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Alzheimer's β-amyloid peptide specifically interacts with and is degraded by insulin degrading enzyme

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Abstract

Cerebral deposition of β-amyloid peptide (βA) is a hallmark of Alzheimer's disease. Concentration of βA could play a critical role in the rate of amyloid deposition. It is therefore of considerable importance to identify proteases involved in processing of βA. ^{125}I-labeled synthetic βA specifically cross-linked to a single protein with M_r = 110,000 in cytosol fractions from rat brain and liver. This protein was identified as insulin degrading enzyme (IDE) since the labeling of the 110 kDa protein was completely blocked by an excess of insulin, and anti-IDE monoclonal antibodies precipitated the labeled protein. Purified rat IDE effectively degraded βA.

Key words: Alzheimer's disease; β-Amyloid; Insulin degrading enzyme; Oxidative modification; Protein degradation; Brain; Rat

1. Introduction

Alzheimer's disease amyloid is composed of a 39–43 residue β-amyloid protein (βA) [1–3] that is derived from a set of larger transmembrane precursor proteins (βAPPs) [4,5]. Growing evidence is accumulating that processing of βAPP may occur intracellularly [6–8] and that βA is a normal product of cell metabolism [9–11]. A recent finding that cultured human neurons generate βA intracellularly before it is released into the medium [12] suggests the possibility that βA accumulation in Alzheimer's brain could result from defects in intracellular proteolytic machinery responsible for its breakdown. Thus, protease(s) involved in processing of βA is a potential target for pharmacological treatment of the disease.

We considered that among intracellular proteases which could participate in degradation of βA, the insulin degrading enzyme (IDE; EC 3.4.22.11) is a very likely candidate. IDE is a highly selective protease involved in the breakdown of certain peptide hormones [13–15]. IDE does not have a strict specificity for any amino acid residue [16]. It has been suggested, however, that its specificity is based on recognition of specific secondary structures rather than specific peptide bonds [16]. It is interesting to note that although the IDE substrates, insulin, atrial natriuretic peptide and glucagon, are greatly different in primary structure, they probably share a common structural motif allowing them to form amyloid fibrils under certain conditions in vitro [17] and in vivo [18,19]. In amyloid fibrils, different proteins have been shown to be organized in a cross-β conformation [17]. Therefore, IDE specificity could be based on recognition of β-structures either present on the surface of the substrate molecules or adopted upon binding to the protease. This possibility suggests that IDE might also degrade βA.

Here we show that βA is a substrate for rat liver IDE. Furthermore, we demonstrate that IDE is the only protein that can be specifically cross-linked to βA in crude extracts of rat brain and liver, suggesting for a role for IDE in the cellular processing of βA.

2. Materials and methods

2.1. Materials

The following materials were obtained: [^{125}I]insulin (1.2 kCi/mmol) from Dainabott (Tokyo, Japan), βA_{1-28} and βA_{1-40} from Bachem California (USA). Monoclonal antibodies to IDE (9B12) were kindly provided by Dr. R. Roth (Stanford University, USA) or purified from ascites fluid provided by Dr. K. Shii (Kobe University, Japan) on protein G-Agarose. βA_{1-28} was radiolabeled by the chloramine-T method using Na^{125}I (Amersham, England) and separated from unlabeled peptide and free iodine by reverse-phase HPLC. Bovine serum albumin was added immediately to the eluted fractions. Then, trifluoroacetic acid (TFA) and acetonitrile were removed under a gentle stream of N_2.

2.2. Degradation of βA_{1-40} and βA_{1-28} by IDE

IDE was purified from the cytosol fraction of rat liver in four chromatographic steps (i.e. DEAE-Toyopearl, hydroxylapatite, pentyl-Agarose, TSK 3000 SW HPLC). The preparations of IDE were homogeneous as analyzed by SDS-PAGE (Kurochkin and Goto, submitted). Freshly dissolved peptide solutions were used. Prior to degradation assay, synthetic peptides were centrifuged at 15,000 × *g* for 10 min. No evidence of precipitates was observed. The enzyme (0.2 μg) was incubated either with 1.2 nmol (5 μg) of synthetic βA_{1-40} or 3.4 nmol (11 μg) of βA_{1-28} in 70 μl of 50 mM Tris-HCl buffer (pH 7.0) at 37°C. At various times, degradation was stopped by the addition of 0.7 ml of 0.06% TFA, and the entire sample was injected into the RP-318 reverse-phase HPLC column (particle size 5 μm, column dimensions 250 × 4.6 mm) (Bio-Rad). The loaded column was eluted with a 0–80% linear

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gradient of acetonitrile in 30 ml of 0.06% TFA at a flow rate of 0.5 ml·min^{-1}. Elution was monitored by the absorbance at 220 nm.

2.3. Cross-linking conditions

[^{125}I]Insulin (0.2 pmol) together with 50 μg of cytosol fraction (prepared by centrifugation of tissue homogenates at 100,000 × g for 60 min) was incubated at 0°C in the absence or presence of 10^{-5} M of unlabeled peptides in a total volume of 50 μl PBS (20 mM sodium phosphate, pH 7.4, and 150 mM NaCl). After 30 min, 3 μl of disuccinimidyl suberate (8.8 mM in 25% dimethylsulfoxide) was added [23]. The reaction was continued for 30 min at 0°C and was stopped by addition of 14 μl of denaturing buffer containing 5% SDS and 25% 2-mercaptoethanol. Different from [^{125}I]insulin cross-linking, [^{125}I]βA_{1-28} (0.2 pmol) was pre-incubated with 50 μg of cytosol fractions (50 μl) for 10 min at 24°C following incubation with cross-linker for 15 min at the same temperature. In addition, the mixture additionally contained 2 mM 1,10-phenanthroline and 5 mM EDTA to inhibit IDE activity. Samples were then heated at 100°C for 3 min and analyzed by SDS-PAGE on 8% separation gel [20]. The gel was stained, destained, dried and exposed to Image Plate (BAS 2000; Fuji, Japan). When [^{125}I]βA_{1-28} was cross-linked with purified IDE the concentration of disuccinimidyl suberate in the cross-linking mixture was 1 mM.

2.4. Immunoprecipitation of cross-linked protein

[^{125}I]βA_{1-28} was cross-linked to a cytosolic fraction from rat brain. The reaction was stopped by the addition of 1 μl of 1 M Tris-HCl (pH 7.4) per 50 μl of the mixture. Then aliquots (25 μl) of the mixture were incubated with various concentrations of either anti-IDE monoclonal antibody 9B12 or normal mouse IgG overnight at 4°C in 100 μl of PBS containing 0.2% BSA, 1 mM PMSF and 0.01% NaN_3. The immune complex was then precipitated with 30 μl of protein G-PLUS-Agarose (Oncogene Science Inc.). The supernatants and washed immunoprecipitates were analyzed by electrophoresis and autoradiography.

2.5. Oxidative modification of lysozyme

Oxidative modification was carried out by a metal-catalized oxidation system with hen egg white lysozyme at a concentration of 5 mg/ml in a buffer containing 25 mM ascorbate, 0.1 mM $FeCl_3$, 1 mM EDTA, 100 mM NaCl and 50 mM potassium phosphate, pH 7.5. Incubation was performed at 37°C with shaking for 4 h. At the end of the incubation period the sample was gel-filtrated on a Sephadex G-25 column equilibrated with 50 mM Tris-HCl, pH 8.5, at room temperature. The protein fraction separated from the $FeCl_3$/ascorbate system was used for experiments.

3. Results and discussion

In our experiments, we used a synthetic full-length βA (βA_{1-40}) and a shorter peptide corresponding to residues 1–28 of βA (βA_{1-28}). βA_{1-40} is known to have a high β-sheet content at physiological pH [21]. Monomeric βA_{1-28} in solution is a mixture of α-helices and random coils [21] but it readily assumes a β-pleated conformation at high concentrations, forming amyloid fibrils morphologically resembling those seen in Alzheimer's neurite plaques [22].

Endogenous IDE in cytosols from different tissues could be specifically cross-linked to [^{125}I]insulin [23]. Addition of βA_{1-28} to the cross-linking mixture prevented the labeling of IDE (Fig. 1a, lane 3), indicating that βA_{1-28} interacts with the enzyme. When purified rat IDE was incubated with synthetic βA_{1-40} and βA_{1-28}, the peptides were effectively hydrolyzed (Fig. 2a,b). The generation of at least eleven proteolytic fragments from βA_{1-28} (Fig. 2b) supports the view [16] that IDE is non-specific in hydrolyzing particular peptide bonds. These experi-

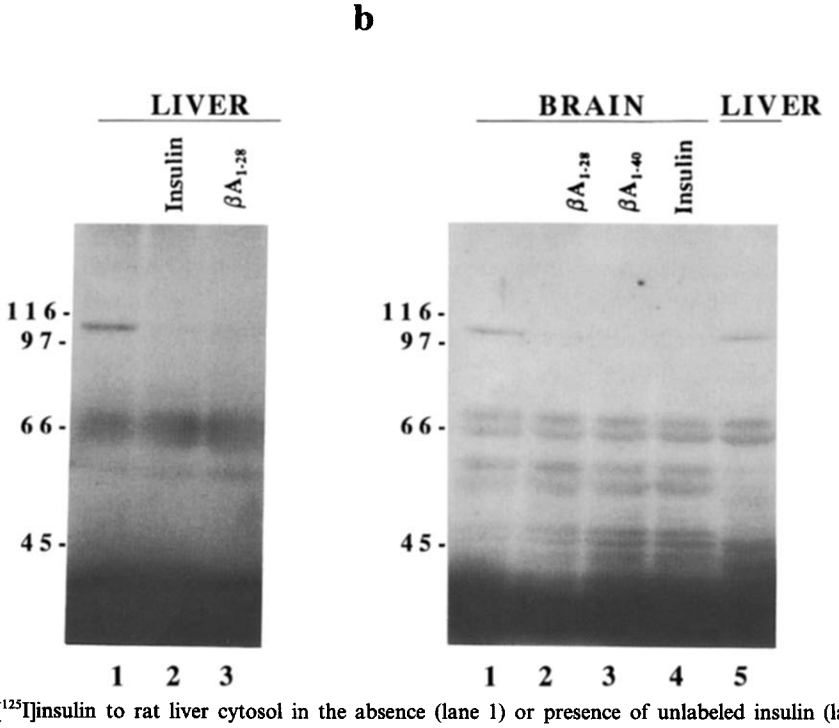

Fig. 1. (a) Cross-linking of [^{125}I]insulin to rat liver cytosol in the absence (lane 1) or presence of unlabeled insulin (lane 2) or βA_{1-28} (lane 3). (b) Cross-linking of [^{125}I]βA_{1-28} to rat brain or liver cytosols in the absence (lanes 1, 5) or presence of unlabeled βA_{1-28} (lane 2), βA_{1-40} (lane 3) or insulin (lane 4). The radioactivity in the 66,000 molecular weight range corresponds to bovine serum albumin present in the [^{125}I]insulin and [^{125}I]βA_{1-28} preparations. The radioactive band observed at the dye front is due to the non-cross-linked ^{125}I-labeled peptides. The numbers on the left indicate $M_r \times 10^{-3}$.

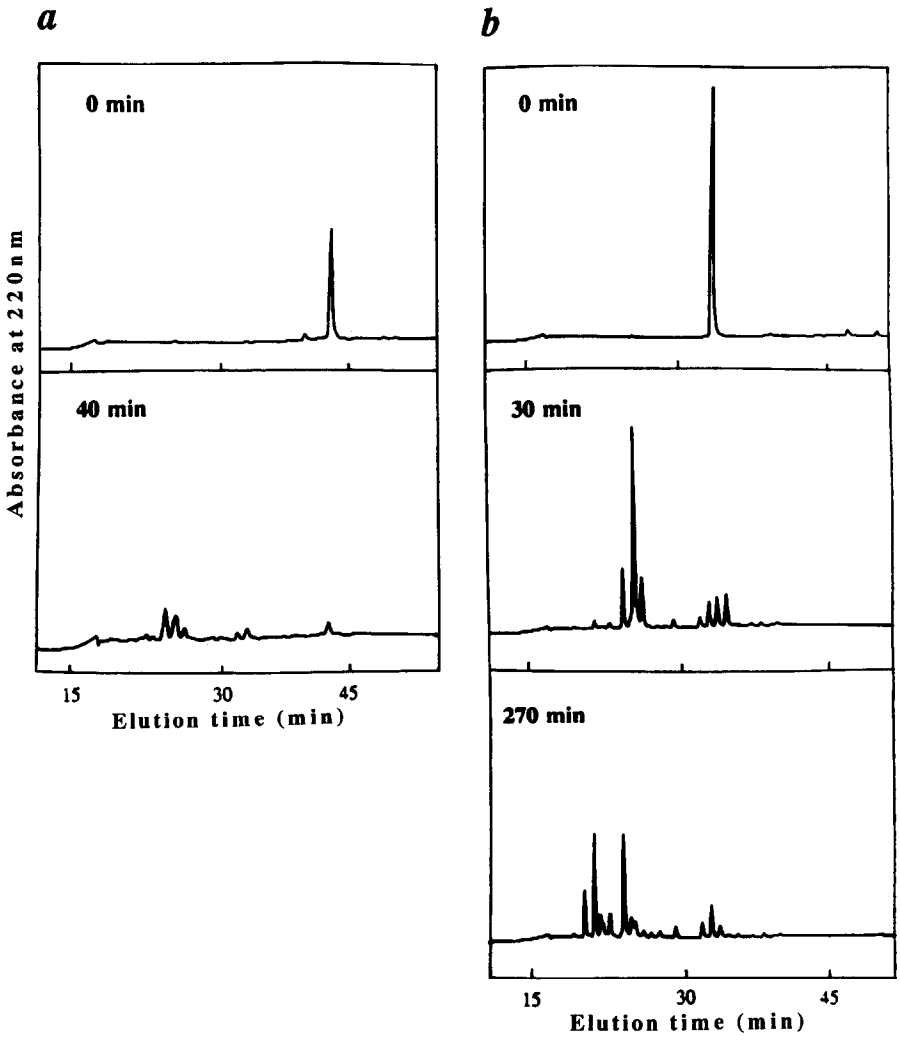

Fig. 2. HPLC profiles of βA_{1-40} (a) and βA_{1-28} (b) after incubation with IDE for indicated periods of time.

ments, however, do not exclude the possibility that other cytosolic proteases participate in the breakdown of βA. Therefore, we performed covalent cross-linking of radioiodinated βA in the presence of tissue extracts. In the presence of either brain or liver cytosol fractions [^{125}I]βA_{1-28} was specifically cross-linked only to one protein of 110,000 kDa (Fig. 1b, lanes 1,5). This specificity was confirmed by the fact that the 110 kDa labeled band was abolished by the addition of excess of unlabeled peptide (Fig. 1b, lane 2). In addition, cross-linking of [^{125}I]βA_{1-28} to the 110 kDa protein was inhibited by the addition of unlabeled full-length β-amyloid peptide, βA_{1-40} (Fig. 1b, lane 3) or insulin (Fig. 1b, lane 4) to the mixture. When the cross-linking mixture did not contain metal chelators known to inhibit the hydrolyzing but not the substrate-binding activity of IDE [24], no ^{125}I-labeled 110 kDa band was observed (data not shown). This fact, in addition to proof of the highly specific interaction of βA with IDE, demonstrates the high susceptibility of the peptide to IDE action, that the interaction of βA with the protease results in such rapid hydrolysis that the cross-linking reaction has no time to occur. Although the molecular weight of the cross-linked band coincided with that of IDE, convincing evidence of its identity as IDE was obtained by immunoprecipitation experiments. Anti-IDE monoclonal antibody 9B12 [13] precipitated the [^{125}I]βA_{1-28}·110 kDa protein complex from brain cytosol fractions (Fig. 3). No bands were seen precipitating with normal mouse IgG (data not shown).

The finding that βA is a highly specific substrate for IDE raises the possibility that factors which may affect the activity of this protease could cause an increase in the overall amount of βA and as a result lead to the formation of insoluble amyloid deposits. Among these factors are other substrates which could compete for binding to the enzyme. Recent studies suggest that IDE is likely to be involved in the removal of oxidatively damaged proteins ([25]; Kurochkin and Goto, submitted) the progressive accumulation of which in aged brain is well documented [26]. Oxidized but not native lysozyme inhibited cross-linking of [^{125}I]βA_{1-28} to purified IDE (Fig. 4), suggesting that abnormal proteins accumulating in aged

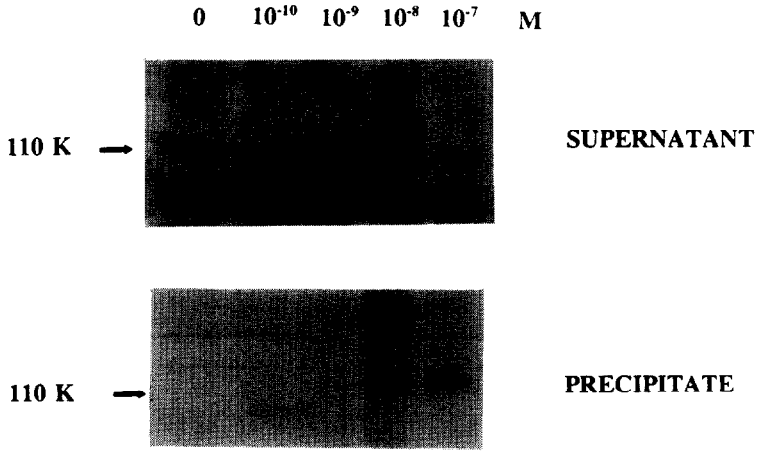

Fig. 3. Immunoprecipitation of the cross-linked 110 kDa protein with anti-IDE monoclonal antibody 9B12. The numbers on the top indicate the concentration of the antibody.

brain may inhibit the degradation of βA. Alternatively, over-production of βA from the precursor protein would cause accumulation of abnormal proteins and as a result lead to the degeneration of nerve cells.

We must also take into account a possible effect of pH on IDE activity. Decreased pH values (pH 6.6) are found in the brain from patients who have died from Alzheimer's disease [27]. We have demonstrated that binding of βA to IDE is highly sensitive to a reduction in pH. The binding activity was reduced by 89% at pH 6.4 and by 63% at pH 6.6 compared with that at pH 7.4 (data not shown). Thus, slight acidification of the cytoplasm may alone significantly promote accumulation of βA by inhibition of its binding to IDE.

The fact that a naturally occurring 16 kDa fragment containing the βA and the C-terminus of βAPP adopts the tertiary structure of βA and has amyloidogenic properties [28] raises the possibility that IDE could be involved in its degradation and of other immediate precursors for βA. It remains unclear where βAPP processing occurs in neurons. Both the endosomal–lysosomal [6,7] and secretory [8] pathways are suggested to be involved in the generation of βA. Although IDE is primarily a cytosolic enzyme, it may be incorporated into the endosome during vesicle formation and degrade internalized receptor-bound insulin before endosome acidification [29]. Identification of the subcellular compartment where IDE may come into contact with βA remains to be determined.

Our results suggest an important role for IDE in the genesis of Alzheimer's disease and raise the interesting possibility of using this highly specific protease as a therapeutic agent to slow the progress of the disease by reducing βA concentration.

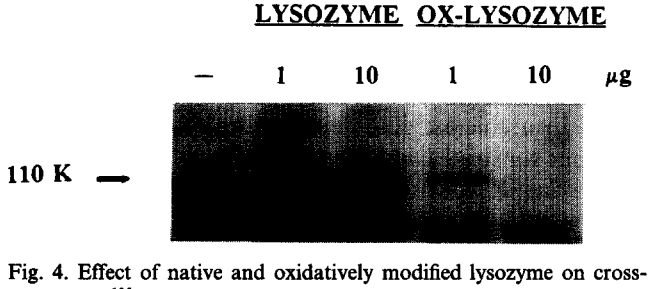

Fig. 4. Effect of native and oxidatively modified lysozyme on cross-linking of [^{125}I]βA_{1-28} to IDE. Purified IDE (10 ng) was cross-linked with [^{125}I]βA_{1-28} in the absence or presence of indicated amounts of either native or oxidatively modified lysozyme and then analyzed by SDS-PAGE and autoradiography.

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