Neurochemical Research, Vol. 25, No. 2, 2000, pp. 247–255

Degradation of Soluble Amyloid β-Peptides 1-40, 1-42, and the Dutch Variant 1-40Q by Insulin Degrading Enzyme from Alzheimer Disease and Control Brains

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(Accepted September 2, 1999)

Insulin degrading enzyme (IDE) is a metalloprotease that has been involved in amyloid β peptide (Aβ) degradation in the brain. We analyzed the ability of human brain soluble fraction to degrade Aβ analogs 1-40, 1-42 and the Dutch variant 1-40Q at physiological concentrations (1 nM). The rate of synthetic ^{125}I-Aβ degradation was similar among the Aβ analogs, as demonstrated by trichloroacetic acid precipitation and SDS-PAGE. A 110 kDa protein, corresponding to the molecular mass of IDE, was affinity labeled with either ^{125}I-insulin, ^{125}I-Aβ 1-40 or ^{125}I-Aβ 1-42 and both Aβ degradation and cross-linking were specifically inhibited by an excess of each peptide. Sensitivity to inhibitors was consistent with the reported inhibitor profile of IDE. Taken together, these results suggested that the degradation of Aβ analogs was due to IDE or a closely related protease. The apparent Km, as determined using partially purified IDE from rat liver, were 2.2 ± 0.4, 2.0 ± 0.1 and 2.3 ± 0.3 μM for Aβ 1-40, Aβ 1-42 and Aβ 1-40Q, respectively. Comparison of IDE activity from seven AD brain cytosolic fractions and six age-matched controls revealed a significant decrease in Aβ degrading activity in the first group, supporting the hypothesis that a reduced IDE activity may contribute to Aβ accumulation in the brain.

KEY WORDS: Amyloid β peptide; Alzheimer's disease; insulin degrading enzyme; Dutch variant.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the accumulation of extracellular amyloid and intracellular neurofibrillary tangles in the cerebral cortex. The major component of amyloid deposits is the 40–43 residue amyloid β peptide (Aβ), a proteolytic product of the amyloid β precursor protein (AβPP) (reviewed in 1). Compelling evidence implicates Aβ deposition in AD pathogenesis. First, mutations in or around the Aβ region of AβPP are linked to early onset AD (reviewed in 2); hereditary cerebral hemorrhage with amyloidosis of the Dutch type (HCHWA-D) is caused by the deposition in cortical and meningeal vessels of Aβ bearing a $A\beta PP_{693}$ Glu → Gln substitution (Aβ 1-40Q) (3). Moreover, patients with Down syndrome show an enhanced expression of AβPP (encoded on chromosome 21) due to an increased gene dosage, resulting in the gradual development of AD lesions (4).

Aβ is a normal cellular product and circulates in biological fluids at low nanomolar levels in a soluble

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Abbreviations: Aβ, amyloid β peptide; AD, Alzheimer's disease; IDE, insulin degrading enzyme; AβPP, amyloid β precursor protein; TCA, trichloroacetic acid.

0364-3190/00/0200–0247$18.00/0

form (5). While Aβ 1-40 is the major species of soluble Aβ in body fluids, Aβ 1-42 is the major component of brain fibrillar deposits (6).

The mechanisms of Aβ generation from AβPP have been widely studied. However, the turnover and degradation of Aβ in the brain is poorly understood. It is possible that abnormal expression or activity of Aβ degrading enzyme(s) may contribute to the elevation in cerebral levels of Aβ. Metallo (7,8,9,10), aspartyl (11,8), thiol (8) and serine (12) proteases have been reported to degrade Aβ. Recent work indicates that insulin-degrading enzyme (IDE) may be related to Aβ clearance in chinese hamster ovary and murine microglial cells (13), rat (14) and human brain (15). Moreover, this protease has been found associated to senile plaques in AD brains (16). IDE is a neutral thiol metalloprotease implicated in the degradation of insulin after internalization by cells (reviewed in 17). It is highly conserved in evolution (18), developmentally regulated (19) and expressed in all tissues (19,20). Besides insulin, this protease also degrades insulin-like growth factor II (21), transforming growth factor α (22), atrial natriuretic peptide (23) and β-endorphin (24), indicating a possible role in the cellular regulation of peptide hormones levels.

We studied the degradation of the relevant Aβ isoforms in cerebral amyloidosis, Aβ 1-40, Aβ 1-42 and Aβ 1-40Q, at physiological concentrations (~1 nM). Next, we compared the degree of proteolysis of synthetic Aβ peptides and insulin by normal and AD brain samples. Our results showed that IDE or a protease with similar characteristics was able to bind and degrade synthetic Aβ analogs peptides at a similar rate and that its activity was impaired in AD patients as compared to normal controls.

EXPERIMENTAL PROCEDURE

Materials. Synthetic Aβ 1-40 and 1-42 were obtained from Bachem (Torrance, CA). The Dutch isoform Aβ 1-40Q was synthesized in W. M. Keck Facility at Yale University and kindly provided by Blas Frangione. Porcine insulin and Aβs were iodinated by the chloramine-T method using $Na^{125}I$ (New England Nuclear). The radiolabeled peptides were purified by reverse-phase HPLC (μBondapack Waters C18 column 3.9 × 300 mm) at a flow rate of 1 ml/min using a linear gradient of water, 0.1% trifluoroacetic acid to 95% acetonitrile, 0.1% trifluoroacetic acid over 50 min. Specific activity was 360 μCi/μg for ^{125}I-insulin and 10–20 μCi/μg for ^{125}I-Aβ analogs. Anti-p15, a immunopurified IgG developed in rabbit against residues 940–953 of human and rat IDE (18,25) was generously provided by Dr. Daniel Udrisar (Universidade Federale de Pemambuco, Brazil).

Subjects. Frontal cortex from postmortem brain tissue of seven patients with diagnosis of Alzheimer's disease confirmed by histopathological analysis and six age-matched neurologically normal controls (56–87 years old) were used in this study. Tissue was obtained from the National Neurological Research Specimen Bank (Los Angeles, CA) and kept at –80°C. Postmortem delay was less than 12 h in all cases.

Preparation of Cytosolic Fractions. Tissue cytosolic fractions were prepared by homogenization in ice-cold PBS, pH 7.4 and centrifugation at 100,000 *g* for 60 min at 4°C. Samples were stored at –80°C until use. As a model of human neurons we used the human neuroblastoma cell line IMR-32 (ATCC). Cells were grown in a complex medium containing 10% fetal bovine serum as described (26). Cells were washed three times, harvested and the cytosolic fraction was prepared as described above.

Partial Purification of Rat IDE. Liver was obtained from Wistar rats (250–300 g) and immediately homogenized in ice-cold 20 mM Tris-HCl buffer, pH 7.4 (buffer A), containing 1 mM PMSF, 10 μg/ml soybean trypsin inhibitor, 1 μg/ml pepstatin and 1 μg/ml leupeptin. All subsequent steps were done at 0–4°C. The homogenate was centrifuged at 8000 *g* for 10 min. The post-debris supernatant was centrifuged at 100,000 *g* for 60 min. The cytosolic fraction was fractionated by ammonium sulfate precipitation. The 30–60% fraction was dialyzed against buffer A and applied to DEAE-Sephadex A50 column equilibrated with the same buffer. The column was washed with buffer A containing 100 mM NaCl and eluted with 250 mM NaCl in the same buffer. Fractions containing insulin-degrading activity were pooled, brought to 60% ammonium sulfate and centrifuged. The pellet was resuspended and applied to a pentylagarose hydrophobic column equilibrated with 45% ammonium sulfate in 10 mM imidazole-HCl buffer, pH 7.1. The column was eluted with a discontinuous gradient of 28% and 23% ammonium sulfate in the same buffer. The majority of the degrading activity eluted in 23% ammonium sulfate. These fractions were pooled, precipitated with 60% ammonium sulfate and desalted in Econo Pac (BioRad) equilibrated with 50 mM phosphate buffer, pH 7.4. Purification steps of IDE were monitored by specific activity using ^{125}I-insulin and TCA method, SDS-PAGE and immunoblotting (see below). A final estimated enrichment of 2,700 fold was obtained (Camberos et al, manuscript in preparation).

Degradation Assays. Insulin and Aβ degradation were routinely measured by trichloroacetic acid (TCA) precipitation. Fractions were incubated at 37°C for 30 min with ^{125}I-labeled ligand (~1.5×10^4 cpm) in 100 μl of 50 mM phosphate buffer, pH 7.4, containing 1% BSA. The reaction was stopped by the addition of 0.9 ml of TCA 5%. After 15 min at 4°C, the samples were centrifuged at 1800 *g* for 5 min at 4°C and supernatant and pellet were separated and counted in a Packard gamma counter. Degradation was calculated as the increase in TCA-soluble ^{125}I fragments as compared to control (no enzyme present). In experiments with inhibitors, these were incubated with fractions for 15 min at 4°C before the addition of substrate and then assayed as described above. All concentrations were chosen so that the extent of degradation was linear with time and protein.

Aβ degradation was also assessed by Tris-Tricine SDS-PAGE. For this assay, 50 μg of human brain cytosolic fraction ware incubated with ^{125}I-Aβ (~1.5×10^4 cpm, 1 nM) in 50 μl of 50 mM phosphate buffer, pH 7.4, containing 0.1% BSA at 37°C for 1 h. In control assays, IDE was inhibited with 1mM 1,10-phenantroline. The samples were subjected to 12.5% Tris-Tricine SDS-PAGE (27) and the gels were stained, dried and autoradiographed using Kodak X-OMAT AR film and a Dupont Cronex Lightning Plus intensifying screen.

Cross-Linking. Since affinity labeling of IDE by ^{125}I-insulin in rat brain is well characterized (28), both human and rat brain tissue

were used for cross-linking experiments. 100 μg of proteins were incubated with ^{125}I-insulin, ^{125}I-Aβ 1-40 or ^{125}I-Aβ 1-42 (~2×10^5 cpm) plus 1 mM 1,10-phenantroline, in the presence or absence of 4×10^{-6} M of unlabeled peptides, in 50 μl of 50 mM phosphate buffer, pH 7.5, 100 mM NaCl. After 60 min at 4°C, 3 μl of disuccinimidyl suberate (8.25 mM in DMSO; final concentration 0.46 mM) were added. After additional 15 min at 0°C, the reaction was stopped by the addition of 20 μl of denaturing buffer containing 0.25 M Tris-HCl, pH 7.4, 7.5% SDS, 375 mM dithiothreitol and the samples were subjected to 10% SDS-PAGE. The gels were stained, dried and exposed as described above.

Immunoblotting of IDE. 100 μg of human brain cytosolic proteins were subjected to 10% SDS-PAGE and transferred to nitrocellulose membranes for 120 min at 100 V. The membrane was blocked for 2 h at room temperature with 5% nonfat milk in 20 mM Tris-HCl, pH 7.5, 0.5 M NaCl and then incubated overnight at 4°C with anti-p15 IgG (3 μg/ml) in the absence or presence of p15 (1 μg/ml). After washing in the same buffer containing 0.05% Tween-20, the bound antibody was detected using alkaline phosphatase-conjugated goat anti-rabbit IgG (BioRad) according to manufacturer's protocols. The data from immunoblots were quantitated using NIH Image software program.

RESULTS

We studied the susceptibility to proteolysis of Aβ using ^{125}I-labeled Aβ. Quality control of the radiolabeled substrates showed that Aβ analogs had similar HPLC elution profiles and sedimentation rates as compared to unlabeled Aβs (data not shown) (29). ^{125}I-Aβs degradation was measured by the increase in radioactive soluble fragments after TCA precipitation of intact substrate. Although this method underestimates the degradation, it has been previously used by others (13) and gives results comparable to more sensitive tests such as receptor binding assay (30). As shown in Fig. 1A, human brain cytosolic fractions were able to degrade synthetic ^{125}I-Aβ 1-40, ^{125}I-Aβ 1-42 and ^{125}I-Aβ 1-40Q at ~1 nM. Analysis of degradation by Tris-Tricine SDS-PAGE and autoradiography were consistent with the TCA precipitation results (Fig. 1B).

It has been previously demonstrated that human brain soluble fraction is capable of degrading synthetic Aβ 1-40 in the μM range at neutral pH. Under these conditions, the main Aβ 1-40 degrading activity is due to IDE, as demonstrated by immunodepletion using a monoclonal antibody to human IDE (15). In order to further characterize the Aβ degrading activity observed, the effect of different inhibitors was tested. Degradation of Aβ analogs was unaffected by the serine protease inhibitor PMSF, soybean trypsin inhibitor, leupeptin, or the catepsin D inhibitor pepstatin, but it was strongly inhibited by the chelating agent 1,10 phenanthroline, bacitracin and the sulfhydryl agent N-ethylmaleimide

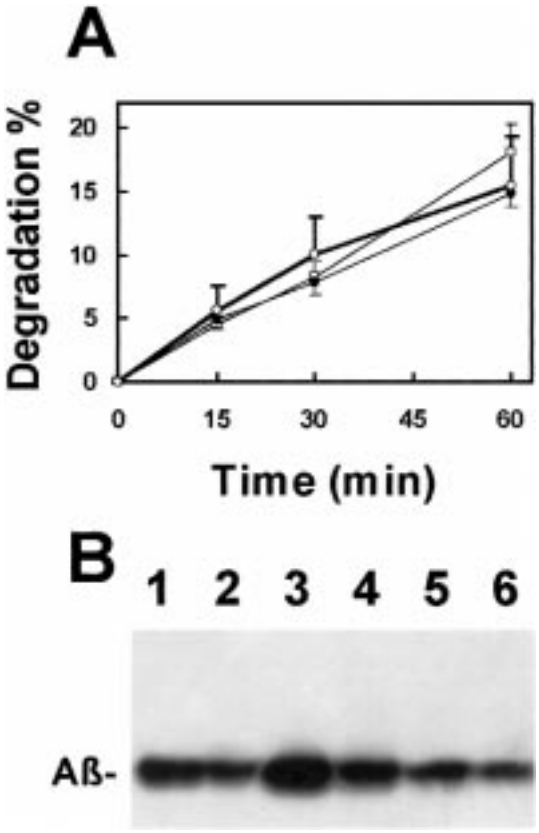

Fig. 1. Degradation of ^{125}I-Aβ analogs at nanomolar levels by human brain cytosolic fraction. **A:** 50 μg of human brain cytosolic fraction were incubated with 1nM ^{125}I-Aβ 1-40 (□), Aβ 1-42 (●) or Aβ 1-40Q (○) for the indicated times. Degradation was determined by TCA precipitation as described in Methods and expressed as the percentage of total Aβ degraded. Results are the mean ± SEM of three experiments performed in duplicate; **B:** 50 μg of human brain cytosolic fraction were incubated for 30 min at 37°C with 1 nM ^{125}I-Aβ 1-40 (lanes 1 and 2), ^{125}I-Aβ 1-42 (lanes 3 and 4) or ^{125}I-Aβ 1-40Q (lanes 5 and 6) in presence (lanes 1, 3 and 5) or absence (lanes 2, 4 and 6) of 1 mM 1,10-phenantroline. Degradation was assessed by Tris-Tricine SDS-PAGE and autoradiography.

(Table I). This inhibitor profile is characteristic of IDE, which is a well known thiol-dependent metalloprotease. The same inhibitor profile was obtained with all studied Aβ analogs and it was in agreement with a previous report of Aβ 1-40 degradation by IDE (15). Moreover, in all cases the degradation was specifically inhibited by an excess of unlabeled insulin and there were no differences in degradation patterns if growth hormone was added in the reaction (Table I). It has been described that matrix metalloproteinases (MMPs) 2 and 9 (gelatinases A and B) are capable of degrading Aβ (7,9). However, these proteinases seem not to be responsible for the Aβ degradation in our experiments, since their activity is completely inhibited by

Table I. Effect of Various Agents on ^{125}I-Aβ 1-40, ^{125}I-Aβ 1-42, and ^{125}I-Aβ 1-40Q Degradation by Human Brain Cytosolic Fraction

		Inhibition of degradation %		
Agent	Concentration	Aβ 1-40	Aβ 1-42	Aβ 1-40Q
N-ethylmaleimide	1 mM	98.68	90.54	100.00
Bacitracin	1 mg/ml	100.00	99.80	100.00
1,10-Phenantroline	1 mM	100.00	98.15	100.00
PMSF	1 mM	29.19	23.87	26.19
Soybean trypsin inhibitor	10 μg/ml	20.51	6.12	1.30
Leupeptin	10 μg/ml	10.34	0.56	1.50
Pepstatin A	10 μg/ml	27.30	18.03	10.33
Insulin	1 μM	91.57	82.11	94.71
Growth hormone	1 μM	8.10	10.37	0.00
Gelatin	0.1%	14.98	ND	ND

^{125}I-Aβ degradation was determined by TCA precipitation as described in Methods. Results are the mean ± SEM of three experiments performed in duplicate. ND, not determined.

1mM EDTA and it is not affected by sulfhydryl agents in contrast with our observations (Table I). Moreover, gelatin, a high affinity substrate of these MMPs, was unable to compete with Aβ (Table I).

Experiments of affinity labeling to ^{125}I-insulin using rat brain soluble fraction showed only one specific labeled band at a molecular mass of ~110 kDa and the binding was completely inhibited by an excess (4×10^{-6} M) of unlabeled insulin (Fig. 2A). This reactivity has been previously identified as IDE by Shii et al

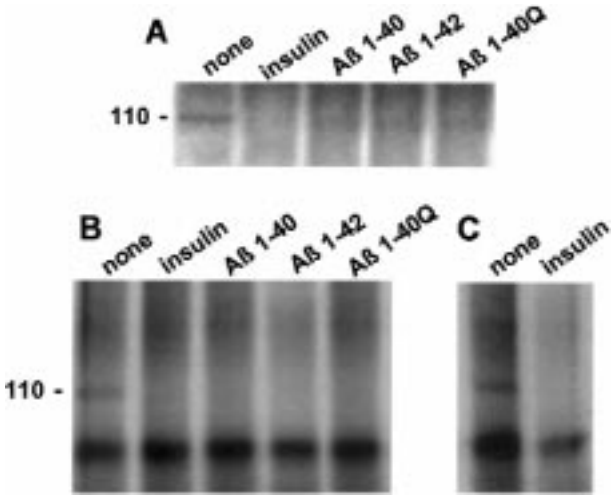

Fig. 2. Cross-linking of ^{125}I-insulin, ^{125}I-Aβ 1-40 and ^{125}I-Aβ 1-42 to rat brain cytosolic fraction. 100 μg of rat brain cytosolic fraction were cross-linked to ^{125}I-insulin (**A**), ^{125}I-Aβ 1-40 (**B**) or ^{125}I-Aβ 1-42 (**C**) in the presence or absence of the indicated unlabeled peptides (4×10^{-6} M), subjected to 10% SDS-PAGE and autoradiographed.

(28). At the same concentration, unlabeled Aβ 1-40, Aβ 1-42 and Aβ 1-40Q partially inhibited the cross-linking to ^{125}I-insulin. Moreover, IDE was affinity labeled with either ^{125}I-Aβ 1-40 or ^{125}I-Aβ 1-42 (Fig. 2B and C, respectively) and the cross-linking was blocked by an excess of unlabeled insulin, Aβ 1-40, Aβ 1-42 or Aβ 1-40Q. When affinity labeling was done using human brain soluble fraction, similar results were obtained, yet, the intensity of the 110 kDa band was very low as compared to rat brain (not shown). Taken together, these results (i.e. the sensitivity to inhibitors, binding to a protein of 110 kDa, competition with insulin in Aβ binding and degradation, absence of competition with gelatin and the previous observation that IDE was a major enzyme implicated in Aβ degradation by human brain soluble fraction (15)), suggested that the degradation of different Aβ analogs was due to IDE or a similar protease. Our attempts to deplete cytosolic fraction from IDE activity with our anti-p15 were unsuccessful, possibly because this antibody is not competent to immunoprecipitate native IDE (Pérez A., unpublished observations).

With regard to the substrate specificity of IDE, similar rates of degradation between Aβ analogs were observed (Fig. 1). IDE from other sources, such as neuroblastoma cytosolic fraction, rat liver cytosolic fraction and partially purified rat IDE, showed the same specificity (Table II). However, the specific activity of IDE varied significantly between different sources. In human brain cytosolic fraction from control subjects, Aβ degradation was relatively low, whereas cytosol from rat liver had a much higher activity. As expected, partially purified IDE from rat, which exhibits

Table II. Degradation of ^{125}I-Aβ 1-40, ^{125}I-Aβ 1-42, and ^{125}I-Aβ 1-40Q by Human Brain Cytosolic Fraction from Control Subjects and Other Sources

	^{125}I-Aβ degradation (fmol/min/mg protein)[a]			
Source	Aβ 1-40	Aβ 1-42	Aβ 1-40Q	insulin
human brain cytosolic fraction	5.37 ± 0.90	5.65 ± 0.83	5.91 ± 2.18	0.34 ± 0.06
neuroblastoma cytosolic fraction	10.33 ± 1.46	11.63 ± 2.37	12.32 ± 2.40	0.71 ± 0.07
rat liver cytosolic fraction	100.90 ± 4.09	91.12 ± 6.13	84.73 ± 18.66	3.63 ± 0.05
purified rat IDE	3182 ± 536	3076 ± 248	2638 ± 310	ND

[a] ^{125}I-peptide degradation was determined by TCA precipitation as described in Methods. Results are the mean ± SEM of at least three experiments performed in duplicate. ND, not determined.

95% amino acid identity to human IDE (25), showed the highest specific activity of all preparations tested (Table II).

In order to further elucidate the substrate specificity of IDE towards Aβ analogs, we studied the ability of increasing amounts of unlabeled Aβ 1-40, Aβ 1-42 and Aβ 1-40Q to compete with ^{125}I-insulin degradation. Aβ 1-40 and Aβ 1-42 were found to inhibit ^{125}I-insulin degradation in a dose-dependent manner with similar specificity, but Aβ 1-40Q showed a lower effectiveness (Fig. 3), possibly due to a higher aggregation of our Aβ 1-40Q stock solution. In fact, as much as 25% of our preparation of Aβ 1-40Q was precipitated by centrifugation at 13.000 *g* (not shown). As expected, the inhibitory potency of all studied Aβ species was lower than insulin (Fig. 3).

Analysis of the degradation of ^{125}I-Aβ analogs by partially purified rat IDE using the TCA precipitation assay yielded an apparent Michaelis Menten constant (Km) estimated by the Lineweaver-Burk method of 2.2 ± 0.4, 2.0 ± 0.1 and 2.3 ± 0.3 μM for Aβ 1-40, Aβ 1-42 and Aβ 1-40Q, respectively (Fig. 4). Again, insulin had the highest specificity for the enzyme, with a Km of 113 nM (not shown). These values were similar to values found by others using different assays for Aβ 1-40 (15) and insulin (17,24). Furthermore, we compared the IDE activity of brain cytosolic fractions from seven AD and six age-matched control subjects. The Aβ degrading activity in cytosolic fraction from AD brains was approximately 50% as compared to control brains for Aβ 1-40, Aβ 1-42 and Aβ 1-40Q. Similar differences were observed when insulin degradation was tested

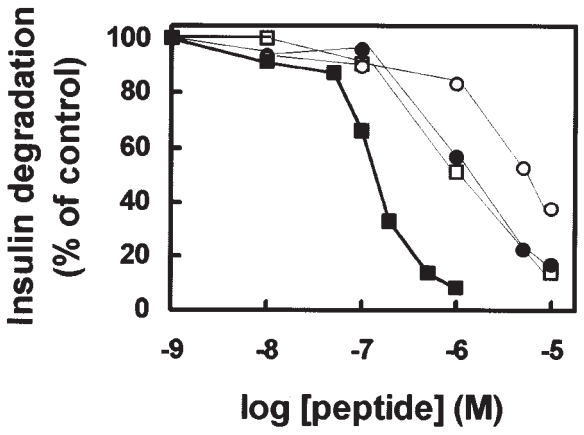

Fig. 3. Competitive inhibition of different Aβ analogs on ^{125}I-insulin degradation. 50 μg of human brain cytosolic fraction were incubated with ^{125}I-insulin in the presence of the indicated concentrations of unlabeled insulin (■), Aβ 1-40 (□), Aβ 1-42 (●) or Aβ 1-40Q (○). Control degradation is the amount of degradation in the absence of competitor peptide. Degradation was determined by TCA precipitation as described in Methods. Results are the mean of three experiments performed in duplicate.

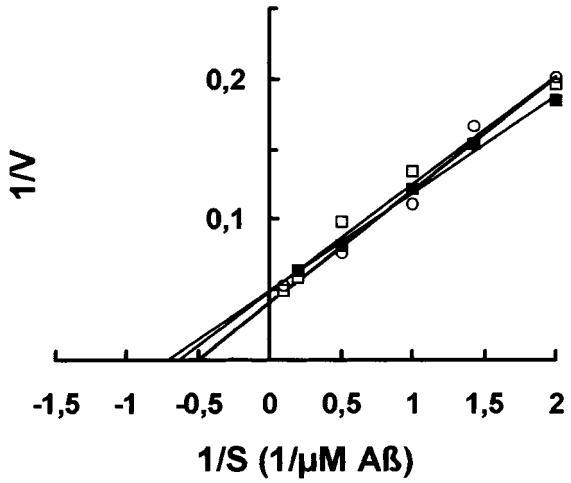

Fig. 4. Lineawever-Burk plot of Aβ degradation by IDE. 160 ng of purified rat IDE were incubated with ^{125}I-Aβ 1-40 (□), ^{125}I-Aβ 1-42 (●) or ^{125}I-Aβ 1-40Q (○) in the presence of various concentrations (0.5–10 μM) of unlabeled Aβ 1-40, Aβ 1-42 or Aβ 1-40Q (S), respectively. Velocity (V) is expressed as μmol of Aβ degraded in 30 min, as determined by TCA precipitation. Results are the mean ± SEM of five experiments performed in duplicate.

Table III. Degradation of ^{125}I-Aβ 1-40, ^{125}I-Aβ 1-42, and ^{125}I-Aβ 1-40Q by Human Brain Cytosolic Fraction from Control and Alzheimer Subjects

	^{125}I-peptide degradation (%)[a]			
Source	Aβ 1-40	Aβ 1-42	Aβ 1-40Q	insulin
Control	8.07 ± 1.91	6.09 ± 1.24	6.63 ± 3.39	7.99 ± 2.33
Alzheimer	3.71 ± 0.98*	2.97 ± 0.70*	2.07 ± 0.82*	5.65 ± 1.42*

[a] ^{125}I-peptide degradation was determined by TCA precipitation as described in Methods. Results are the mean ± SEM (Controls, n = 6; AD, n = 7) of three experiments performed in duplicate.
*$p < 0.05$ (Student's *t*-test)

(Table III). The inhibitor profile was identical to Table I (not shown) indicating that the difference between AD and controls corresponds to IDE-like activity.

Next, immunoblot analysis was performed to determine if the differences found in IDE activity between control and AD brains was due to differences in IDE levels. When immunopurified antibodies to IDE sequence (anti-p15) were used, a single immunoreactive band was detected in both control and AD brain cytosolic fractions, at a molecular mass of ~44 kDa (Fig. 5). This band may represent a carboxyl-terminal fragment of IDE, as previously described (18). This immunoreactivity was completely adsorbed after the addition of an excess of p15 to antibody solution, supporting the specificity of the reaction (data not shown). Different efforts to obtain detectable immunoreactivity to intact IDE, such as the addition of a cocktail of protease inhibitors during cytosol extraction, were unsuccessful. However, anti-p15 antibody was able to detect full length IDE in rat liver (Fig. 5), a fraction with Aβ degrading activity 20-fold higher, as compared to human brain (Table II) and human IDE from erythrocytes (not shown). Probably, the intact IDE levels in aged human brain samples were very low, in agreement with the low levels of degrading activity obtained, and the sensitivity of our immunoblot analysis was not sufficient to detect the full length 110 kDa IDE band. After normalization by total protein content, densitometric analysis showed that the level of immunodetected 44 kDa fragment was higher in control than in AD brains ($p < 0.05$ by Student's *t*-test) (not shown).

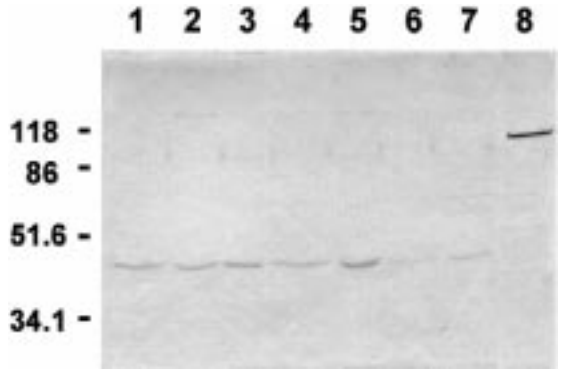

Fig. 5. Immunoblot analysis of human brain cytosolic fractions with anti-p15 antibody. Each lane contains 100 μg protein of brain cytosolic fractions from controls (lanes 1, 2 and 3) and Alzheimer (lanes 4, 5, 6 and 7) subjects. Rat liver cytosolic fraction (lane 8) is shown for comparison. The positions (in kDa) of prestained molecular mass markers are indicated on the left.

DISCUSSION

Several well-characterized proteases have been reported to degrade Aβ in vivo or in vitro, including MMP-2 (7), MMP-9 (9), lysosomal cathepsin D (11) and neutral endopeptidase (10). However, none of them appears to be responsible for the activity observed in our experiments. Inhibitor profile, competition and cross-linking experiments support that the Aβ degrading activity in human brain cytosolic fraction was due to IDE, consistent with a previous report (15).

It has been shown that IDE degrades small peptides of unrelated sequence (21–24). The absence of a consensus cleavage sequence indicates that the specificity of IDE is determined by the three-dimensional structure rather than the primary structure of the substrate (17). It has been postulated that some IDE substrates with the potential to aggregate as amyloid fibrils such as insulin, atrial natriuretic peptide, glucagon and Aβ share a common structural amyloidogenic motif that may be the recognition determinant for IDE (31). This sequence in Aβ corresponds to residues 17–24, which appear to be crucial for the formation of amyloid fibrils (32) and the main site of interaction

with IDE (15). While the residues 17–24 are the same in both Aβ 1-40 and Aβ 1-42, Aβ 1-40Q contains Gln → Glu at position 22. Although this amino acid substitution yields an Aβ analogue that has a higher content of β-sheet and an increased ability to form amyloid (33), it seems not to affect the interaction with IDE. The ability of IDE to recognize and degrade amyloidogenic peptides of unrelated sequences lead us to propose that IDE may act as an "amyloidase" by preventing the accumulation of amyloidogenic peptides.

IDE substrates can be divided according to their degradation efficiency into two main groups. The first group includes high affinity substrates with Km ~0.1 μM, such as insulin, transforming growth factor α, atrial natriuretic peptide and insulin-like growth factor II, for which the in vivo association with IDE has been reported (34,35,23). The second group comprises substrates with lower affinity and Km ≥ 2 μM, such as glucagon, epidermal growth factor, insulin growth factor I, β-endorphin and Aβ analogs (30,35,24). Although the relevance of in vivo remotion of Aβ isoforms by IDE remains to be established, their relatively high Km do not preclude these peptides of being physiological substrates for IDE. This is the case of some neuropeptide-degrading enzymes, with similar low affinities for their physiological substrates (36). Moreover, local Aβ peptide concentrations may reach relatively high transient levels to permit the interaction with IDE. On the other hand, IDE was detected in human cerebrospinal fluid (13), supporting a possible interaction between Aβ and IDE in the extracellular compartment.

Distinct roles have been assigned to Aβ 1-40 and Aβ 1-42 in AD. Aβ 1-42 is the major component of the senile plaques (6), it is overproduced by cells carrying specific mutations causing familial AD (2) and shows a higher rate of amyloid fibril formation in vitro (37). Moreover, its concentration is elevated in the soluble fraction of AD brains as compared to Aβ 1-40 (38). Despite the similar affinity of IDE for Aβ analogs, Aβ 1-42 may partially escape proteolytic degradation by IDE and other proteases because of self-aggregation (39,13,40).

The significant decrease of IDE activity in AD brain soluble fraction as compared to age-matched controls was the most notable finding in this work. However, the impossibility of detecting intact IDE prevented us to conclude whether IDE levels were diminished in AD brains. Two factors may account for the relatively low levels of IDE activity in our samples: the postmortem delay and aging. Supporting the latter, lower IDE activity in rat tissues related to aging has been shown (41). However, these factors cannot explain the differences in IDE activity between control and AD brains, since both postmortem delay and age were similar between groups.

Our finding raises the question of whether the lower IDE activity in AD brain is a primary defect or the result of a neurodegenerative process. Recently, IDE immunoreactivity has been described in cortical and subcortical neurons from human brain, but not in glia (16). Neuronal IDE immunostaining was stronger in AD brains, as compared to control brains, particularly in neurons associated with senile plaques. Remarkably, extracellular deposits of IDE in senile plaques were also found. These observations are consistent with our results of lower IDE activity in the cytosolic fraction of AD brains, and may reflect the abnormal localization of IDE to an insoluble compartment (i.e. fibrillar deposits), as reported for regulatory subunits of the proteasome (42). Alternatively, the decreased activity of IDE may be related to cell loss (particularly neurons) in AD brains.

An impaired Aβ degradation as a mechanism that contributes to Aβ accumulation in the brain is an attractive hypothesis, since Aβ overproduction seems not to be involved in non familial, sporadic AD (see (43) for discussion). Supporting this, the expression and/or activity of different proteases related to Aβ processing or degradation appears to be altered in AD brains: the levels of cathepsin D are increased, probably related to regenerative or repair processes (44); the amount of the latent form of neuronal MMP-9 is increased and thus the lack of enzyme activation may contribute to Aβ accumulation (9) and thimet oligopeptidase, which degrades acetyl-EVKMDAEF-NH_2 (residues 593–600 of AβPP), is diminished (45).

The wide tissue distribution of IDE, its high degree of evolutionary conservation and its association with growth, development and cellular differentiation (19,46) suggest that this protein plays an important role in cellular functions in addition to its proteolytic activity. IDE has been proposed as an intracellular receptor for growth factors (47). Hamel et al have shown that a direct interaction between IDE and insulin results in the inhibition of the 20S proteasome proteolytic activity, suggesting that such interaction regulates cellular protein turnover (48). Moreover, Kupfer et al have reported that IDE increases DNA binding of steroid receptors and that this effect can be reversed by insulin (49). It remains to be addressed whether these IDE-modulated processes participate during brain aging in normal and pathological conditions such as AD. In this context, the study of brain IDE expression, activ-

ity and interactions may contribute to unravel mechanisms of neuronal degeneration that are not directly related to Aβ fibrillar deposition.

ACKNOWLEDGMENTS

We thank Rubén laccono for technical assistance in densitometric analysis. This study was supported by a grant from Alberto J. Roemmers Foundation.

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