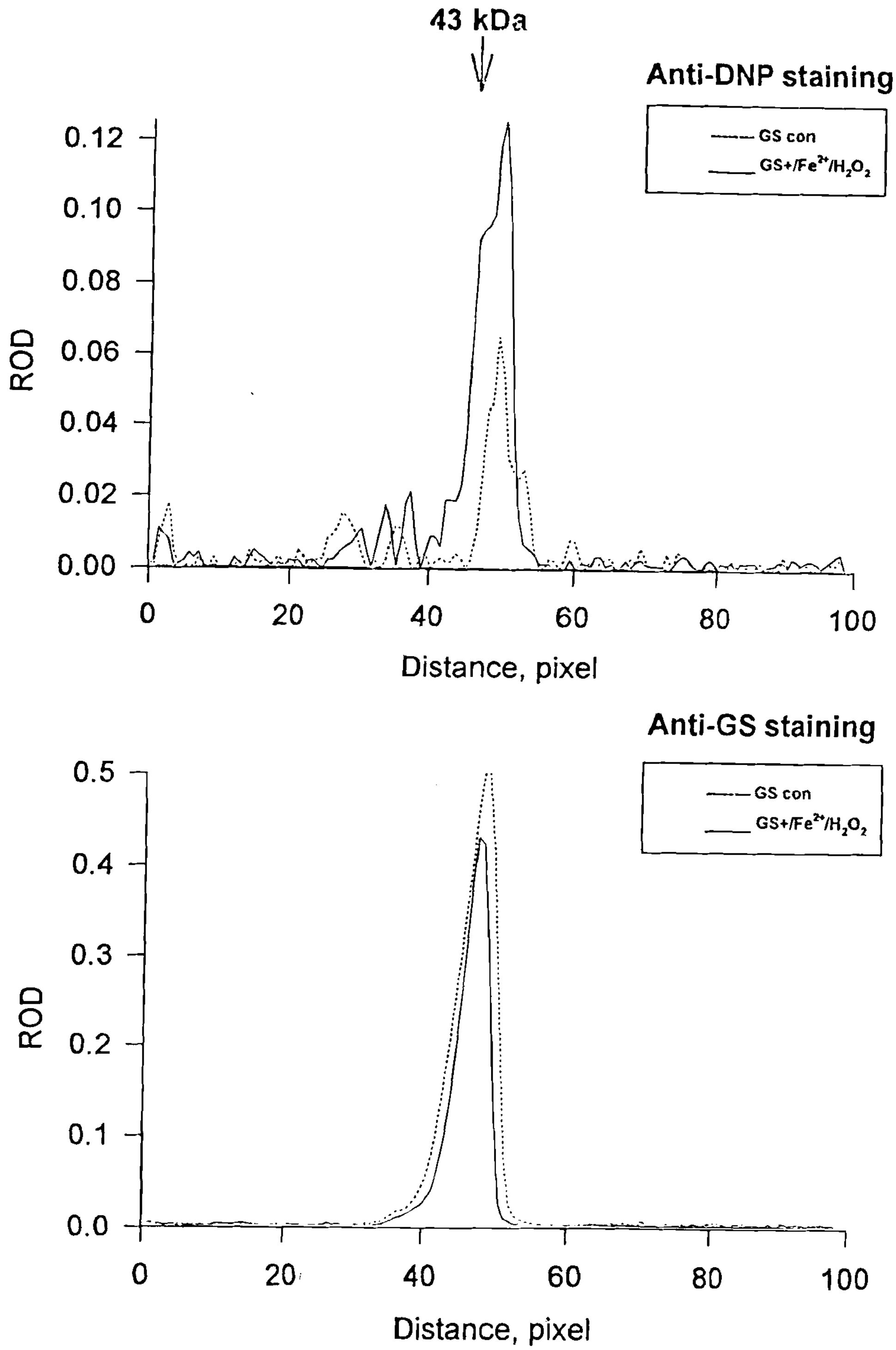

FIGURE 5 Typical scan of anti-DNP- and anti-GS-stained Western blots of GS co-incubated with iron/peroxide. For each trial the Western analysis was repeated 3 times and the number of trials was 3.

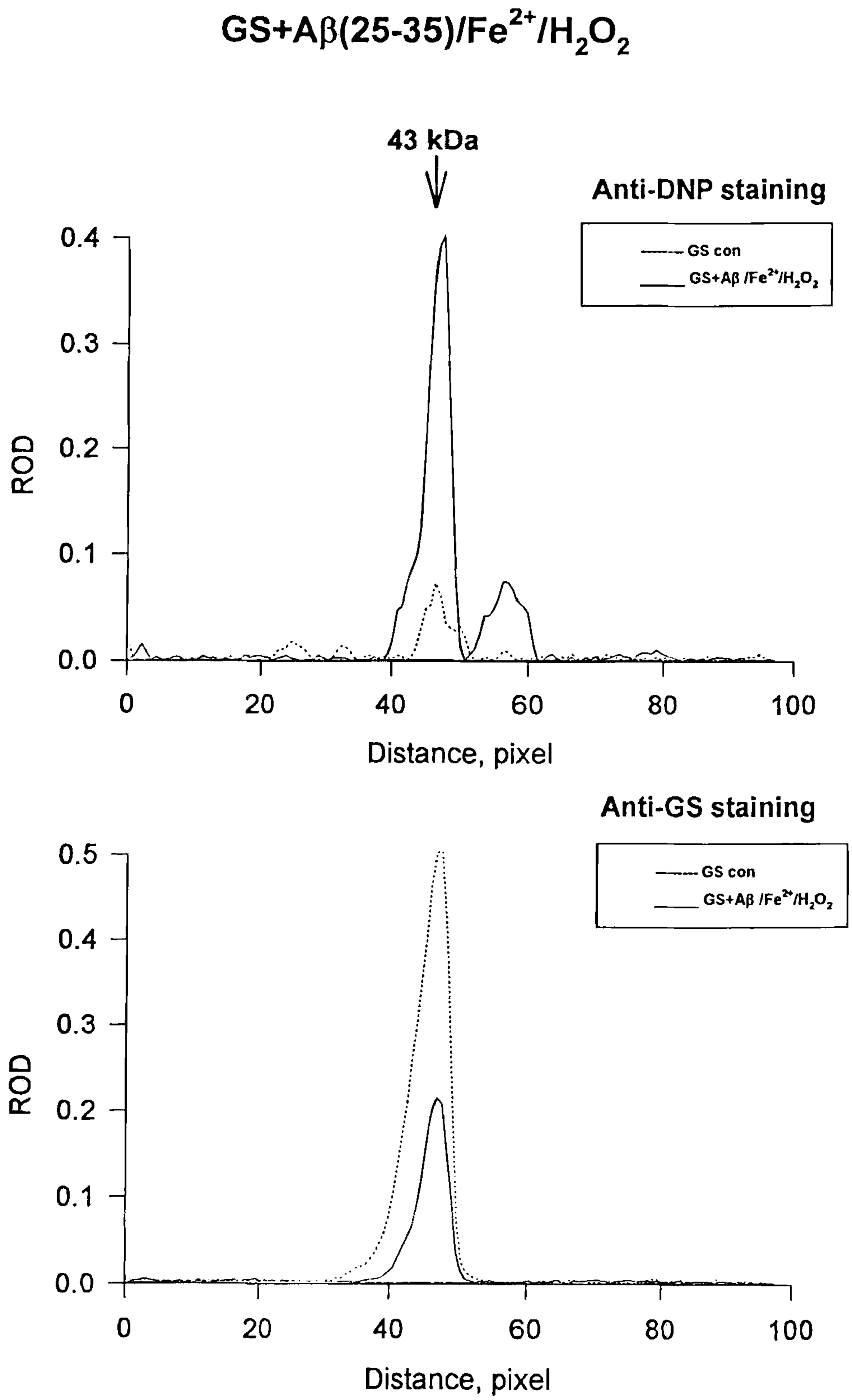

FIGURE 6 Typical scans of anti-DNP- and anti-GS-stained Western blots of GS co-incubated with "toxic" Aβ(25–35) + iron/peroxide mixture. For each trial the Western analysis was repeated 3 times and the number of trials was 3.

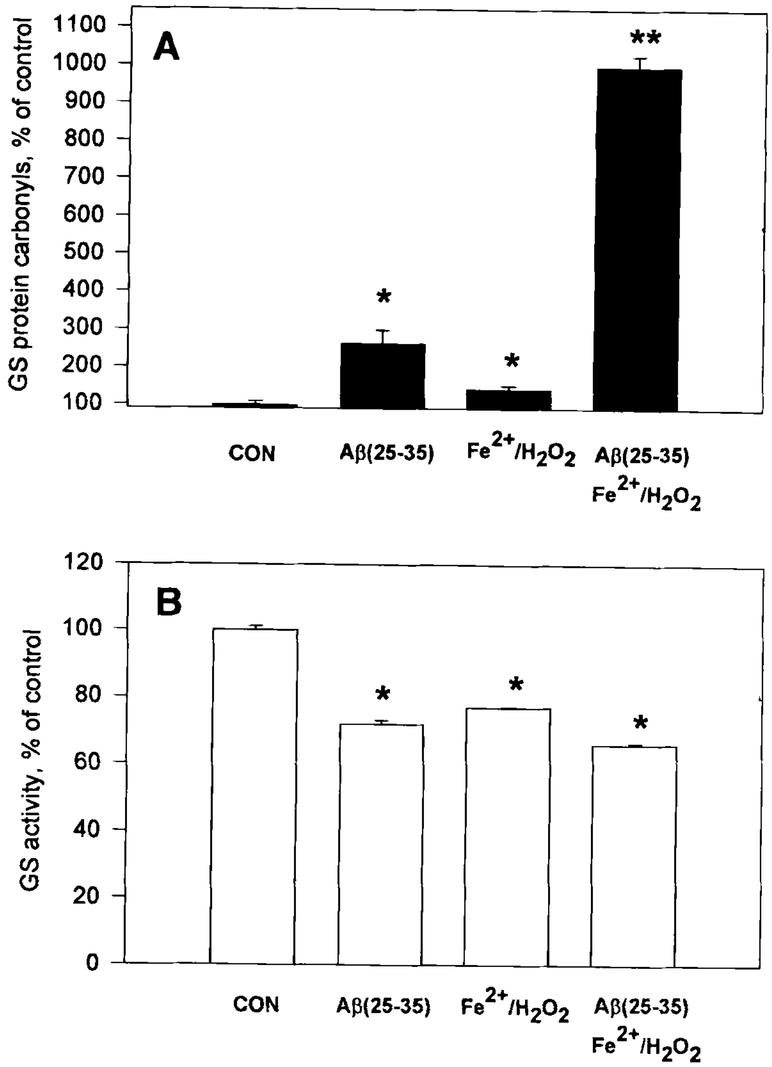

FIGURE 7 The relative changes of GS protein carbonyl content (**A**) and the enzyme activity (**B**) in the samples of GS co-incubated for 1 hour with "toxic" Aβ(25–35), iron/peroxide mixture, or Aβ(25–35) + iron/peroxide together. **A.** GS protein carbonyl content (the density of Anti-DNP stain) was normalized to the immunoreactive GS content (the density of Anti-GS stain) and expressed as a % of control ± SEM. Results are presented as an average for 3 different trials. For each trial Anti-DNP staining were performed 3 times and averaged. *$p < 0.01$ vs. control, ANOVA followed by Dunnett's test. **$p < 0.005$ vs. control, ANOVA followed by Dunnett's test. **B.** GS activity data are given as a % of control (GS alone after 1 hour incubation). The data presented in this figure were obtained using Aβ(25–35) from the lot #01014008 (QCB) (see also Table I). *$p < 0.01$ vs. control, ANOVA followed by Dunnett's test.

inactivation.[33,37] Attempts to inhibit Aβ-radical formation with different chelators were unsuccessful, but it was effectively prevented by sparging buffer with nitrogen.[12] Here we report that the presence of iron and/or peroxide is not essential for the ability of "toxic" Aβ(25–35) to cause protein oxidation, but the treatment of GS with the peptide, iron and peroxide together significantly stimulates the protein carbonyl formation. This results are consistent with the reports about the increase of Aβ toxicity toward neuronal cells in presence of iron or peroxide.[39]

EPR-detectable Aβ/PBN reaction products are stable nitroxides or hydronitroxides formed from

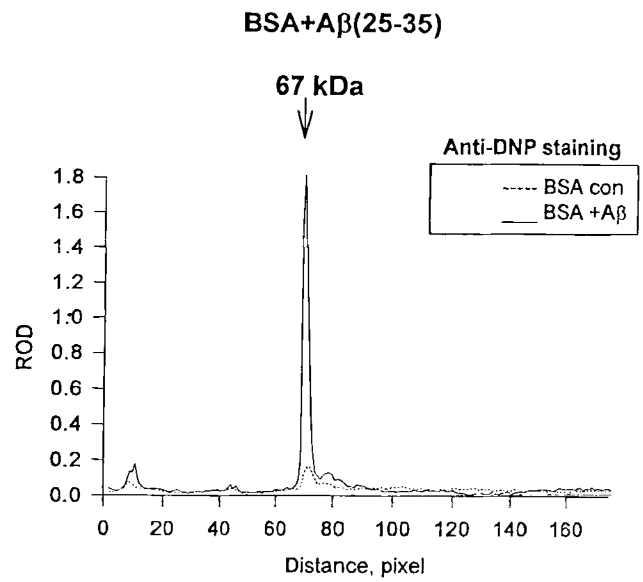

FIGURE 8 Typical scan of Anti-DNP-stained Western blot of BSA co-incubated with "toxic" lot of Aβ(25–35). Number of trials was 3.

peptide-mediated cleavage of the PBN nitrone bond.[14] This pattern of reactivity is consistent with the hypothesis of a peptidyl peroxy radical species.[14,40] The chemical mechanism by which Aβ generates peptidyl peroxy radicals in the solution is unclear. The formation of a quasi-stable radical center in the polypeptide molecule would require H abstraction by OH· from the amino acid residues, such as leucine, isoleucine, lysine, proline or valine.[40] It was proposed that hydroxyl radical could be generated in synthetic Aβ-preparations during synthesis and lyophilization procedures.[14] This would explain how peptidyl radical centers could be formed without the addition of any extra OH·-generators. Our results demonstrate that the presence of an additional source of OH· significantly increases the prooxidant ability of "toxic" Aβ(25–35). It is possible that in AD brain hydrogen peroxide produced by activated microglia and increased free iron concentration[41] may play a role of OH·-generating system, which enhances prooxidant properties of Aβ peptides and inflates its neurotoxicity.

The treatment of GS with beta peptide caused almost the same decrease of enzyme activity as the treatment with iron/peroxide, but the increase of protein carbonyl content was higher with Aβ. The inactivation of bacterial GS by MCO was shown to be site-specific.[30,42] According to the "site-specific" free radical mechanism of metal-catalyzed inactivation of bacterial GS,[38] Fe^{2+} binds to a divalent cation binding site of the enzyme. Oxidation of the protein-bound Fe^{2+} then generates one or more forms of activated oxygen, which react with residues at the site of generation. Therefore, the increase in carbonyl content correlates well with the modification of amino acid residues essential for GS activity (histidine in the metal-binding site) and with the grade of the inactivation. The introduction of carbonyls into the side chains of other than histidine amino acids is more slow. Though sheep-brain GS is much different from bacterial GS, it is likely that the inactivation of mammalian enzyme by iron/peroxide system goes according to the same mechanism. In our experiments a 1.3-times decrease of GS activity after iron/peroxide treatment was accompanied by a 1.4 increase of GS protein carbonyl content. In contrast to the inactivation of GS caused by iron/peroxide system, the interaction of GS with

Aβ(25–35) could be not site-specific. Aβ-generated radicals might react with different amino acid residues of GS, not necessarily essential for the enzyme activity.

Recently it was observed that the interaction of GS with Aβ(1–40) drastically decreases the ability of the enzyme to react with the sulfhydryl-specific thiosulfonate spin label MTS. This result could reflect a loss of GS-resident thiols due to their oxidation by Aβ-generated free radicals and/or a collapse of enzyme structure into a more compact arrangement following the Aβ-treatment with concomitant decrease in accessibility of thiol groups.[36] Together with the increase of protein carbonyl content, the decrease of reactive SH-groups is very common for oxidatively modified proteins. Thus, the inactivation of GS by Aβ peptides *in vitro* is a consequence of the direct oxidation of amino acids residues of the enzyme.

The activity of GS in brain tissue of AD patients was shown to be much lower in brain regions rich in Aβ-containing senile plaques.[43] GS was found to be present in CSF,[44] and its content was reported to be increased in AD.[45] It is conceivable that GS can be released from astrocytes, and that the released enzyme can interact with Aβ peptide *in vivo*. GS isolated from the brain of an Alzheimer's disease-afflicted subject exhibited enzyme structural compromise similar to that seen in an experimental hydroxyl free radical oxidative stress treatment.[36] Changes in GS structure after co-incubation with Aβ resembled structural changes in GS purified from AD brain.[36] The fact that GS in AD brain might be subjected to oxidative modification allows to suggest that Aβ-mediated oxidative damage can contribute to the decrease of GS activity in AD.

Aβ can bind to a variety of protein components of brain tissue, CSF or plasma. In the present study we also report that Aβ(25–35) can oxidize BSA as well as GS. This result suggests that Aβ-derived peptidyl radicals might induce the oxidative modification of a wide spectrum of proteins able to interact with β-amyloid. It was proposed by several authors that Aβ-peptide induces neuronal cell damage via oxidative mechanism.[20,46–48] Direct oxidation of proteins by Aβ-generated free radicals might contribute to the increase of protein carbonyl content observed in AD brain autopsies and in cultured neurons treated with toxic Aβ.[48] Together with Aβ-mediated lipid peroxidation, disruption of Ca^{2+} homeostasis, mitochondrial disfunction and activation of oxidative stress-related signaling pathways, direct oxidative modification of brain proteins by Aβ-radicals may be a part of the molecular basis of oxidative stress in AD.

Acknowledgments

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