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Oxidative modification and down-regulation of Pin1 in Alzheimer's disease hippocampus: A redox proteomics analysis

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Abstract

Alzheimer disease (AD) is characterized neuropathologically by intracellular neurofibrillary tangles (NFT) and of extracellular senile plaques (SP), the central core of which is amyloid beta-peptide (Aβ) derived from amyloid precursor protein (APP), a transmembrane protein. AD brain has been reported to be under oxidative stress that may play an important role in the pathogenesis and progression of AD. The present proteomics study is focused on identification of a specific target of protein oxidation in AD hippocampus that has relevance to the role of oxidative stress in AD. Here, we report that the protein, Pin1, is significantly down-regulated and oxidized in AD hippocampus. The identity of Pin1 was confirmed immunochemically. Analysis of Pin1 activity in AD brain and separately as oxidized pure Pin1 demonstrated that oxidation of Pin1 led to loss of activity. Pin1 has been implicated in multiple aspects of cell cycle regulation and dephosphorylation of tau protein as well as in AD. The in vivo oxidative modification of Pin1 as found by proteomics in AD hippocampus in the present study suggests that oxidative modification may be related to the known loss of Pin1 isomerase activity that could be crucial in AD neurofibrillary pathology. Taken together, these results provide evidence supporting a direct link between oxidative damage to neuronal Pin1 and the pathobiology of AD.

Keywords: Alzheimer's disease; Tau protein; Peptidyl prolyl *cis–trans* isomerase (PPIase); Hippocampus; Oxidative stress

1. Introduction

Alzheimer disease (AD) brain is characterized neuropathologically by intracellular neurofibrillary tangles (NFT), extracellular senile plaques (SP) [consisting of a core of amyloid beta-peptide (Aβ), surrounded by dystrophic neurites], and synapse loss. Aβ is derived from amyloid precursor protein (APP, a transmembrane protein) [26]. NFT are largely composed of hyperphosphorylated tau and neurofilament proteins, both of which are important members of the neuronal cytoskeleton [22].

Hippocampal nuclei are intimately involved in memory processing, and this area of the AD brain is heavily involved pathologically in this dementing disorder [5]. AD brain has been reported to be under oxidative stress that may play an important role in the pathogenesis and progression of AD, manifested by protein oxidation, lipid peroxidation, DNA

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oxidation, advanced glycation end products, and ROS formation [9,10,38]. ROS can facilitate different kinds of protein oxidation [52]. Protein carbonyls (aldehydes and ketones, PCO) can arise from direct oxidation of aminoacid side chains (His, Pro, Arg, Lys, and Thr, etc.), by oxidative cleavage of proteins via the α-amidation pathway, or by Michael addition reactions of α-, β-unsaturated aldehydes, such as 4-hydroxy-2-nonenal, malondialdehyde and 2-propenal (acrolein), derived from lipid peroxidation [11]. PCO are the most commonly used markers of protein oxidation. Moreover, elevated levels of PCO are generally associated not only with oxidative stress, but also with disease-resident protein dysfunction [23]. Protein oxidation has been shown to play a role in the pathophysiology of AD [9,10,23,43,48].

The present study was focused on identification of specific targets of protein oxidation in AD hippocampus using a proteomics approach by which we previously demonstrated a role of oxidative stress in mechanisms of neurodegeneration [7,8,12–15]. Previously we have used proteomics methods (reviews: [7,8]) to successfully identify specific targets of protein oxidation in the inferior parietal lobule (IPL) of AD brain. Oxidatively modified proteins were classified into those involved in pathways of energy metabolism, excitotoxicity, proteasomal dysfunction, phospholipid asymmetry, cholinergic dysfunction, and neuritic abnormalities, consistent with biochemical and pathological alterations in AD. In all the cases examined thus far, oxidative modification of proteins is associated with loss of function [14,23,31,54], suggesting a possible link between oxidative stress of key proteins and mechanisms for neurodegeneration in AD brain. Identification of oxidatively modified proteins is crucial for establishing a relationship between oxidative modification and neuronal death and may help lead to development of a therapeutic approaches to protect the brain against oxidatively mediated degenerative processes.

In the current study, a similar approach used in IPL applied to AD hippocampus revealed oxidation of a key protein known to be of involved in function in AD [34].

2. Materials and methods

2.1. AD and control brains

Frozen hippocampal samples were obtained from six AD patients and from six age-matched controls. The Rapid Autopsy Program of the University of Kentucky Alzheimer's Disease Research Center (UK ADRC) resulted in extremely short postmortem intervals (PMIs) (Table 1). Indeed, the mean PMI for both AD and control brain was less than 3 h. This short PMI is an advantage in redox proteomics, since oxidative modification likely reflects the intrinsic situation in AD brain rather than PMI-related artifacts. All AD subjects displayed progressive intellectual decline and met NINCDS-ADRDA Workgroup criteria for the clinical diagnosis of probable AD [40]. Hematoxylin–eosin and modified Bielschowsky staining and 10-D-5, ubiquitin, and α-synuclein immunohistochemistry were used on multiple neocortical, hippocampal, entorhinal, amygdala, brainstem, and cerebellum sections for diagnosis. Some AD patients were also diagnosed with dementia with Lewy bodies, but the results of this study were no different from AD patients with or without the presence of Lewy bodies. Control subjects, who underwent annual mental status testing as a part of the UK ADRC normal volunteer longitudinal aging study, did not have a history of dementia or other neurologic disorders. All control subjects had test scores in the normal range. Neuropathologic evaluation of control brains revealed only age-associated gross and histopathologic alterations.

Table 1
Demographic characteristics of subjects

Sample ($n=6$)	Age (years)	Gender (M/F)	Post-mortem interval (h)
Control	85.8 ± 4.1	4/2	2.9 ± 0.23
AD	84.5 ± 5.2	4/2	2.1 ± 0.47

2.2. Sample preparation

Brain samples were minced and suspended in 10 mM HEPES buffer (pH 7.4) containing 137 mM NaCl, 4.6 mM KCl, 1.1 mM KH_2PO_4, 0.6 mM $MgSO_4$ and proteinase inhibitors: leupeptin (0.5 mg/ml), pepstatin (0.71 μg/ml), type II S soybean trypsin inhibitor (0.5 lg/ml), and PMSF (40 lg/ml). Homogenates were centrifuged at 14,000 × *g* for 10 min to remove debris. Protein concentration in the supernatant was determined by the BCA method (Pierce, Rockford, IL, USA).

2.3. Two-dimensional electrophoresis

One hundred and fifty micrograms of samples were incubated at room temperature for 30 min in four volumes of 10 mM 2,4-dinitrophenylhydrazine (DNPH) in 2 M HCl for protein carbonyl derivatization/oxyblots or 2 M HCl for gel maps and mass spectrometry analysis, according to the method of Levine et al. [33], followed by precipitation of proteins by addition of ice-cold 100% trichloroacetic acid (TCA) to a final concentration of 15% for 10 min on ice. Precipitates were centrifuged for 2 min at 14,000 × *g* at 4 °C. The pellet was washed with 500 μl of 1:1 (v/v) ethyl acetate/ethanol three times. The final pellet was dissolved in rehydration buffer (8 M urea, 2 M thiourea, 2% CHAPS, 0.2% (v/v) biolytes, 50 mM dithiothreitol (DTT), and bromophenol blue). Samples were sonicated in rehydration buffer on ice three times for 20 s intervals and were applied to a ReadyStrip IPG (pH 3–10) (Bio-Rad). The strip was then actively rehydrated at 50 V for 16 h in protean IEF cell (Bio-Rad). Isoelectric focusing was performed at 20 °C as follows: 800 V for 2 h linear gradient, 1200 V for 4 h slow gradient, 8000 V for 8 h linear gradient, and 8000 V for 10 h rapid gradient. The strips were stored at −80 °C until second dimension electrophoresis was performed. Gel strips were equilibrated

for 10 min in 50 mM Tris–HCl (pH 6.8) containing 6 M urea, 1% (w/v) sodium dodecyl sulfate, 30% (v/v) glycerol, and 0.5% dithiothreitol, and followed by re-equilibration for 10 min in the same buffer containing 4.5% iodoacetamide in place of dithiothreitol prior to electrophoreses in the second dimension. Linear Gradient Precast criterion Tris–HCl gels (8–16%; Bio-Rad) were used to perform second dimension electrophoresis. Precision protein standards (Bio-Rad) were run along with the sample at 200 V for 65 min.

2.4. SYPRO Ruby staining

The gels were fixed in a solution containing 10% (v/v) methanol, 7% (v/v) acetic acid for 20 min and stained overnight at room temperature with agitation in 50 ml of SYPRO Ruby gel stain (Bio-Rad, Hercules, CA, USA). The gels were placed in deionized water overnight and scanned.

2.5. Oxyblot

For immunoblotting analysis, DNPH derivatized samples were electrophorized as described in sample preparation followed by transfer to a nitrocellulose membrane (Bio-Rad) using the Transblot-Blot SD Semi-Dry Transfer Cell at 45 mA per gel for 2 h. The membranes were blocked with 3% bovine serum albumin (BSA) in phosphate buffered saline containing 0.01% (w/v) sodium azide and 0.2% (v/v) Tween 20 (PBST) at 4 °C for 1 h. The membranes were incubated with anti-2,4-dinitrophenylhydrazone (DNP) polyclonal antibody (1:100) in PBST for 2 h at room temperature with gentle rocking. Following washing, the blots three times in PBST for 5 min each, the anti-rabbit IgG alkaline phosphatase secondary antibody (1:3000) in PBST was incubated 2 h at room temperature. The membranes were washed in PBST three times for 5 min and developed using Sigma-Fast [5-bromo-4-chloro-3-indolyl-phosphate/nitroblue tetrazolium (BCIP/NBT)] tablets. Blots were dried and scanned with Adobe Photoshop.

2.6. Western blotting

Proteins were first separated by 2D gel electrophoresis followed by protein transfer to nitrocellulose membrane. Blot was stained with Ponceau S and scanned with Adobe Photoshop. Membrane was then washed with PBST to remove the stain. The membrane was blocked with 3% bovine serum albumin (BSA) in PBST for 1 h at 4 °C. The membrane was incubated with anti-Pin1 polyclonal antibody (Stressgen, CA) (1:1000) for 2 h in 1% BSA for 2 h at room temperature, and then the membrane were washed three times in PBST for 5 min each. An anti-rabbit IgG alkaline phosphatase secondary antibody was diluted 1:3000 in PBST and incubated with the membranes for 1 h at room temperature. The membranes were washed in PBST three times for five minutes and developed using Sigmafast Tablets (BCIP/NBT substrate).

2.7. Image analysis

The gels and nitrocellulose membranes were scanned and saved in TIFF format using Scanjet 3300C (Hewlett Packard, Palo Alto, CA, USA). PDQuest software (Bio-Rad, Hercules, CA, USA) was used to compare protein expression and protein oxidation in the 2D-gels and 2D-oxyblots, respectively, between control and AD subjects. Protein expression was measured using SYPRO Ruby-stained gels that were scanned using a UV transilluminator ($\lambda_{ex} = 470$ nm, $\lambda_{em} = 618$ nm, Molecular Dynamics, Sunnyvale, CA, USA). Oxyblots, used to measure carbonyl immunoreactivity, were scanned with a Microtek Scanmaker 4900. Average mode of background subtraction was used to normalize intensity value, which represents the amount of protein (total protein on gel and oxidized protein on oxyblot) per spot. After completion of spot matching, the dataset was obtained from the normalized intensity of each protein spot from the control and AD gels or oxyblots. The latter were obtained by the following procedure: the spot intensity values obtained on the blots are divided by the spot intensity value on the gels to get the level of specific protein carbonyls. These, in turn, are analyzed for significance using Student's *t*-test.

2.8. Trypsin digestion

Samples were prepared according to the method described by Thongboonkerd et al. [57]. Based on the data obtained from image analysis, the protein spot that is the subject of this paper and that showed a significant increase in oxidation in AD versus control was excised from gel with a clean razor blade and transferred into a clean 1.5 ml microcentrifuge tubes. The gel pieces were washed with 0.1 M ammonium bicarbonate (NH_4HCO_3) for 15 min at room temperature under a flow hood, followed by addition of acetonitrile and incubation at room temperature for 15 min. The solvents were removed and the gel pieces were allowed to dry. The gel pieces were incubated with 20 μl of 20 mM DTT in 0.1 M NH_4HCO_3 and incubated for 45 min at 56 °C. The DTT solution was removed and 20 μl of 55 mM iodoacetamide (IA) in 0.1 M NH_4HCO_3 was added and incubated for 30 min in the dark at room temperature. The liquid was drawn off and the gel pieces were incubated with 200 μl of 50 mM NH_4HCO_3 at room temperature for 15 min. Acetonitrile was added to the gel pieces for 15 min at room temperature. The solvents were removed and the gel pieces were allowed to dry for 30 min. The gel pieces were rehydrated with 20 ng/μl modified trypsin (Promega, Madison, WI, USA) in 50 mM NH_4HCO_3. The gel pieces were chopped into small pieces and placed in a shaking incubator overnight (∼18 h) at 37 °C.

2.9. Mass spectrometry

Mass spectra of the sample were determined by a Tof-Spec 2E (Micromass, UK) MALDI-TOF mass spectrometer in reflectron mode. One microliter of tryptic digest was mixed

with 1 μl α-cyano-4-hydroxy-*trans*-cinnamic acid (10 mg/ml in 0.1% TFA:ACN, 1:1, v/v) directly on the target and dried at room temperature. The sample spot was then washed with 1 μl of 1% TFA solution for approximately 60 s. The TFA droplet was gently blown off the sample spot with compressed air. The resulting diffuse sample spot was recrystallized (refocused) using 1 μl of a solution of ethanol:acetone:0.1% TFA (6:3:1 ratio). Reported spectra are a summation of 100 laser shots. External calibration of the mass axis, used for acquisition and internal calibration using either trypsin autolysis ions or matrix clusters, was applied post acquisition for accurate mass determination.

The MALDI spectra used for protein identification from tryptic fragments were searched against the NCBI protein databases using the MASCOT search engine (http://www.matrixscience.com). Peptide mass fingerprinting used the assumption that peptides are monoisotopic, oxidized at methionine residues and carbamidomethylated at cysteine residues [8,13–15]. Up to one missed trypsin cleavage was allowed. Mass tolerance of 150 ppm was the window of error allowed for matching the peptide mass values. Probability-based MOWSE scores were estimated by comparison of search results against estimated random match population and were reported as $-10\log_{10}(p)$, where p is the probability that the identification of the protein is not correct. MOWSE scores greater than 59 were considered to be significant ($p < 0.05$). Protein identification was in the expected size and p*I* range based on position in the gel.

Previous proteomics analysis of AD brain proteins from our laboratory were verified in some cases by immunochemical methods [13]. Thus, we are confident in the correct identity of the protein that is the subject of this paper and we also provided an experimental evidence to support our identification of the reported protein.

2.10. Protein oxidation

Fifty microliters of purified Pin1 protein (2 mg protein/ml stock solution as a generous gift from Prof. Ping Lu) was incubated with 30 μM $FeSO_4$/1 mM H_2O_2 in a final volume of 100 μl for 3 h, the latter producing hydroxyl free radicals as described previously [25]. The samples were then spun at 12,000 rpm for 5 min and the pellet was washed twice with HEPES buffer. And finally suspended in HEPES buffer.

2.11. Petidyl-prolyl cis–trans isomerase assay

PPIase activity was measured by a protocol modified from Kofron et al. [27], Schutkowski et al. [46], and Yaffe et al. [59]. Typically, the 100 μl assay mixture consisted of 97 μl of HEPES buffer (32 mM, pH 7.8), 1 μl of purified Pin1 protein or 2 μl of brain homogenate (2 mg/ml) [in which case only 96 μl of HEPES were used], and 1 μl (60 mg/ml) of chymotrysin solution (St. Louis, MO). The reaction was started by addition of 1 μl (25 mg/ml) Suc-AEPF-pNA (Bachem, Switzerland) to the peptide stock solution in DMSO, and the absorbance of *p*-nitroaniline was followed at 395 nM for 10 min.

2.12. Statistics

The means of protein levels and protein specific carbonyl levels were analyzed by two-tailed Student's *t*-tests. A value of $p < 0.05$ was considered statistically significant. A similar statistical analysis is usually used for proteomics data analysis [12,28,39]. Only those specifically oxidatively modified proteins in AD brain that were significantly different from control brains were subjected to proteomics analysis. As discussed extensively by Maurer et al. [39], the proteome expression data with only several hundred protein spots are much smaller than microarray data sets with at least several thousand genes. There are no statistical tools commercially available currently tailored for proteome expression data. The situation with respect to the current results is even more dramatic, since there were only 18 proteins whose specific carbonyl levels were elevated in AD brain over the intrinsic oxidation level in control brain. Of these, we were able to identify with statistical confidence 6, one of which (Pin1) is the subject of this paper. Consequently, with this low number of proteins, as discussed by Maurer et al. [39], microarray algorithms and statistical approaches are not applicable for proteomics, so we relied on Student's *t*-test as is often used [3,39].

3. Results

Comparison of AD and control hippocampus protein oxidation levels was carried out by first identifying carbonylated proteins via anti-DNP immunochemical development of proteins transferred to a nitrocellulose membrane, or 2D-oyxblot analysis (Fig. 1B and D), followed by comparison of 2D blots with 2D gels. Individual protein spots were matched between the 2D-PAGE maps and the 2D-oxyblots and the carbonyl immunoreactivity of each spot was normalized to the protein content in the 2D-PAGE. The oxyblot of AD brain showed more oxidized protein spots compared to that of control hippocampus (Fig. 1D). Several protein spots were determined to be more oxidized in AD hippocampus compared to control. Based on the PD Quest analysis, six proteins in AD brain were found to show a significantly different protein oxidation, and analysis of five of these proteins will be published elsewhere. However, given the potential importance of Pin1 in AD pathology [45,60], we report in this communication the results for the spot representative of this protein in AD hippocampus, which is rich in NFT and is one of the most affected areas of AD brain. Based on the data obtained from the MASCOT database, this protein is successfully identified as Pin1 [also called PPIase, Table 2]. Pin1 was found to exhibit a significant increase in protein carbonylation in AD hippocampus (Fig. 1D) compared with age-matched control (Fig. 1B). Using MASCOT, the probability-based MOWSE

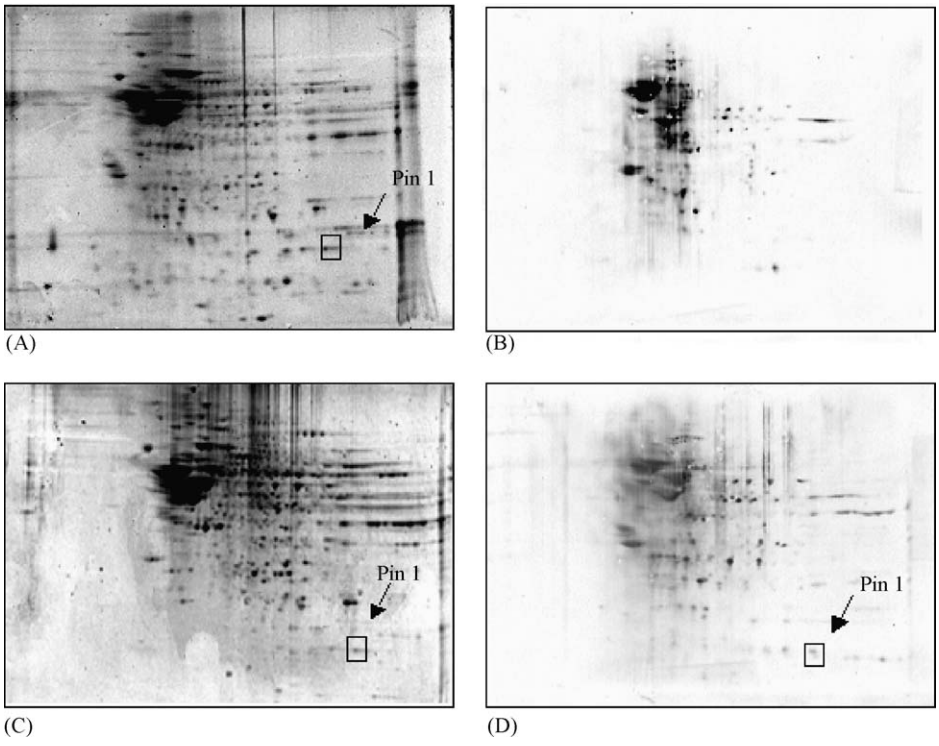

Fig. 1. Sypro Ruby-stained gels of hippocampal proteins from control (A) and AD (C), and 2D-oxyblots of hippocampal proteins from control (B) and AD (D). A box is drawn around the Pin1 on both gels and blots. Relative changes in carbonyl immunoreactivity, after normalization of the carbonyl intensities to the protein content, were significant for Pin1. See text.

Table 2
Proteomics characteristics

Protein	PPIase
Number of peptides matched of the identified protein	5/22
Percentage coverage of the matched peptides	32
p*I*, Mr	7.82, 18 kDa
Mowse Score	60

score was 60, with 5/22 peptide matches and 32% sequence coverage. The increase in carbonylation compared to control was significant for Pin1 ($135.7 \pm 55\%$ control, $p < 0.05$). Pin1 protein expression was found to be significantly decreased in AD hippocampus ($40.2 \pm 8.2\%$ of control, $p < 0.03$). To verify the correct identification of Pin1, we performed Western blot analysis. Fig. 2B shows the localization of Pin1 protein observed as an immunopositive spot that is positioned as reported by mass data to be oxidized Pin1.

Generally, oxidative modification of proteins leads to loss of activity [23,31,54]. To investigate whether oxidative modification of Pin1 led to loss of activity, the enzyme function in AD and control brain was measured. Pin1 activity was found to be significantly ($p < 0.01$) reduced in AD hippocampus compared to that of control (Fig. 3A). In addition, in vitro oxidation of purified Pin1 protein with Fe^{+2} and H_2O_2 resulted in a significant ($p < 0.0001$) decrease in Pin1 activity (Fig. 3B).

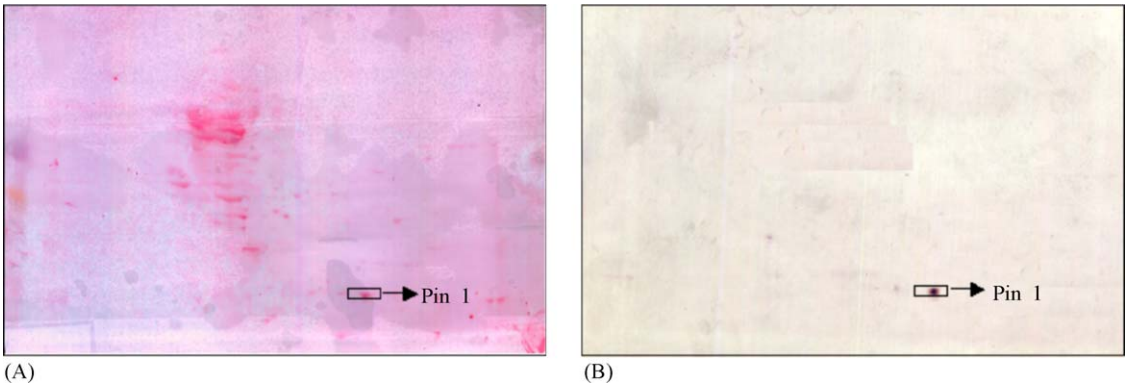

Fig. 2. Western blot analysis. (A) Ponceau stained blot and (B) blot probed with anti-Pin1 antibody. A box is drawn around the Pin1 protein.

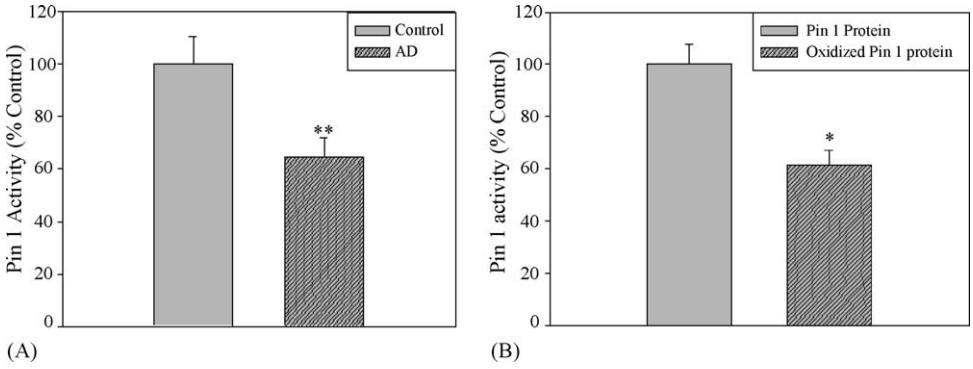

Fig. 3. Pin1 enzyme activity in in vivo and in vitro samples. (A) Pin1 activity in control and AD hippocampus. (B) In vitro oxidation of purified Pin1 protein by Fe^{+2} and H_2O_2 decreases the activity of Pin1 protein. $^{*}p < 0.0001$, $^{**}p < 0.001$.

4. Discussion

Alzheimer's disease is characterized pathologically by the presence of abundant NFTs together with senile plaques and synapse loss in the brains of affected individuals [4,17,18,32,45,50,56,60]. NFTs are composed of paired helical filament (PHF), which contain a highly phosphorylated form of the microtubule-associated protein tau [2,29,41]. Tau is an important component of neuronal cytoskeleton and found primarily in axons. Normal tau promotes tubulin polymerization and stabilizes microtubule (MT) structures, whereas hyperphosphorylated tau reduces its affinity for MTs and destabilizes MT-structures [6,16,21]. This destabilization results in the disruption of vital cellular processes, such as axonal transport mechanisms and loss of cell shape, and leads to the degeneration of affected neurons [37,53]. The microtubule binding region of tau lies in its C-terminal half, including the three or four internal repeats. The basic and proline-rich regions flanking the repeats help to target tau to the microtubule, while the acidic N-terminus does not bind to the microtubule. These binding functions of tau are regulated by phosphorylation. Tau phosphorylated at certain sites can detach from microtubules so that these cytoskeletal proteins become more labile and dynamic. The hyperphosphorylation of tau protein could be due to unbalanced kinase and phosphatase activities and the availability of their substrate molecules in a given system or may be due to inhibition of Pin1 [peptidyl prolyl *cis–trans* isomerase, PPIase] [20,58]. Pin1 regulates the phosphorylation and dephosphorylation of tau protein and has been shown to be down-regulated in AD brain [34,55].

Peptidyl-prolyl isomerases are chaperone enzymes that alter the peptide bond between a given amino acid and a proline, changing it from the *cis* to the *trans* conformation and vice versa [46]. This modification can cause dramatic structural modifications, which can affect the properties of targeted proteins. The ubiquitous PPIase Pin1, conserved from yeast to human, has been shown to be necessary for entry into mitosis [35,47]. The yeast homologue, Ess1, is essential for cell survival [19]. Pin1 possesses a WW domain, which specifically recognizes pSer-Pro and pThr-Pro motifs in which the first amino acid is phosphorylated. Pin1 binds to many proteins implicated in cell cycle regulation (e.g. p53, Myt1, Wee1, and Cdc25C) [42,60]. Pin1 also targets tau, a protein forming part of the neuronal cytoskeleton, which is hyper-phosphorylated in patients suffering from AD [29]. Pin1 could, therefore, be involved in the pathogenesis of AD.

In the present study, Pin1 was found to be oxidatively modified in AD hippocampus and shows a significantly diminished activity. Both in vivo and in vitro oxidation of Pin1 protein significantly reduced the Pin1 activity, further supporting prior studies that showed oxidatively modified proteins have decreased activity [23,31,54]. The observed decreased activity of Pin1 protein might be responsible for the increased accumulation of phosphorylated tau protein in AD hippocampus, a brain region rich in NFTs. Recent studies demonstrated that Pin1 is co-localized with phosphorylated tau [24,45] and exists in inverse relationship to the expression of tau in Alzheimer's disease [34]. We, in the present study, and others [34,36] observed a decrease in the expression of Pin1 in AD hippocampus compared to that of control.

Pin1 also has been shown to protect the cell against age-related neurodegeneration [34] and to restore the function of tau protein in AD [36]. Pin1 is unique, and its oxidative modification conceivably could be among the initial events that trigger tangle formation and neurotoxicity.

Oxidative damage is reported in the aging brain [44,51] but is more severe in AD [10,38]. PCO content was reported to be elevated 42% in AD hippocampus and by 37% in AD inferior parietal lobule, which are rich in Aβ-containing SP and NFT, relative to AD cerebellum, a brain region that shows little degenerative changes in AD and no oxidative damage [23,30]. Therefore, the oxidation of Pin1 in severely affected region of the brain may increase the vulnerability of the hippocampus, leading to the progression of disease.

Oxidative modification of proteins in vivo may affect a variety of cellular functions involving proteins: receptors, signal transduction mechanisms, transport systems and enzymes. Identification of such modified proteins is crucial for establishing a relationship between oxidative mod-

ification and neuronal death and potentially will help in the development of therapeutic targets to protect the brain against degenerative processes. Previously, we have identified specific targets of protein oxidation that are involved in energy metabolism, protein degradation pathways, excitotoxicity, phospholipid asymmetry, cholinergic dysfunction, neuritic abnormalities, and interneuronal communications [1,7,12,13,15]. Pin1 has been implicated in multiple aspects of cell cycle regulation [35], a process recently suggested to be abnormal in AD [49]. The in vivo carbonylation of the Pin1 protein as observed in AD hippocampus in the present study suggests that oxidative modification might have resulted in loss of Pin1 isomerase activity that could be crucial in AD neurofibrillary pathology. It is tempting to speculate that Pin1 oxidative modification results in accumulation of phosphorylated tau protein leading to tangle formation and progression of AD. Studies to test this idea are underway.

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