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A Novel Isoform of a Kallikrein-like Protease, TLSP/Hippostasin, (PRSS20), Is Expressed in the Human Brain and Prostate

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cDNAs encoding two splicing variants of a serine protease, termed hippostasin, were isolated by a PCR-based cloning strategy. The difference of 5′ nucleotide sequence resulted in the variation in the amino terminal ends of the two, brain and prostate, types of human hippostasin. The longest ORF of the brain-type was 250 amino acids with a putative signal peptide, while that of the prostate-type was 282 amino acids. Homology search using the amino acid sequence revealed that prostate-type hippostasin was identical to TLSP (PRSS20), which is expressed in human primary keratinocytes (1). Transient expression analysis showed that both brain- and prostate-type TLSP/hippostasin were secreted into the conditioned medium as about 40 kDa proteins. Human TLSP/hippostasin showed 47% and 45% identity to trypsinogen II and kallikrein, respectively. In fact, the recombinant human TLSP/hippostasin efficiently cleaved Bz-Phe-Arg-4-methylcoumaryl-7-amide, a kallikrein substrate, and weakly cleaved other substrates for kallikrein and trypsin. Northern blot analysis detected a 1.3 kb band in the whole brain and a 1.4 kb band in the prostate and the lung. *In situ* hybridization revealed that it was expressed preferentially by the pyramidal neurons in the human hippocampus and secretory epithelial cells in the prostate. These results indicated that TLSP/hippostasin is involved in the functions of the human central nervous system and prostate and that it is a multifunctional protease present in various organs.

Key Words: **alternative splicing; brain; hippocampus; prostate; serine protease.**

Serine proteases have various roles in the central nervous system (CNS) and affect plasticity, neuronal cell death and the development of the brain (reviewed in 2, 3). However, the proteolytic functions in the CNS may be highly specific and restricted, since they are non-reversible. The specificity of the functions partially depends on the transcriptional regulation. Motopsin (PRSS12) is a serine protease, which is composed of a proline-rich domain, a kringle domain and three scavenger receptor cysteine-rich domains (4). This enzyme appears to be secreted into the extracellular environment, and activated by a furin-like protease. The expression of motopsin is restricted to motor neurons (5). Another protease, neurosin (PRSS9), is expressed predominantly by activated microglias (6). It is also secreted into the extracellular environment, and then suitably processed by digestion of the proenzyme fragment (7).

The production of brain-specific isoforms is another means by which the specificity of the proteolytic functions in the CNS is achieved. Recent reports showed that some serine proteases have isoforms specific to the CNS. We reported a novel isoform of human neuropsin, which is preferentially expressed in the adult brain (8). A smaller isoform of human motopsin was identified in the testis (9). A novel isoform of granzyme K is expressed specifically in the mouse brain (10), although this enzyme was originally isolated as a serine protease of natural killer cells and T cells. These variations are caused by alternative splicing and/or tissue-specific transcriptional initiation. The reports would

The nucleotide sequences reported in this paper have been entered into the DDBJ/GenBank/EMBL databases with the Accession Nos. AB013730 and AB041036.

Abbreviations used: CNS, central nervous system; ECM, extracellular matrix; EST, expression sequence tag; MCA, 4-methylcoumaryl-7-amidide; RACE, rapid amplification of cDNA ends; SRCR, scavenger receptor cysteine-rich domain; tPA, tissue-type plasminogen activator; PSA, prostate specific antigen.

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TABLE 1

Sequences of PCR Primers

Name	Sequence	Name	Sequence
DP-S	GTGCTCACNGCNGCBCAYTG	Primer 5	GGACTCAAGAGAGGAACCTG
DP-A	AGCGGNCCNCCDSWRTCVCC	Primer 6	CTGCCTTGCTCCACACCTG
Primer 1	TCAAGCCCCGCTACATAGTT	Primer 7	AACTGCAGGAACCAAACACCAAGTGG
Primer 2	ATCATGCTGGTGAAGATGGC	AP1	CCATCCTAATACGACTCACTATAGGGC
Primer 3	TTCTCACACTTCTGGTGCTC	AP2	ACTCACTATAGGGCTCGAGCGGC
Primer 4	ATGGTGTCTGTGATGTTGCC	Adaptor primer	GGCCACGCGTCGACTAGTAC

suggest that other serine proteases have brain-specific isoforms caused by alternative splicing. Recently, a trypsin-like serine protease, TLSP/hippostasin (PRSS20), has been reported to be expressed in human primary keratinocytes (1). In an effort to isolate novel serine proteases in the human brain, we identified a novel splicing variant of TLSP/hippostasin in the human hippocampus. The localization and enzymatic activity of human TLSP/hippostasin are described.

MATERIALS AND METHODS

cDNA cloning of human TLSP/hippostasin cDNAs. Poly(A)$^+$ RNA from human hippocampus and prostate (Clontech, Palo Alto, CA) was reverse-transcribed by using BcaBEST polymerase (Takara Shuzo, Shiga, Japan) according to the instruction manual. Degenerated PCR with DP-S and DP-A primers was performed as described previously (5). The PCR products from human hippocampal and prostate cDNAs were sequenced using an automatic sequencer (DSQ-1000, Shimadzu Co, Kyoto, Japan) after the cloning into a pGEM-T Easy vector (Promega Co., Madison, WI). For the rapid amplification of cDNA ends (RACE) of human TLSP/hippostasin, specific primers were synthesized (Table 1). For 3′ RACE, human hippocampal and prostate polyA$^+$ RNAs were reverse transcribed using oligo dT with an adaptor primer sequence at the 5′ end; GGCCACGCGTCGACTAGTAC$(T)_{17}$. The cDNA was first amplified between forward primer 1 and the adaptor primer, then reamplified using forward primer 2 and the adaptor primer. 5′ RACE was performed using a Marathon cDNA amplification kit (CLONTECH) according to the instruction manual. In brief, nested PCR with AP2 and primer 3 was performed using products of PCR with primer 4 and AP1 as a template. To isolate the full length clones, primer 5, 6 and 7 were designed according to the sequence of 5′ and 3′ RACE products. The combination of primer 5 and 7, and of primer 6 and 7 was used for the amplification of brain- and prostate-type TLSP/hippostasin cDNA, respectively.

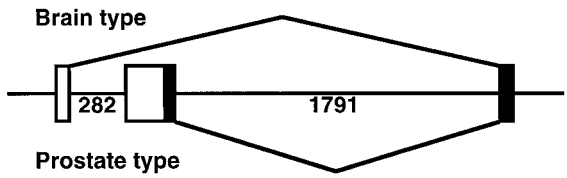
Transient expression of human TLSP/hippostasin in COS7 cells. Full-length cDNAs encoding the brain- and prostate-types of human TLSP/hippostasin were subcloned into a pcDNA3.1/Myc-His A vector (Invitrogen, Carlsbad, CA) at the *Eco*RI site; however, human TLSP/hippostasin did not fuse with the Myc-His tag. One μg of plasmid DNA was transfected into COS7 cells using LipofectAMINE (Gibco BRL, Life Technologies, Inc., Rockville, MD) according to the instruction manual. On the 2nd day after transfection, the conditioned medium was recovered in SDS sample buffer (62.5 mM Tris-Cl, pH 6.8, 2% β-mercaptoethanol, 2% SDS, 10% glycerol, 0.01% bromophenol blue), and analyzed by Western blotting using anti-human TLSP/hippostasin antibody. Anti-TLSP/hippostasin antibody was raised in rabbits against a synthetic peptide (IEHQKCENAYPGNI) according to the standard protocol (11).

Northern hybridization. Northern hybridization against human tissues was carried out using commercially available membranes (Clontech). The cDNAs encoding the full length of human TLSP/hippostasin ORF were labeled by the random labeling method using a Takara BcaBEST labeling kit (Takara Shuzo Co. Ltd., Shiga, Japan). Hybridization was carried out in ExpressHyb hybridization solution (CLONTECH) at 60°C overnight, and the final wash was performed in 0.1 × SSPE and 0.1% SDS at room temperature for 10 min. The radioactivity was detected using an FLA-2000 image analyzer (Fuji Photo Film Co. Ltd., Tokyo, Japan).

In situ hybridization. Human brain and prostate tissues were collected with informed consent. *In situ* hybridization was carried out as described previously (5). After fixation in phosphate-buffered 4% paraformaldehyde, tissue sections were cut on a cryostat at 20 μm. Hybridization was performed at 50°C for 12 h in a buffer containing 50% formamide, 0.3 M NaCl, 60 mM sodium citrate, 10% dextran sulfate, 20 mM Tris-Cl, 5 mM EDTA, 1× Denhardt's solution, 0.2% SDS, 500 mg/ml yeast tRNA, and digoxygenin (DIG)-labeled RNA probe to full length brain-type TLSP/hippostasin. The sections were rinsed in 2× SSC, 50% formamide, and treated with 10 mg/ml RNase A. Then they were treated with blocking solution (Roche Diagnostics Co., Basel, Switzerland) and incubated with alkaline phosphatase-conjugated anti-DIG antibody. The signals were visualized by incubating with 4-nitroblue tetrazolium chloride (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP).

Expression and purification of recombinant TLSP/hippostasin in a baculovirus expression system. To obtain the recombinant active TLSP/hippostasin, a cDNA fragment encoding a putative mature enzyme of human TLSP/hippostasin was fused with trypsinogen signal peptide followed by histidine hexamer. The chimera cDNA was inserted into pFastBAC1 vector (Gibco BRL) and was expressed in insect cells using a Bac-To-Bac baculovirus expression system (Gibco BRL). The recombinant protein with a histidine tag is secreted into the conditioned medium in this system, can be easily purified with a chelating column, and finally can be activated by removing the histidine hexamer with enterokinase. After 3–5 days infection with the baculovirus expressing the recombinant protein, the conditioned medium was recovered, adjusted to a pH of 8.0 with 1 M NaOH, and centrifuged at 10,000*g* for 10 min. The supernatant was incubated with 1 ml of chelating resin (Qiagen Inc., Chatsworth, CA) in base buffer (10 mM Tris-Cl, pH 8.0, 100 mM NaCl) for 3 h at room temperature. The suspended resin was transferred to an open column and washed with 10 mM immidazole in base buffer. The recombinant TLSP/hippostasin was eluted with base buffer containing 50 mM immidazole.

Enzyme assay. The purified recombinant TLSP/hippostasin was preincubated with recombinant enterokinase (EK max, Invitrogen) immobilized on NHS-Sepharose (Amersham-Pharmacia Biotech) for 30 min at room temperature. Thirty microliters of the activated enzyme was incubated with 100 μl of the indicated substrate peptides (Peptide Inst. Inc., Osaka, Japan) in 10 mM Tris-HCl (pH 8.0). After 5 and 15 min, the fluorescence (excitation at 380 nm, emission at 460 nm) was measured by a plate reader (cytofluor 2300, Millipore, Bedford, MA).

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Prostate type AGGGCTGGCTGCTGTCAGAACCTAGGCCCTCCCTGCCTTGCTCCACACCTGGTCAGGGGA  60

Prostate type GAGAGGGGAGGAAAGCCAAGGGAAGGGACCTAACTGAAAACAAACAAGCTGGGAGAAGCA 120

Brain     type CAGGCTTGGAAAGCAGGAGAGCTCAGCCTACGTCTTTAATCCTCCTGCCCACCCCTTGGA
Prostate type GGAATCTGCGCTCGGGTTCCGCAGATGCAGAGGTTGAGGTGGCTGCGGGACTGGAAGTCA 180
                                   M  Q  R  L  R  W  L  R  D  W  K  S

Brain     type TTCTGTCTCCACTGGGACTCAAGAG
Prostate type TCGGGCAGAGGTCTCACAGCAGCCAAGGAACCTGGGGCCCGCTCCTCCCCCCTCCAGGCC 240
               S  G  R  G  L  T  A  A  K  E  P  G  A  R  S  S  P  L  Q  A

Prostate type ATGAGGATTCTGCAGTTAATCCTGCTTGCTCTGGCAACAGGGCTTGTAGGGGGAGAGACC 300
               M  R  I  L  Q  L  I  L  L  A  L  A  T  G  L  V  G  G  E  T
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FIG. 1. The structure of the 5′ end of brain- and prostate-type TLSP/hippostasin. (Upper) Exon/intron structure of the first and second exons. Brain-type specific exon was located 282 bp upstream of prostate-type specific exon 1. (Lower) The nucleotide sequences of the 5′ end of human TLSP/hippostasin cDNAs. The first initiation codon in each type is represented by white letters. Prostate-type specific exon contains an initiation codon, while brain-type specific exon is noncoding. An arrowhead means the start position of the common 2nd exon.

RESULTS

cDNA cloning of a novel splicing variant of human TLSP/hippostasin. The sequence analyses of the degenerated PCR and RACE products revealed two isoforms of the transcripts encoding a serine protease. Originally, we designated this protease as hippostasin, for hippocampal and prostatic trypsin like serine protease, since it was first identified in the hippocampus and was shown to be markedly expressed in the prostate (Fig. 2, see below). RT-PCR using as forward primer 5; GGA CTC AAG AGA GGA ACC TG for the shorter type or 6; CTG CCT TGC TCC ACA CCT G for the longer type, and reverse primer 7; AAC TGC AAG GAA CCA AAC ACC AAG TGG, showed that the longer type existed in the prostate while the brain expressed the shorter type (Fig. 2B). We named the longer and shorter type prostate- and brain-type, respectively. The nucleotide sequence of human prostate-type hippostasin was identical to that of the brain-type except for the upstream sequence from the 205th A (Fig. 1). This difference caused the different N-terminal leader sequences of the longest hippostasin open reading frames (ORFs). The longest ORF of the brain-type hippostasin was 250 amino acids while that of the prostate-type was 282 amino acids. Thus, the prostate-type hippostasin contained 32 more amino acids in the amino terminus (Fig. 1).

A homology search revealed that the prostate-type hippostasin was identical to TLSP (GenBank Accession No. AB012917). Furthermore, a comparison with the sequence of the TLSP gene (AF164623) indicated that an exon for the brain-type was located 282 bp upstream of the prostate-type specific exon (Fig. 1). We concluded that both types were generated from a single gene by alternative splicing.

Organ distribution of TLSP/hippostasin. To study the organ distribution of human TLSP/hippostasin mRNA, northern hybridization against human tissues was carried out. In addition to the hippocampus and the prostate, organs such as the lung, heart and testis, expressed TLSP/hippostasin (Fig. 2A). However, the TLSP/hippostasin mRNA appeared to vary in size. The signals in the human brain and heart were 1.3 kb, while those in the lung and prostate were 1.4 kb. This difference of mRNA size appeared to reflect the difference of the nucleotide sequence of the brain- and prostate-types. RT-PCR clearly showed the existence of both prostate-type and brain-type TLSP/hippostasin in the corresponding organs (Fig. 2B).

In situ hybridization revealed that hippocampal pyramidal cells expressed TLSP/hippostasin mRNA (Fig. 3). No glial cells in any portion of the human brain showed positive signals. In the prostate, TLSP/hippostasin was expressed in the epithelial cells. The higher intensity of the signal in the prostate than the hippocampus was consistent with the expression level shown by northern hybridization (Fig. 3). The sense probe showed no signals against either tissue (not shown).

Transient expression of human TLSP/hippostasin in COS7 cells. RT-PCR using primer sets specific for prostate-type or brain-type TLSP/hippostasin suggested that both splicing variants are functional (Fig. 2B). To confirm this, prostate- or brain-type human

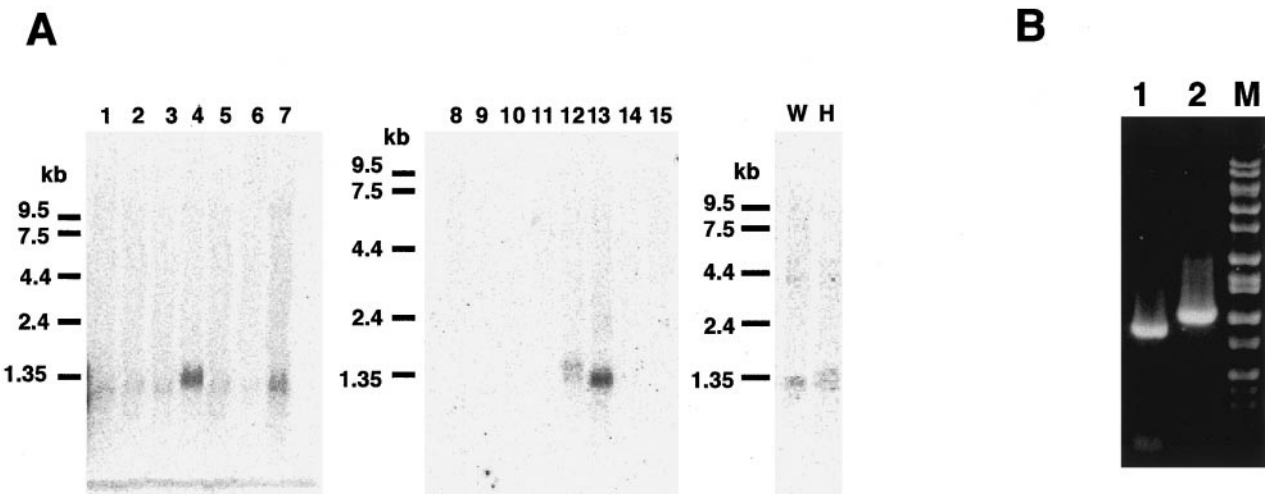

FIG. 2. Distribution of human TLSP/hippostasin mRNA. (A) The organ distribution of human TLSP/hippostasin mRNA. Poly(A)$^+$ RNAs (2 μg) on a human multiple tissue blot (Clontech) were hybridized to the ORF of human brain-type TLSP/hippostasin cDNA. 1, kidney; 2, skeletal muscle; 3, liver; 4, lung; 5, placenta; 6, brain; 7, heart; 8, blood leukocyte; 9, colon; 10, intestine; 11, ovary; 12, testis; 13, prostate; 14, thymus; 15, spleen; W, whole brain; H, hippocampus. (B) Typing of TLSP/hippostasin in the brain and prostate. RT-PCR was carried out using forward primer 5 for brain-type or forward primer 6 for prostate-type and reverse primer 7. The brain expressed brain-type (lane 1), while the prostate-types were expressed in the prostate (lane 2). M, size markers 10,000 bp, 8,000 bp, 6,000 bp, 4,000 bp, 3,000 bp, 2,000 bp, 1,550 bp, 1,400 bp, 1,000 bp, 750 bp, 500 bp, 400 bp, 300 bp.

TLSP/hippostasin was transiently expressed in COS7 cells, and the conditioned media of the cells were analyzed by Western blot analysis using anti-human TLSP/hippostasin antiserum. Both types of TLSP/hippostasin were secreted into the medium and were of similar molecular weight, 40 kDa and 44 kDa (Fig. 4). This heterogeneity may be caused by heterogeneity in the glycosylation of 4 possible glycosylation sites, since the secreted protein was larger than the predicted molecular mass of 28,362 Da. These sizes were similar to those of the recombinant active enzyme fragment produced in the baculovirus system. In contrast, no bands appeared when the conditioned medium of vector-transfected cells was analyzed.

Enzyme activity of human TLSP/hippostasin. A putative active fragment of human TLSP/hippostasin, Ile^{54}–Asn^{282}, fused with a signal peptide with a histidine tag followed by an enterokinase recognition site was constructed in order to obtain the recombinant

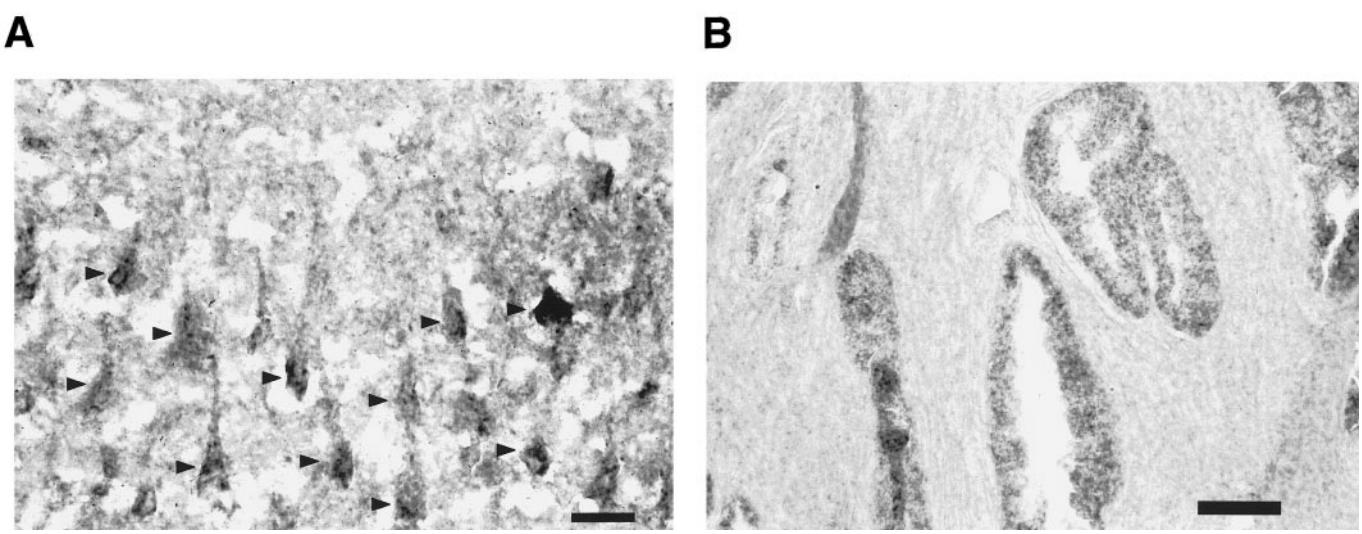

FIG. 3. *In situ* hybridization of TLSP/hippostasin in the hippocampus and prostate. Human tissues were hybridized with DIG-labeled antisense-RNA probe. (Left) Only pyramidal neurons were stained weakly in the hippocampus. Bar, 50 μm. (Right) Secretory luminal epithelium of the prostate was stained. Bar, 25 μm.

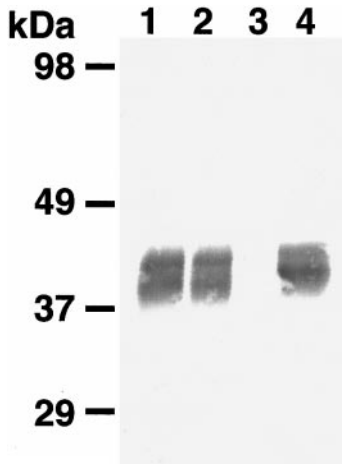

FIG. 4. Transient expression of human TLSP/hippostasin by COS cells. The conditioned medium of COS cells transfected with human TLSP/hippostasin cDNA was analyzed by Western blotting using anti-TLSP/hippostasin serum. 1, brain-type human TLSP/hippostasin; 2, prostate-type human TLSP/hippostasin; 3, pcDNA3.1 vector; 4, chimera protein of TLSP/hippostasin produced by a baculovirus expression system.

active TLSP/hippostasin. Using a baculovirus expression system, the recombinant chimera protein was secreted to the conditioned medium and was purified by chelating column chromatography (Fig. 5A). SDS-polyacrylamide gel electrophoresis and Western blot analysis showed that the recombinant protein was eluted by 50 mM imidazole. In our system, the purified protein was activated after the removal of the artificial leader peptide by enterokinase. The purified TLSP/hippostasin hydrolyzed synthetic peptide only when it was pre-incubated with enterokinase immobilized on NHS-Sepharose (Fig. 5B). This confirmed that the enzyme activity was derived from the recombinant TLSP/hippostasin. To study the substrate specificity, the purified TLSP/hippostasin activated by enterokinase was incubated with some synthetic peptides (Fig. 5C). Human TLSP/hippostasin cleaved Z-Phe-Arg-4-methylcoumaryl-7-amide (MCA) the most preferentially (substrate 10), and Pro-Phe-Arg-MCA less effectively (substrate 6). Both peptides are substrates for kallikrein. Among the other substrates, human TLSP/hippostasin showed weak enzyme activity against Boc-Gln-Ala-Arg-MCA and Pyr-Gly-Arg-MCA, which are substrates for trypsin or plasminogen activator.

DISCUSSION

We isolated two types of cDNAs encoding a serine protease, TLSP/hippostasin, from human tissues. This enzyme showed significant homology to trypsinogen and kallikrein (47% and 45% identity, respectively) and contained the essential triad with all conserved cysteine residues, suggesting that it has trypsin- and/or kallikrein-like activity. The multiple alignment of TLSP/hippostasin, kallikrein and trypsinogen suggests that Ile^{54} of human TLSP/hippostasin is the amino terminal residue of the active enzyme. The recombinant human TLSP/hippostasin from Ile^{54} to Asn^{282} showed kallikrein-like enzyme activity, supporting this speculation. Namely, the purified recombinant human TLSP/hippostasin cleaved Z-Phe-Arg-MCA, known as a substrate for kallikrein, cathepsin B and cathepsin L.

It is interesting that hippocampal pyramidal neurons expressed TLSP/hippostasin mRNA (Fig. 3), since

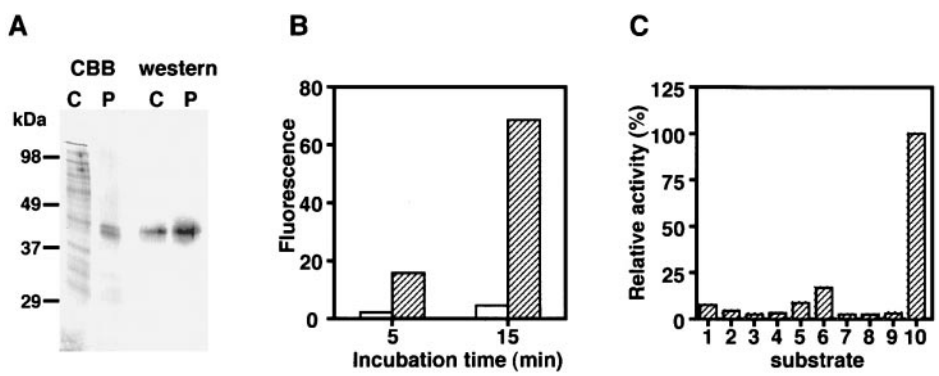

FIG. 5. Enzyme activity of recombinant human TLSP/hippostasin produced by the baculovirus expression system. (A) The purified recombinant human TLSP/hippostasin was analyzed by Coomassie brilliant blue staining following SDS-polyacrylamide gel electrophoresis (left panel) and Western blot analysis using anti-human TLSP/hippostasin serum (right panel). C, conditioned medium of the infected cells; P, purified TLSP/hippostasin. (B) The activation of the recombinant TLSP/hippostasin by recombinant enterokinase. The purified recombinant TLSP/hippostasin was incubated with Boc-Gln-Ala-Arg-MCA at 37°C after pre-incubation with (hatched column) or without enterokinase (open column). The fluorescence was measured at 5 or 15 min. (C) The substrate specificity of human TLSP/hippostasin. The activated recombinant TLSP/hippostasin was incubated with various substrates at 37°C for 1 h. The enzyme activity was shown as relative to that for Z-Phe-Arg-MCA. 1, Boc-Gln-Ala-Arg-MCA; 2, Boc-Phe-Ser-Arg-MCA; 3, Bz-Arg-MCA; 4, Boc-Val-Leu-Lys-MCA; 5, Pyr-Gly-Arg-MCA; 6, Pro-Phe-Arg-MCA; 7, Boc-Val-Pro-Arg-MCA; 8, Z-Arg-Arg-MCA; 9, Arg-MCA; 10, Z-Phe-Arg-MCA.

evidence has been accumulating from animal models that some serine proteases are involved in the formation of memory (3, 12–14). And this is the first report to identify a trypsin-like protease in the human hippocampal pyramidal neuron. Both brain- and prostate-types of TLSP/hippostasin were found to be secreted into the conditioned medium by a transient expression analysis, suggesting that they are functional in the extracellular environment. The sequence of X-Phe-Arg-X is found in molecules related to the neuronal plasticity, such as the extracellular region of the neuronal cell adhesion molecule (P13592, P13593), in extracellular matrix protein (ECM) such as laminin (P07942) and tenascin (P24821), and at the junction of the A and B chains of tPA (SWISS-PROT Accession No. P00750). It was reported that the extracellular breakdown of cell adhesion molecules mediated by serine proteases contributes to the formation of stable potentiation (15). The ability to cleave these molecules and localization of TLSP/hippostasin should be investigated to elucidate the physiological functions of TLSP/hippostasin in the CNS. Concerning the prostate, TLSP/hippostasin mRNA was localized in secretory luminal epithelium (Fig. 3), suggesting that TLSP/hippostasin is secreted to the seminal fluid. It is known that seminal fluid contains prostate specific antigen (PSA) and glandular kallikrein (hK2) among members of the kallikrein family. However, the substrate specificities of these kallikrein-like proteases are quite different (16). hK2 preferentially cleaves the C-terminus of arginine, whereas PSA digests the C-terminus of tyrosine and leucine residues. hK2 purified from seminal fluid is known to show kininogenase activity (17). hK2 activates PSA by the digestion of the proenzyme sequence of PSA (18). Since the substrate specificity of TLSP/hippostasin was similar to that of hK2, TLSP/hippostasin may digest these proteins in the seminal fluid. Furthermore, Northern hybridization showed that the heart, the lung and the testis also expressed TLSP/hippostasin mRNA in addition to the brain and prostate. This fact suggests that TLSP/hippostasin is multifunctional in various organs.

We found a novel splicing variant of TLSP/hippostasin in the human hippocampus. Comparison between the sequences of genomic DNA and cDNAs showed that a brain-type specific exon is located 282 bp upstream of the prostate-type specific exon (Fig. 1). In a transient expression assay, brain- and prostate-type TLSP/hippostasin secreted into the conditioned medium were of the same molecular mass, 40 kDa and 44 kDa (Fig. 4), suggesting that both types of protein are digested at the same sites by signal peptidase. We have reported that two types of human neuropsin, known to be a serine protease involved in neuronal plasticity, are generated by alternative splicing (8). Type 2 neuropsin is inserted with 45 amino acids in the leader sequence and is expressed in the adult brain, while type 1 is distributed in several organs such as placenta and skin. However, splicing variants have not been found for mouse neuropsin suggesting that the alternative splicing of human neuropsin reflects specific functions in the human brain. In contrast, the alternative splicing event in TLSP/hippostasin appears to be more general, since cDNAs encoding both splicing variants of TLSP/hippostasin were isolated from mouse organs (in preparation).

Some serine proteases other than neuropsin have splicing variants specific to the CNS. Motopsin (PRSS12) is a serine protease whose expression in the CNS is restricted to motoneurons (5). This protease has a kringle structure and three scavenger receptor cysteine-rich domains, suggesting interaction with other proteins (4). These domains appear to be essential for the functions in the CNS, since a small isoform lacking them is expressed in the testosterone-producing cells of the testis (9). Very recently, it was reported that a novel splicing variant of carboxypeptidase B is expressed in the human brain and that the novel variant cleaves amyloid β precursor protein by endopeptidic manner (19). These reports indicate that splicing variants may have proteolytic functions specific to the CNS. Furthermore, the 5′ end may affect the stability of the mRNA and/or translational efficiency. An isoform of mouse granzyme A, a serine protease implicated in the cytolysis process, with a less hydrophobic leader peptide caused by alternative splicing has been reported (20). The mRNA for this isoform is less efficiently translated *in vitro* than the isoform mRNA encoding the typical signal peptide. The alternative splicing and the use of the alternative transcriptional initiation sites produce two types of human cathepsin B mRNA that differ in their leader peptide. The subcellular distribution of the truncated from is different from the lysosomal pattern shown by the full length form of cathepsin B (21). Concerning TLSP/hippostasin, the two types of the secreted enzyme have the same molecular mass, and the efficiency of the secretion appeared to be similar (Fig. 3). The variation in TLSP/hippostasin may contribute to the intracellular trafficking of the mRNA or the translational product.

Here, we described a novel splicing variant of human TLSP/hippostasin in the human hippocampal neurons. The localization and the transcriptional regulation of the enzyme should be investigated to understand the physiological functions of TLSP/hippostasin in the human organs.

ACKNOWLEDGMENTS

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