Zyme, a Novel and Potentially Amyloidogenic Enzyme cDNA Isolated from Alzheimer's Disease Brain*

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The generation of the β amyloid peptide is thought to be the result of processing of the amyloid precursor protein (APP)1 by one or more proteases. After the deduced amino acid sequence of APP was revealed, a number of laboratories initiated studies to purify and characterize the N-terminal cleaving enzyme of amyloid β-protein (Aβ), termed β-secretase (1). The cleavage of the Met⁵⁰⁶-Asp⁵⁰⁷ bond of the full-length APP generates the N-terminal amino acid of Aβ, which was first shown by Glenner and Wong (2) to be aspartic acid. β-Secretase is yet an unidentified protease.

Several themes and strategies influenced the direction of investigation of β-secretase. The first strategy was to follow a traditional biochemical purification. Assays were utilized in which short peptide substrates were substituted for the large transmembrane precursor protein (1). Any enzyme capable of making a methionine (M)/aspartic acid (D) cleavage could be designated a potential β-secretase. The second theme, since the amino acid that surrounded the N terminus of Aβ was found to be a methionine, was some laboratories have rationalized that a search for an enzyme with chymotrypsin-like specificity (a specificity for cleavage of substrates containing a neutral hydrophobic residue at the S1 subsite) was necessary (3–7).

To facilitate the second approach, we have developed a method to identify chymotrypsin-like enzymes. The amino acid sequences of several chymotrypsin-like enzymes were compared, and regions of at least four amino acids of homology were identified. Specifically, the deduced amino acid sequences of rat and mouse mast cell proteases, human cathepsin G, granzymes, and chymotrypsin were compared, and consensus sequences were identified. The motifs TAAHC, DIMLL, and GDSGGPL contain the active histidine (H), aspartic acid (D), and serine (S), which are part of the active site/charge-relay system of serine proteases. Oligonucleotides directed toward these active site regions were used as primers for the polymerase chain reaction amplification of relevant cDNAs.

A novel serine protease, given the name Zyme, has been identified using this technology from Alzheimer’s disease brain. The Zyme cDNA sequence is highly homologous to human trypsinogen s I-IV and human kallikreins (like prostate-specific antigen) and is tissue-specific for brain, kidney, and salivary gland. Zyme sequence is conserved in many mammalian species but is not detected in rats, mice, and hamsters. It is expressed predominantly in normal and AD adult brain and is not detected in human fetal brain. The Zyme gene maps to chromosome 19q13.3. When Zyme cDNA was co-expressed with the APP 695 cDNA in 293 cells (human embryonic kidney cells), amyloidogenic fragments were detected by Western blot analysis using antibody to the C terminus of APP. The co-transfected cells release an altered pattern of Aβ products. Zyme can be immunolocalized to perivascular cells in primate and AD brain tissue. Since perivascular cells are thought to be the progenitor cells to microglial cells, the potential role for Zyme in AD pathology may evolve from its activity in activated microglial cells known to surround neuritic plaques.

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1 The abbreviations used are: APP, amyloid precursor protein; AD, Alzheimer’s disease; Aβ, amyloid β-protein; PCR, polymerase chain reaction; bp, base pair(s); kb, kilobase(s); GAPDH, glyceraldehyde-3-phosphate dehydrogenase; DMEM, Dulbecco’s minimal essential medium; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; FISH, fluorescence in situ hybridization; apoE, apolipoprotein E; P3, residues 17–42 of Aβ.
EXPERIMENTAL PROCEDURES

Materials—All enzymes, commercially available plasmids, and cell culture media were purchased from Life Technologies, Inc. unless otherwise indicated. All radioisotopes were purchased from NEN Life Science Products. Tricine gels and buffers for SDS-polyacrylamide gel electrophoresis were purchased from ISS Inc. and Novex. Reagents for the ECL-Western blot analysis were purchased from Amersham Corp. Reagents for the polymerase chain reaction (PCR) were obtained from Perkin-Elmer. Human poly(A)+ messenger RNA was purchased from CLONTECH. Brain tissue (ad and normal) and APP C-terminal rabbit antiserum (B2X6) were the kind gifts of Athena Neurosciences. Brain tissue was obtained from normal adult female rhesus monkeys.

Methods—Library screening, 32P-labeling, ligations, restriction digests, the kinase reaction, and all other molecular biology techniques were performed as described in Sambrook et al. (8) unless otherwise stated.

Identification of Homologous Regions of Chymotrypsin-like Proteases—LINEUP and PRETTY algorithms of the Wisconsin Genetic Computer Group Program Package (9) were used to identify consensus sequences of some chymotrypsin-like enzymes. Human cathepsin G, granzymes, chymotrypsin, and mouse and human mast cell protease amino acid sequences were aligned, and the following consensus sequences were revealed: HIGG, HSFRSPYMA, CGGFIL, TAAHC, DIIML, TLREV, GDSGGPL, and VAHGI. Only TAAHC, DIIML, and GDSGGPL (active site homologous regions) were used for PCR primer design.

PCR Identification of Protease cDNA and Cloning—The sequence of the PCR primers for molecular enzymology experiments were essentially based on Lathe’s rule (10). TAAHC = GTG (A/O/TG ACA GCT GCC CTC, TAAHC (forward) and TAAHC (reverse) CAG CTG/CTG CAG CAT GTC, and GDSGGPL = CAG GGG GCC GCC GGA GTC GCC. Additional bases were added to increase the length of the PCR primer. Total RNA was extracted from human brain superior frontal gyrus using RNAgent’s Total RNA Isolation Kit (Promega), and complementary DNA was generated using random hexamers essentially as described by Gerard et al. (11). PCR amplification was performed at 94 °C for 1 min, 52–55 °C for 1 min, and 72 °C for 3 min for 30 cycles using the TAAHC and GDSGGPL primers. PCR products were analyzed by Southern blot hybridization to an internal probe (DIIMLL primer). PCR products bands that were 450–500 bp and hybridized to the internal probe were excised, and a second round of PCR amplification was conducted using the TAAHC probe and the DIIMLL (reverse) probe. PCR products that were 160–180 bp in size were subcloned into pUC18 and pGEM3Z, transformed into DH5α, and then sequenced. Clone 56Z contained an open reading frame containing active site homologous regions consistent with a serine protease and will hereby be referred to as Zyme. DNA sequence from clone 56Z was used as template for subsequent hybridization experiments.

Construction of cDNA Library—A cDNA library was constructed using AD brain mRNA with the Superscript Lambda System for cDNA Synthesis and Lambda Cloning (Life Technologies, Inc.). PCR primers representing the unique DNA sequence of clone 56Z (ZYMIE 3, 5′-AACCGAATTCGAGGTCTCGG-3′, and ZYMIE 4, 5′-ATGCGTG- CCGCGATCGATCGAGGC-3′) were used to create an α-32P-labeled probe for library screening essentially as described by Schwalter and Sommer (12). This probe will be subsequently referred to as Zyme 3–4. Out of 1 × 106 phage plated, one clone of 1451 bp was isolated. DNA sequence was determined by Lark Sequencing Technologies, Inc.

Cloning of Zyme Genomic Clone and Chromosome Localization—A human chromosome 19 genomic library was purchased from ATCC (No. 57711) with the Escherichia coli host strain LE392 (No. 33572). The Zyme 3–4 radiolabeled probe was generated as described under “Experimental Procedures.” A genomic clone was isolated by conventional screening of 2 × 106 phage (8). DNA from the positive clone, 66zyme, containing an insert of 4.2 kb was digested from Charon 21A using HindIII and subsequently subcloned into pUC-18. The 4.2-kb fragment was then random primed for synthesis of a probe used for chromosome localization by fluorescence in situ hybridization (FISH) analysis by BIOS Laboratories (New Haven, CT).

In Vitro Transcription—A Zyme cDNA fragment spanning nucleotides 169–336 was subcloned into the SpeI cloning site of pGEM-3Z. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) template for in vitro transcription was purchased from Ambion Inc. For in vitro transcription of suitable antisense cRNA, pGEM-3Z was linearized with HindIII. Template cDNA was gel purified after separation on a 1% Tris borate/EDTA-agarose gel using the Prep-a-gene (Bio-Rad). In vitro transcription was performed according to the manufacturer protocol (Promega) except that 50 μCi of [32P]UTP at 400 Ci/mmol were used in the absence of cold UTP. Transcription reactions were incubated at 37 °C for 60 min and then the cDNA template was removed by the addition of 1 unit of RNase-free DNase. Unincorporated [32P]UTP was removed by size fractionation over a Sephadex G-50 RNA column, and the in vitro transcribed probe was quantitated using a Beckman liquid scintillation counter.

RNase Protection Analysis—RNase protection assays were performed according to the manufacturer instructions (Ambion Inc.) with minor modifications. Twenty μg of total RNA were incubated with the radiolabeled cRNA probes in hybridization buffer (80% deionized formamide, 100 mM sodium citrate, pH 6.4, 300 mM sodium acetate, pH 6.4, and 1 mM EDTA), heated for 3 min at 95 °C, and then hybridized overnight at 42 °C. Single-stranded RNA and free probe were digested by 200 units/ml RNAse A and 200 units/ml RNAse T1 for 30 min at 37 °C. The RNase was inactivated with the addition of 300 μl of inactivation/precipitation buffer. The samples were precipitated by incubating at −20 °C for 2 h, followed by centrifugation at 10,000 × g for 15 min at 4 °C. The protected fragments were resuspended in loading buffer, heated to 95 °C for 3 min, electrophoresed on Tris/borate/EDTA-polyacrylamide/urea gels, dried, and exposed to x-ray films at −80 °C.

Manuscript Expression Vectors and DNA Transfection—The 1.4-kb Zyme cDNA was cloned into the eukaryotic expression vector pGEMCI (Invitrogen). The expression vectors containing APP 695 and APP 751 have been previously described (13). Cultured 293 cells (ATCC No. CRL 1573) were transfected in 6-well dishes using the calcium phosphate precipitation method of Chen and Okayama (14) in medium containing Ham’s F-12/DMEM (Dulbecco’s modified Eagle’s medium, Life Technologies, Inc.), 1:1, with 5% fetal calf serum. After transfection, medium was changed, and cells were incubated for 72 h at 37 °C in a 5% carbon dioxide, 95% air atmosphere. Cell pellets and culture media were harvested in Tricine sample buffer in preparation for Western blot analysis.

Recombinant Zyme Activation, Purification, and Substrate Specificity—Human 293 cells transfected with a plasmid containing the 1.4-kb Zyme cDNA were subjected to selection by growth in G418 (400 μg/ml) for 3 weeks, after which time stable transformants were isolated. Clone 567 was used as a source of Zyme protease medium. Zyme has a putative activation peptide, EEQNK, analogous to the activation peptide of other serine proteases. Auto-activation of the enzyme was accomplished by concentrating the cell culture supernatants approximately 10-fold and then leaving the concentrate at 4 °C for approximately 1 week before purification. Purification of Zyme from the concentrated cell culture supernatants was achieved by reversed-phase high pressure liquid chromatography (C-8, Aquapore RP-300, 0.45 × 25 cm, Waters Associates, Milford, MA) using a linear gradient of acetic acid/acetonitrile. Generally, the gradient increased at a rate of 1% acetonitrile/min. Fractions containing Zyme were located by SDS-polyacrylamide gel electrophoresis, collected, lyophilized, and stored at −20 °C (15).

Western Blot Analysis—Western blot analysis was conducted essentially as described by Johnstone et al. (16). Rabbit anti-Zyme antibody (No. 274) was prepared by immunization with a peptide representing residues 72–88 of the deduced amino acid sequence of Zyme (Research Genetics, Huntsville, AL).

Metabolic Labeling, Immune Precipitation, and Gel Fluorography—Twenty-four h after transfection, cells were incubated for 2 h in methionine-free, serum-free DMEM and labeled overnight with 300 μCi/ml [35S]Met (NEN Life Science Products) in serum-free DMEM. Cell culture medium was subjected to immune precipitation with the monoclonal antibody 4G8 (Sembach et al. 17). Monoclonal 4G8 is reactive to amino acid residues 17–24 of β amyloid peptide. Immune complexes were boiled in reducing SDS sample buffer, electrophoresed on a 12.5% Tris-Tricine gel (Daiichi) and subjected to autoradiography.

Immune Localization of Zyme—Rhesus monkey brain (n = 3) was perfusion fixed and frozen. Human AD brain was immersion fixed, cryoprotected, and frozen. Four separate cortical regions were analyzed using 22 kDa phase antibody 274 and an immunoperoxidase stain (ABC kit). For double immunostaining, sections of AD cortex were first labeled with Zyme antisera (1:500) using an avidin-biotin detection system. Subsequently, sections were incubated with monoclonal antibody to von Willebrand’s factor VIII (1:10; Boehringer Mannheim) or to HLA-DR (prediluted; Biogenex Labs, San Ramon, CA). The monoclonal was visualized using an alkaline phosphatase-fast red detection system. Sections were mounted in 70% glycerol and examined using a Nikon Microphot microscope equipped with Nomarsky optics.
RESULTS AND DISCUSSION

Molecular Enzymology—We have designed PCR primers to the active site amino acid sequence of those enzymes that are thought to be chymotrypsin-like and have identified serine proteases. Our primers reflected codon usage (Lathe's rules (10)) rather than degeneracy. The first subcloned PCR product was identical in sequence to human tissue plasminogen activator, serine protease (data not shown). The second PCR product represented the unique Zyme sequence. Only 1 phage in 10^6 was identified in our human brain cDNA library.

The Zyme transcript consists of 1451 bp, and the open reading frame encodes a protein of 244 amino acids (Fig. 1A). Residues surrounding the presumed initiation codon at AUG (CGGCCAUGA) are consistent with the Kozak consensus sequence (CC(G/A)GCCAUGG) (18, 19). One polyadenylation site is seen at 1245 bases. The active site homologous sequences are shown as TAAHC, DIMLL, and GDSGGPL. The chymotrypsin-like consensus sequences, IIGG and PHSRPYMA, which were not a part of the active site, were not conserved. Amino acid residue 136 is a possible site for Asn-linked glycosylation.

A hydropathic analysis of Zyme in Fig. 1B shows an extremely hydrophobic region representing the first 20 residues, a pre-
Dendrogram of multiple pair wise alignments of other human serine proteases. The PILEUP algorithm was used to generate the plot of Zyme-related enzymes. GenBank(TM)/EBI accession numbers are as follows: cathepsin G, P06868; granzyme B, P08311; complement factor D, P00746; granzyme A, P12544; glandular kallikrein 2, P20151; prostate specific antigen, P07288; glandular kallikrein 1, P06870; trypsinogen IV, P35030; trypsinogen III, P15951; trypsinogen II, P07478; trypsinogen I, P07477; and chymotrypsin, P40313.

When the amino acid sequence was analyzed using the algorithm FASTA, Xenopus laevis trypsinogen was found to have the highest identity with Zyme (45%). To predict the phylogenetic relatedness of Zyme with other human serine proteases, enzymes identified by FASTA were used in the PILEUP program of the Genetic Computer Group Package software (Fig. 2). The Zyme amino acid sequence was aligned with a group of proteases that does include chymotrypsin, human trypsinogens I-IV, the glandular kallikreins 1 and 2, and prostate-specific antigen. Enzymes like cathepsin G and granzyme A showed less similarity to Zyme.

To better characterize the predicted structural motifs of Zyme, enzymes found to be similar to Zyme were aligned using the ALIGNMENT software of GeneWorks (IntelliGenetics, Inc.). Fig. 3 shows the output alignment. Zyme appears to show homology with several regions of the kallikreins as well as the trypsinogens. In the region shown by a solid bar, residues VPFDDK of trypsin IV represents the activation peptide domain, whereas PLILSR represents the activation peptide of prostate-specific antigen (20). The Zyme-predicted activation peptide shows little similarity to either pro-peptide. 10 of the 12 cysteine residues align well with cysteine residues in the kallikreins and trypsinogens. Two cysteine residues, as noted with stars, are not in alignment and may represent other regions of Zyme-specific interaction. The amino acid sequence surrounding the active site cysteine suggests that Zyme is more closely related to the kallikreins than the trypsinogens. The stippled region indicates an 11 amino acid loop characteristic of the kallikreins not found in the Zyme primary sequence. The adjacent aspartic acid active site (residues 59–63) shows conservation and similarity to both the kallikreins and the trypsinogens. The serine active site (residues 195–205) shows remarkable similarity as well. The above discussion is meant to simply show predictive properties of Zyme based on homology with other proteases.

Evolution and Tissue Distribution—To determine the evolutionary relatedness of Zyme DNA sequence, a blot containing genomic DNA of multiple species was hybridized under stringent conditions (8) with the Zyme 3–4 probe (Fig. 4). The degree of relatedness is shown by the presence of comparable hybridization signals. It appears that bands of human and marmoset DNA gave signals of similar intensity, whereas the bands of cat and dog DNA share weak homology and gave less intense signals. Hybridization with cat and dog may indicate the presence of other related sequences. Note that some species showed no homology with Zyme. The fact that the human probe did not hybridize with several mammalian genomic DNAs may show that Zyme shows species specificity. When the complete Zyme cDNA is used as a probe, some bands are visible in all species under reduced stringency (data not shown).

RNase protection analysis, using a probe consisting of nucleotides 169–336, was conducted to determine the tissue distribution of Zyme transcripts. A survey of several tissues (liver, kidney, pancreas, salivary gland, spleen, and testes) shows that more Zyme transcript is present in brain than other organs (Fig. 5). It is interesting to note that Zyme RNA is not detectable in human fetal brain but is present in the adult brain tissue.

Transcripts from AD and normal brain were compared by RNase protection analysis to a “housekeeping gene,” GAPDH (Fig. 6). The overall ratio of Zyme transcript to GAPDH transcript is higher in AD than in normal cortex. This suggests that Zyme transcripts may be elevated in AD over the level of a major glycolytic enzyme, GAPDH. Later in our discussion of Zyme immunolocalization, we will show that Zyme is present in perivascular and microglia; therefore, perhaps the elevation of Zyme transcription may reflect the changes toward the active state of microglia known to exist in AD pathology (1).

Chromosomal Localization and FISH—The chromosomal localization of Zyme was determined by Southern analysis of DNA from a somatic hamster/mouse cell hybrid panel (BIOS Laboratories). The Zyme 3–4 probe, described under “Experimental Procedures,” hybridized only to human specific bands. Discordance analysis (Table I) allows localization of Zyme to chromosome 19 (the lowest percent discordance is 0.4). FISH further confirmed localization to chromosome 19q13.3 (BIOS Laboratories) (Fig. 7). A total of 85 metaphase cells were analyzed using the 4.2-kb Zyme genomic clone 66zyme as probe. Another marker, E2A (21), was used to co-localize the Zyme gene to the long arm of chromosome 19. The region 19q13.1–19q13.3 is thought to be a locus for familial Alzheimer’s disease (22). The apolipoprotein E gene map location is 19q13.2. Although the apoE 4 is strongly associated with AD in late onset familial AD families, Yu et al. (23) have used linkage analysis to demonstrate that the apoE region did not cosegregate with AD in a collection of late onset AD cases, suggesting that apoE is not the major locus. It is likely that Zyme is part of the locus of tissue-specific kallikreins (19q13.3), such as prostate-specific antigen (24), and yet its role in familial AD cannot be ruled out until tested.

Is Zyme Activity Amyloidogenic?—C-terminal APP fragments were examined as potential amyloidogenic fragments during Zyme co-transfection experiments. For clarity of discussion of our results, we have operationally defined the amyloidogenic fragments by size, encompassing a region large enough to contain Aβ or portions of Aβ and the contiguous C terminus of APP. The resolution of C-terminal fragments using 12.5% Tricine-polyacrylamide gels presents the a-secretase fragment (25) as 10 kDa. A fragment representing the Cys106 residues has an apparent molecular mass of 14 kDa. APP C-terminal
fragments greater than 14 kDa are thought to contain additional N-terminal residues as well. Western blot analysis was conducted using a well characterized C-terminal antibody, BX6 (13).

Co-transfection experiments were performed using APP 695 and APP 751 cDNA and Zyme cDNA in 293 cells. The duration of the transient assay was 48 h, at which time cells and culture media were harvested and analyzed by Western blots using antibody to the C terminus of APP (BX6) or to the Zyme primary sequence (No. 274). The Western blot in Fig. 8 shows that the \( \alpha \)-secretase fragment is detected in cells transfected with either APP 695 or APP 751 cDNA (lanes c and e). When cells are co-transfected with Zyme cDNA and APP 695 cDNA (lane d), a plethora of APP C-terminal fragments are detected at 22, 16, and 12 kDa. A less intense band is seen at 45 kDa. One of the fragments appears to migrate slightly faster than a Cys100 fragment in apparent molecular mass. In cells transfected with Zyme cDNA and APP 751 cDNA, there are less apparent changes in the C-terminal amyloidogenic fragments (lane f). The APP 751 form of APP contains the KUNITZ protease inhibitor domain, and we think that it has either inhibited Zyme activity or interfered in steps leading to the activa-
The culture media of co-transfected cells was analyzed by immune precipitation of \[^{35}\text{S}\]methionine-labeled cells using A\(\beta\)-specific antibody, 4G8 (directed to residues 17–24 of A\(\beta\)) (17) (Fig. 9). Cells transfected with Zyme and APP 695 cDNAs accumulated amyloidogenic fragments in the culture media representing A\(\beta\) (4 kDa), a faster migrating A\(\beta\) (arrow), and P3 (27, 28). P3 is the result of an \(\alpha\)(internal cleavage) and \(\gamma\)(C-terminal cleavage) secretase cleavage of A\(\beta\) or APP. The faster migrating A\(\beta\) shows that one of the A\(\beta\) products resulting from Zyme co-transfection is truncated, and it is likely that the cleavage site is not the Met-Asp site seen with \(\beta\)-secretase.

Zyme, a Potentially Amyloidogenic Enzyme cDNA

Table I

| Chromosome | Percent discordance |
|------------|---------------------|
| 1          | 28                  |
| 2          | 36                  |
| 3          | 36                  |
| 4          | 32                  |
| 5          | 56                  |
| 6          | 28                  |
| 7          | 24                  |
| 8          | 44                  |
| 9          | 44                  |
| 10         | 32                  |
| 11         | 24                  |
| 12         | 32                  |
| 13         | 16                  |
| 14         | 28                  |
| 15         | 40                  |
| 16         | 40                  |
| 17         | 40                  |
| 18         | 40                  |
| 19         | 04                  |
| 20         | 32                  |
| 21         | 20                  |
| 22         | 28                  |
| 23         | 44                  |
| X          | 44                  |
| Y          | 40                  |

The 10-kDa non-amyloidogenic fragment increases in both the APP 695 and APP 751 co-transfected cells. This observation suggests that the \(\alpha\)-secretase cleavage has been enhanced as well in these transfected cells. Similar sized C-terminal fragments (22, 16, and 12 kDa) have been isolated from AD and normal cortical microvessels (26). Fetal microvessels contained little or no 22-kDa amyloidogenic fragment (26). Also, it is important to note that Zyme is not detectable in fetal brain (Fig. 5).
being released from the 293 cell and can be used as a substrate for γ(Aβ C-terminal cleavage) secretase. The significance of the drop of P3 is that during normal processing of APP, a certain proportion of Aβ is subjected to an α-secretase-like cleavage (28), yielding a fragment containing residues 17–42 of Aβ which can be isolated from the AD brain. If the Aβ peptide undergoes α-secretase cleavage, it will prevent the generation of neurotoxic Aβ filaments. Clearly the presence of Zyme alters the processing of Aβ peptide so that the P3 species is reduced and more, Aβ, albeit truncated, material is produced. Higgins et al. (31) have demonstrated that P3 deposition is correlated with AD and is noticeably absent in vascular amyloid deposits. Later in this report we show that Zyme is localized to microves- 

cultures, and its activity to reduce P3 is consistent with the observations of Higgins et al. (31)

A Characterization of Zyme—Aliquots of co-transfected cells and culture media shown in Fig. 10 were also analyzed by Western blot using antibody to Zyme. Fig. 10, lanes b, d, and f, shows the presence of a new 30- and 28-kDa protein and a smaller fragment at 6.5 kDa not seen in mock-transfected cells. The 6.5-kDa fragment appears to be present in transfections where APP C-terminal amyloidogenic fragments are generated. Conversely, APP 751/Zyme cDNA co-transfected cells show a build-up of a higher molecular mass Zyme and an absence of the 6.5-kDa fragment. The 6.5-kDa fragment cannot be detected using pre-absorbed anti-274 antibody (data not shown) and appears to be Zyme-specific. The anti-274 antibody was prepared against residues 72–88 of mature Zyme primary sequence. The detection of this smaller Zyme fragment sug-
The method for identification of proteases using active site homologous regions as probes has been performed by other laboratories (33, 34) to hunt for serine and cysteine proteases. Our study identifies a serine protease that either directly generates amyloidogenic fragments or is a member of a cascade of other potentially amyloidogenic activities. The higher ratio of Zyme transcript to GAPDH RNA in the AD brain and its general relocalization to microglial cells in the AD brain suggest that this enzyme has a different display in a brain disease state. A homologous tissue-specific serine protease, prostate-specific antigen, is highly correlated with disease of the prostate (35, 36), and perhaps Zyme will be found to have a relationship with diseases of the brain. Elucidation of the role of Zyme in the brain awaits further study.

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