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Biosynthesis and Characterization of the Brain-Specific Membrane Protein DPPX, a Dipeptidyl Peptidase IV–Related Protein[1]

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Dipeptidyl peptidase IV-related protein (DPPX) was found to be preferentially expressed in the brain tissue. We isolated two rat cDNA clones encoding DPPX-S and DPPX-L from a brain cDNA library, of which DPPX-L had a longer sequence at the NH2 terminus. The biosynthesis of DPPXs was examined in both *in vitro* and *in vivo* systems. In the cell-free translation system, DPPX-S and DPPX-L were synthesized as 93-kDa and 97-kDa forms, respectively, which are in good agreement with the molecular masses estimated from their primary structure. In COS-1 cells transfected with the cDNAs, DPPX-S and DPPX-L were initially synthesized as 113-kDa and 117-kDa forms, respectively, with high-mannose type oligosaccharides, which were then converted to 115-kDa and 120-kDa forms, mostly with the complex-type sugar chains. Immunofluorescence-microscopic observations confirmed that both DPPXs were expressed on the cell surface. DPPXs were found to have no enzyme activity of DPPIV, even when they were mutated to have the consensus active-site sequence Gly-X-Ser-X-Gly for serine proteases. Immunoblot analysis of samples prepared from various rat tissues demonstrated that DPPX-S, but not DPPX-L, was detectable only in the brain tissue. These results indicate that, of the two isoforms, DPPX-S is preferentially expressed in the brain tissue as the surface glycoprotein without protease activity, although its function remains unknown at present.

Key words: brain-specific protein, dipeptidyl peptidase IV, DPPX, DPPX-L, DPPX-S.

Dipeptidyl peptidase IV (DPPIV) is a serine protease that cleaves NH_2-terminal dipeptides from oligo- and polypeptides with a penultimate prolyl residue (*1*, *2*). DPPIV is an integral membrane glycoprotein localized on the cell surface and enriched in brush borders of small intestine and kidney proximal tubules and in bile canaliculi of liver (*1–4*). The primary structure of DPPIV was determined for rat (*5*) and human (*6*) based on the cDNA sequences. The protein is anchored to the membrane by the NH_2-terminal hydrophobic domain and has the active-site sequence Gly-Trp-Ser-Tyr-Gly at positions 629–633 (*7*), which corresponds to the consensus sequence Gly-X-Ser-X-Gly proposed for the active-site of serine proteases (*8*). DPPIV was identified as the lymphocyte surface antigen CD26 that is involved in T cell activation (*9*) and associated with adenosine deaminase (*10*). Furthermore, the protein is suggested to play a role in fibronectin-mediated interactions of cells with extracellular matrix (*11*). Thus, it is likely that DPPIV is a multifunctional molecule that exerts important functions depending on the expressed cells and tissues, in addition to its catalytic activity as a peptidase (*12*).

In the brain tissue, DPPIV is detected only at a trace level by the enzyme assay (*13*, *14*) and immunocytochemical method (*15*). Wada *et al.* (*16*) tried to isolate cDNA clones for a subtype of glutamate receptor purified from bovine brain. The cDNA clones obtained from the bovine cDNA library, however, did not encode an expected amino acid sequence of the receptor. Instead, the primary structure deduced from the cDNA sequence was found to have 33% identity with that of DPPIV. The predicted DPPIV-related protein (designated DPPX) was suggested to exist as two isoforms, DPPX-S and DPPX- L, which differ in the sequence and length of the NH_2-terminus. Although the presence of DPPXs in the brain was further confirmed by a Northern blot analysis and *in situ* hybridization of mRNA (*16*), they have not been identified and characterized at the protein level. In the present study we have examined the biosynthesis and intracellular transport of DPPXs in COS-1 cells transfected with their cDNAs.

MATERIALS AND METHODS

Materials—cDNA synthesis and sequence kits and mCAP mRNA capping kit were obtained from Stratagene (La Jolla, CA) and Amersham Pharmacia Biotech (Buckinghamshire, UK). Various DNA-modifying enzymes and restriction endonucleases were purchased from Nippon Gene (Toyama) and Takara Shuzo (Kyoto). RNAzol B RNA

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Abbreviations: DMEM, Dulbecco's modified Earle's medium; DPPIV, dipeptidylpeptidase IV; DPPX, DPPIV-related protein; endo H, endoglycosidase H; ER, endoplasmic reticulum.

isolation kit was from Tel-Test (Houston, TX) and Lipofectamine reagent was from GIBCO BRL (Gaithersburg, MD). Rhodamine- or alkaline phosphatase-conjugated goat anti-rabbit IgG antibodies were obtained from DAKO A/S (Denmark).

Cloning and Sequencing of cDNA—A poly(A)$^+$ RNA fraction was prepared from rat brain and used for construction of a cDNA library in λZAPII bacteriophage (*17*). A 726-bp DNA fragment was prepared by polymerase chain reaction (PCR) (*18*) with the following mixed oligonucleotides as primers based on the available sequence (*16*): 5′-ATATATTGAATTCARGGWAARTTYTAYCAYAT-3′, 5′-ATATATTGAATTCARGGSAARTTYTAYCAYAT-3′, and an antisense 5′-TATTCATGCTAGCYTCCATYTGRTCYTCYTC-3′, where R is A or G, W is A or T, Y is T or C, and S is G or C. The 726-bp DNA fragment obtained was inserted into pUC119 vector and the nucleotide sequence was confirmed to be identical with a partial sequence of DPPX (*16*). The DNA fragment labeled with ^{32}P was used as a probe for screening of the cDNA library (5×10^5 clones) by the plaque hybridization method (*19*). cDNA inserts of two clones, pDX1 (4.5 kbp) and pDX2 (4.7 kbp), were characterized by restriction endonuclease mapping and subcloned into pUC118 plasmid vector. The cDNA inserts were sequenced on both DNA strands by the dideoxynucleotide chain termination method (*20*) using the Sequenase DNA-sequencing.

Construction of Expression Plasmid—A single *Eco*RI site in the coding region was destroyed by a silent mutation [Glu^{93} (GAA)→ (GAG)] directed with a synthetic oligonucleotide 5′-ATAAGGAGTTCATCTACA-3′ (*21*). Synthetic oligonucleotides 5′-TGGG-GTGGCGAATTCCGGACAGACCATGA-3′ (for DPPX-S) and 5′-TCCTAACCAGTGAATTCGCGCAGAGGCCGCCCATGG-3′ (for DPPX-L) were used as a primer to generate a new *Eco*RI site in the 5′-noncoding region of each DPPX cDNA. These cDNAs were digested with *Eco*RI and inserted into the *Eco*RI site of the pSG5 expression vector (*22*, *23*). The insert orientation was confirmed by restriction endonuclease mapping. The expression plasmids thus prepared were designated pSDXS (for DPPX-S) and pSDXL (for DPPX-L). Site-directed mutagenesis of the DPPX cDNA was also designed for substitution of Asp^{650} (DPPX-S) and Asp^{706} (DPPX-L) by Ser to generate the consensus active-site sequence of serine proteases (Gly-X-Ser-X-Gly). A 25-mer synthetic oligonucleotide 5′-GTTTGGGAAGTCTTATGGTGGGTAC-3′ was used as a primer for the second strand. The mutant expression plasmids were isolated as described (*22*, *23*) and named pSDXS/DS and pDXL/DS.

Preparation of Anti-DPPX Antibodies—The following two peptides were synthesized: (i) CFRIQDKLPTATAKEDEEED, which corresponds to the COOH-terminal sequence of the two DPPXs; and (ii) ASLYQRFTGKINTSRSFPAC, which is sequence-specific for the NH_2 terminus of DPPX-L. Each synthetic peptide (2 mg) was conjugated with maleimide-activated keyhole limpett hemocyanin (5 mg). The conjugates were used for immunization into rabbits, and antibodies raised were purified from rabbit sera by affinity chromatography through an AffiGel-15 column coupled with each antigen peptide, as described previously (*24*). Unless otherwise indicated, the antibody against the NH_2-terminal peptide (anti-N) was used for immuno-detection of DPPX-L, while that against the COOH-terminal peptide (anti-C) was used for detection of DPPX-S. Another peptide (36 residues) in an internal sequence of DPPX (positions 345–380 in the DPPX-S sequence) was produced as a fusion protein with glutathione S-transferase, for which the corresponding cDNA fragment was inserted into pGEX-3X expression vector (*25*). The fusion protein was used for immunization in rabbits, and antibodies raised were used as anti-In36 antibodies after anti-glutathione S-transferase antibodies were removed by affinity chromatography (*25*).

Transfection and Analysis of Expressed Protein—Each plasmid (20 μg) was transfected into 5×10^6 COS-1 cells using an electroporation apparatus (Gene Pulser, Bio-Rad) as described previously (*24*). The transfected cells were cultured in Dulbecco's modified Earle's medium (DMEM) containing 10% fetal calf serum in 6-cm dishes for 2 days. The cells were labeled for 40 min at 37°C with [^{35}S]methionine (3.7 MBq/dish) in 5 ml of methionine-free Eagle's minimum essential medium, and then chased in the fresh complete medium DMEM. At the indicated times of chases, cells were harvested, lysed, and subjected to immunoprecipitation with the indicated antibodies (*24*). The immunoprecipitates before and after treatment with endoglycosidase H (endo H) (0.1 unit/ml at pH 5.5 and 37°C for 16 h) were analyzed by SDS-PAGE (7.5% gel) and fluorography. When indicated, the transfected cells were treated with tunicamycin (2.5 μg/ml) for 3.5 h and used for metabolic labeling experiments as above.

In Vitro Transcription and Translation—The DPPX cDNA was inserted into the *Eco*RI site of the vector pGEM3. *In vitro* transcription of the DPPX mRNA was carried out with T7 RNA polymerase using the mCAP mRNA capping kit in accordance with the manufacturer's protocol. *In vitro* translation (0.5 μg of mRNA in a reaction mixture of 20 μl) was performed at 30°C for 90 min in a reticulocyte lysate system with [^{35}S]methionine (800 kBq) in the presence or absence of dog pancreas microsome (0.1 A_{250} unit) as described (*24*). The products, when indicated, were digested with trypsin and chymotrypsin (50 μg each/ml) at 0°C for 1 h or with endoH (0.1 unit/ml) at 37°C for 16 h (*24*). All the products were analyzed by SDS-PAGE (7.5% gel) and fluorography.

Immunofluorescence Microscopy—COS-1 cells grown on glass coverslips were transfected with the DPPX expression plasmid (20 μg) using Lipofectamine reagent and cultured for 2 days in DMEM containing 10% fetal calf serum. Cells were fixed and incubated with the indicated rabbit antibodies, then incubated with rhodamine-conjugated goat anti-rabbit IgG antibodies as described previously (*27*). For detection of the surface antigen, the fixed cells were incubated with the indicated rabbit anti-DPPX antibodies for 15 min, while for detection of the intracellular antigen the cells were permeabilized with 0.1% saponin in PBS for 20 min (*28*).

Western Blot Analysis—Various tissues from a male adult Wistar rat were homogenized with 7 volumes of 20 mM Tris-HCl (pH 7.5) containing 50 mM NaCl. Proteins of each homogenate (15 μg) were separated by SDS-PAGE (7.5% gels) and transferred onto an Immobilon membrane filter. The membrane was incubated for 1 h with rabbit anti-In36 IgG (10 μg/ml), then with alkaline phosphatase-conjugated goat anti-rabbit IgG antibodies for 1 h, followed by the enzyme reaction for visualization as described (*27*)

Northern Blot Analysis—Total RNA was extracted from various rat tissues by using the RNAzol B isolation kit. The

RNA (50 μg each) was separated by electrophoresis on 1.2% agarose gel, transferred onto a Hybond-N (Amersham-Pharmacia Biotech) membrane, and hybridized with a ^{32}P-labeled *Nco*I–*Nco*I fragment (340 bases) prepared from the DPPX cDNA.

DPPIV Activity—DPPIV activity was assayed with Gly-Pro-*p*-nitroanilide as a substrate (*2*, *7*).

RESULTS

Predicted Primary Structure of DPPXs—Two independent cDNA clones, pDX1 (4.5 kbp) and pDX2 (4.7 kbp), were obtained from the rat brain cDNA library. The clone pDX1 encodes 803-residue protein with a calculated molecular mass of 91 kDa, which corresponds to DPPX-S, while pDX2 encodes 859-residue protein with a molecular mass of 97 kDa, corresponding to DPPX-L (*16*). A structural difference was found in the NH_2-terminal sequence of the two proteins. DPPX-L has an NH_2-terminal extension of 56 residues that is absent in DPPX-S, followed by a sequence of 19 residues that is completely different from that of DPPX-S. The remaining sequence (784 residues) is completely identical between the two. The DPPXs have 33.1% identity with the entire sequence of DPPIV.

Based on these sequence data, structural features of DPPXs are schematically shown in Fig 1, in comparison with DPPIV, for which the membrane topology has been established (*5*, *7*). Both DPPX-S and DPPX-L are transmembrane proteins with short NH_2-terminal extensions of different lengths. The COOH-terminal major part of DPPXs contains 7 potential sites for *N*-linked glycosylation, while DPPIV has 8 sites. Three of them are aligned at the same positions in DPPXs and DPPIV (indicating by the shaded letters CHO in Fig. 1).

In Vitro Transcription and Translation—The DPPX cDNAs were subjected to *in vitro* transcription and translation (Fig. 2). The plasmid containing the DPPX-S cDNA directed the synthesis of mRNA that was translated into a protein of 93 kDa (lane 1), which is consistent with the predicted mass of DPPX-S (91 kDa). Translation of the mRNA in the presence of microsomes produced a major form of 113 kDa (lane 2). Digestion of the product with endo H in the presence of a detergent resulted in reduction of its molecular mass to 93 kDa (lane 3), indicating that the increase in molecular mass (20 kDa) is due to the addition of oligosaccharide chains. Essentially the same results were obtained for DPPX-L, which was synthesized as 97-kDa (lane 5) and 117-kDa forms in the absence and presence, respectively, of microsomes. The 117-kDa form was converted to the 97-kDa form by treatment with endo H (lane 7). When microsome-dependent products were treated with proteases (trypsin and chymotrypsin) in the absence of a detergent, both DPPX-S and DPPX-L were converted to a form with the same molecular mass (about 112 kDa) (lanes 4 and 8), slightly smaller than the untreated forms. These results support the structural model and membrane topology of DPPXs (Fig. 1). The COOH-terminal major part of the proteins is cotranslationally segregated into and glycosylated in the microsomal lumen. Proteolytic removal of the NH_2-terminal regions exposed at the membrane surface yields DPPXs with the same molecular mass.

Biosynthesis of DPPXs in Transfected Cells—The biosynthesis of DPPXs was examined in COS-1 cells transfected with the cDNA. The cells were metabolically labeled with [^{35}S]methionine, and cell lysates were subjected to immunoprecipitation with the indicated antibodies. As shown in Fig. 3A, anti-C antibodies precipitated 93-kDa and 113-kDa forms of DPPX-S (lane 1) and 97-kDa and 117-kDa forms of DPPX-L (lane 2). In contrast, anti-N antibodies recognized only the two forms of DPPX-L (lane 4), but not those of DPPX-S (lane 3), indicating that anti-N antibodies can be used as those specific for DPPX-L. However, the presence of the two forms for each DPPX was an unexpected result, and we therefore examined the relationship of the two forms (Fig. 3B). When the immunoprecipitates were treated with endo H, each larger form was converted to a single

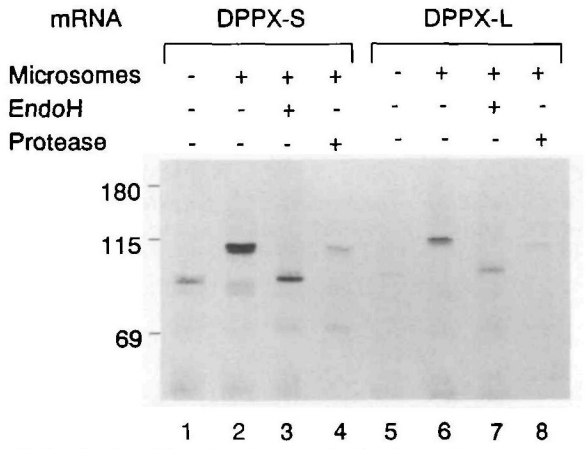

Fig. 2. **Analysis of *in vitro* transcription/translation products of DPPXs.** PGEM3 plasmids bearing the DPPX-S or DPPX-L cDNA were transcribed with T7 RNA polymerase. The transcripts were then subjected to *in vitro* translation with [^{35}S]methionine in the reticulocyte lysate system in the absence or presence of dog pancreatic microsomes. When indicated, the translation products were digested with endo H in the presence of detergent or with proteases (trypsin and chymotrypsin) in the absence of detergent. The products were analyzed by SDS-PAGE (7.5% gel) and fluorography.

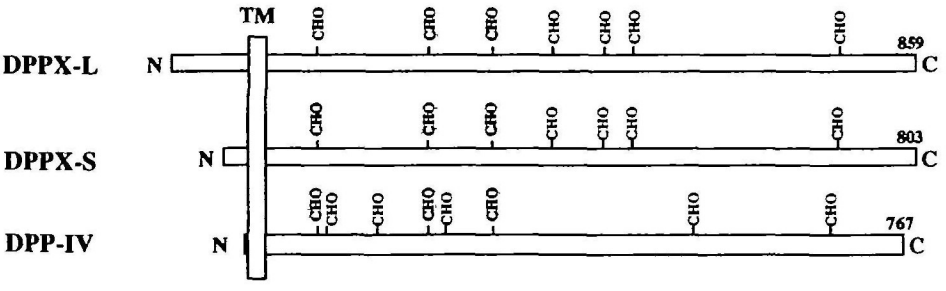

Fig. 1. **Structural diagram of DPPXs and DPPIV.** TM, transmembrane domain; CHO, potential *N*-linked glycosylation sites, of which those in the same positions in the three proteins are shaded. The total number of amino acid residues of each protein is shown at the COOH terminus.

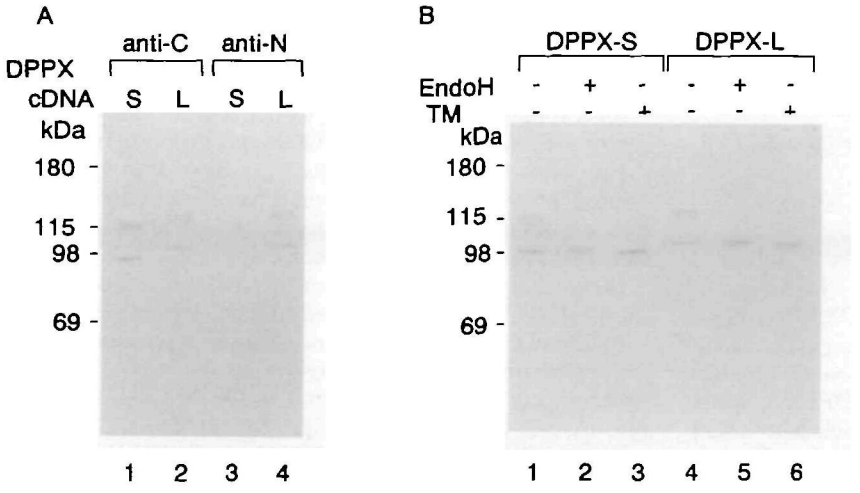

Fig. 3. **Analysis of DPPXs synthesized by transfected cells.** A: COS-1 cells which had been transfected with the DPPX-S (S) or DPPX-L (L) cDNA were labeled for 1 h with [^{35}S]methionine. Cell lysates were prepared and subjected to immunoprecipitation with anti-C or anti-N antibodies. B: transfected cells with or without prior treatment with tunicamycin (TM) were labeled for 20 min with [^{35}S]methionine and used for immunoprecipitation as above. The resultant immunoprecipitates were analyzed as in Fig. 2.

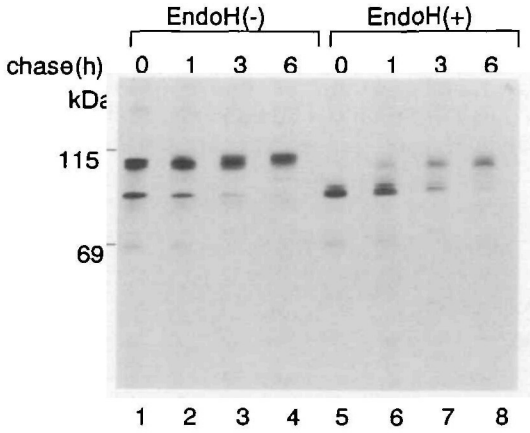

Fig. 4. **Pulse-chase experiments with transfected cells.** Cells transfected with the DPP-S cDNA were labeled with [^{35}S]methionine for 40 min and chased. At the indicated time of chase, cell lysates were prepared and subjected to immunoprecipitation with anti-C antibodies. The immunoprecipitates were analyzed as in Fig. 2.

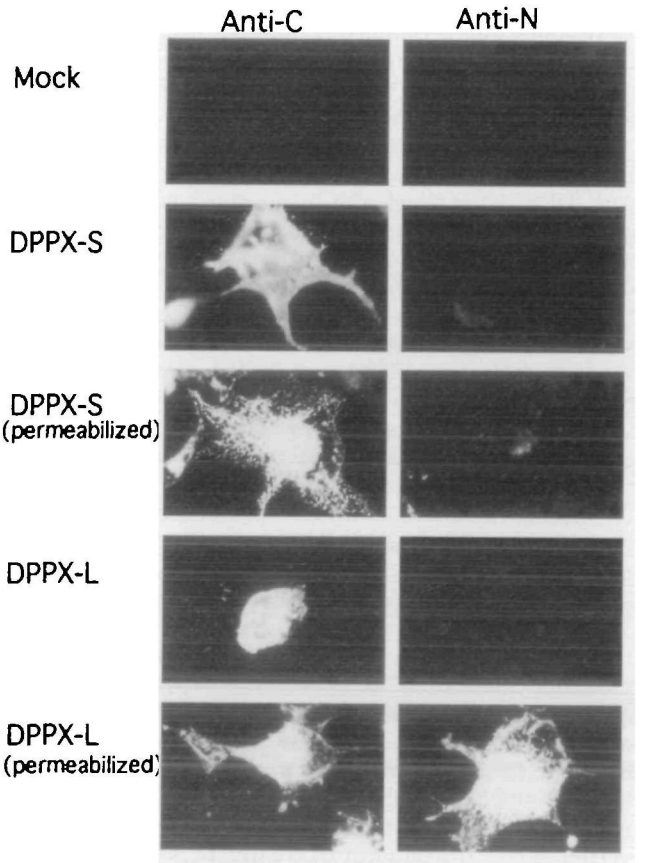

Fig. 5. **Immunofluorescence microscopy of transfected cells.** Cells transfected with the DPPX-S or DPPX-L cDNA were cultured for 2 days and fixed. The fixed cells before or after permeabilized with 0.1% saponin were incubated with rabbit anti-C or anti-N antibodies and then with rhodamine-conjugated goat anti-rabbit IgG antibodies. Cells transfected with the pSG5 vector plasmid with no DPPX cDNA were used as control (mock).

form identical with the smaller form (lanes 2 and 5). In addition, the same smaller form of each DPPX was obtained from cells which had been treated with tunicamycin, an inhibitor of the oligosaccharide synthesis (lanes 3 and 6). Thus, it is evident that the smaller forms are unglycosylated forms, the same as those obtained in the cell-free translation system without microsomes (Fig. 2, lanes 1 and 5). A possible reason for occurrence of the unglycosylated forms will be discussed later. The doublets occasionally observed for the larger forms may be due to different extents of glycosylation, because they disappeared upon treatment with endo H.

Pulse-chase experiments showed that DPPX-S was initially synthesized as the 113-kDa form with endo H-sensitive oligosaccharides (Fig. 4, lanes 1 and 5), which was then converted to a 115-kDa form with endo H–resistant sugar chains (lanes 2–4 and lanes 6–8). The result indicates that the newly synthesized DPPX-S is transported through the Golgi complex where its oligosaccharides are processed from the high-mannose type to the complex type (*29*). It was, however, noted that the mature form obtained after a 6-h chase was converted by endo-H treatment to a molecule (lane 8) slightly smaller than that of the control (lane 4). This suggests the possibility that some of the oligosaccharide chains contained in the mature form are still of the high-mannose type, as observed for the rat complement C3 (*30*). Essentially the same results were obtained for DPPX-

L, which was synthesized as the 117-kDa form and converted to the 120-kDa form by processing of the oligosaccharides (data not shown).

Immunofluorescence-Microscopical Observations—Transfected and unfransfected cells were examined by an indirect immunofluorescence technique (Fig. 5). Untransfected COS-1 cells were not stained with anti-C or anti-N antibodies. Cells transfected with the DPPX-S cDNA were stained with anti-C IgG but not with anti-N IgG. Non-permeabilized cells showed staining over the entire surface, while in permeabilized cells the intracellular distribution of the antigen was concentrated mainly in the endoplasmic reticulum (ER) and Golgi. The ER and Golgi distributions of DPPX-S were confirmed by its co-localization with calnexin (*31*) and with mannosidase II (*32*), respectively (data not shown). Cells transfected with the DPPX-L cDNA were also immunostained with anti-C IgG both before and after permeabilization of the cell membrane. In contrast, anti-N IgG did not react with DPPX-L of the non-permeabilized cells, although the antibodies recognized the antigen in the permeabilized cells (Fig. 5, lower panels at the right). These results confirm the specificity of the antibodies used here and the topology of DPPXs expressed on the cell surface. Detection by anti-C IgG of DPPX-S and DPPX-L in the non-permeabilized cells demonstrates that the COOH terminus of the both proteins is exposed on the cell surface. The cytoplasmically disposed NH_2 terminus of DPPX-L is detectable with anti-N IgG only after the cells are permeabilized.

No DPPIV Activity in the Wild-Type and Mutant DPPXs—DPPIV has the consensus active-site sequence Gly-X-Ser-X-Gly for serine proteases at positions 629–633 and two other active residues, Asp^{709} and His^{741}, involved in the catalytic triad (*5*). In the DPPX sequence the active Ser is substituted by Asp, but the other residues are conserved at the corresponding positions. We examined the enzyme activity of DPPIV in cells transfected with the DPPX cDNA with or without a mutation carrying the substitution Asp→Ser at the active site (Table I). Neither the wild-type DPPXs nor either mutant DPPX exerted a significant activity of the peptidase.

Expression of DPPX in the Brain Tissues—Total RNAs prepared from various rat tissues were analyzed for the presence of the DPPX mRNA by Northern blotting with ^{32}P-labeled cDNA fragment. As shown in Fig. 6, a major RNA of 4.5 kb was found in the brain tissues (lanes 1 and 2). This RNA species was not detectable in other tissues including lung, liver, spleen, kidney, small intestine (mucosa), and testis (lanes 3–8). The size of the major RNA is consistent with those of the DPPX cDNAs (4.5 kb for DPPX-S and 4.7 kb for DPPX-L). However, it remains to be determined whether the 4.5-kb mRNA band detected here contains both of the mRNAs for DPPX-S and DPPX-L or one of them.

We then examined the expression of DPPXs at the protein level in rat tissues by Western immunoblotting. Anti-In36 antibodies were used as the common antibodies reacting with both DPPXs. A major band corresponding to a 115-kDa protein was detected in the brain tissues (Fig. 7, lanes 1–3) but not in other tissues examined (lanes 4–9). The same 115-kDa protein was also detected in the brain tissues by the anti-C IgG, whereas no protein was specifically stained in any tissues when the anti-N antibodies were used for the detection (data not shown). These results indicate that only DPPX-S is preferentially expressed in

TABLE I. **DPPIV activity of wild-type and mutant DPPXs in transfected COS-1 cells.** COS-1 cells were transfected with the indicated plasmid and cultured for 2 days. The cells were harvested, lysed, and assayed for DPPIV activity with Gly-Pro-*p*-nitroanilide as a substrate. Values represent the means ± SE of three separate experiments.

Plasmid	Enzyme activity (μmol/min/mg of protein)
Mock	0.005 ± 0.005
DPPX-S (wild type)	0.005 ± 0.003
DPPX-L (wild type)	0.006 ± 0.002
DPPX-S (Asp^{650}→Ser)	0.001 ± 0.000
DPPX-L (Asp^{706}→Ser)	0.010 ± 0.004
DPP-IV	0.310 ± 0.007

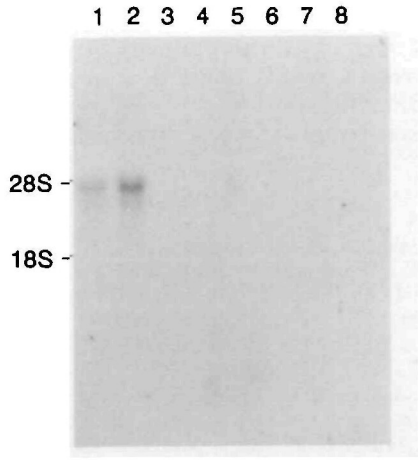

Fig. 6. **Northern blot analysis of DPPX mRNA.** Total RNAs (50 μg each) prepared from the indicated rat tissues were separated by electrophoresis on a 1.2% agarose gel. The RNAs were blotted and hybridized with the ^{32}P-labeled *Nco*I–*Nco*I fragment (340 bp) prepared from DPPX cDNA. Lanes 1–8: cerebrum, cerebellum, liver, lung, spleen, kidney, testis, and small intestine (mucosa), respectively. RNA size marker are 28S and 18S rRNA of rat liver.

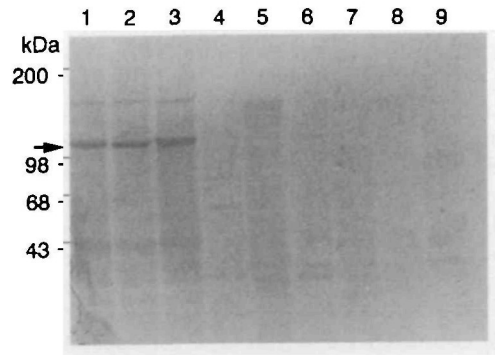

Fig. 7. **Western blot analysis of DPPX.** Proteins (15 μg) of homogenates prepared from the indicated rat tissues were separated by SDS-PAGE (7.5% gel) and transferred onto the Immobilon membrane. The membrane was blocked and incubated with rabbit anti-In36 antibodies and then with alkaline phosphatase–conjugated goat anti-rabbit IgG, followed by the enzyme reaction for color development. Lanes 1–9: cerebrum, cerebellum, brain stem, liver, lung, spleen, kidney, testis, and small intestine (mucosa), respectively. The arrow indicates a position corresponding to the 115-kDa DPPX-S.

brain as a protein detectable under the conditions used here.

DISCUSSION

In the present study we have examined the biosynthesis and modification of DPPXs in both *in vitro* and *in vivo* systems using the cDNA. DPPXs were cotranslationally segregated into the microsomal lumen leaving the NH_2-terminal tails outside the membrane, indicating that a hydrophobic domain near to the NH_2 terminus is an uncleavable signal sequence for translocation across the membrane and functions as an anchor to the membrane, as observed for DPPIV (*5*). DPPXs were also cotranslationally glycosylated, resulting in increase of the molecular mass by 20 kDa, which corresponds to the sum of the masses of oligosaccharides attached to all the 7 potential sites. The glycosylated DPPXs were further modified by processing of the oligosaccharides from the high-mannose type to the complex type of slightly larger molecular mass, which occurs in the Golgi complex (*29*). The complete conversion to the endo H–resistant complex-type oligosaccharides required more than 3 h (Fig. 4), much longer than that of other membrane proteins expressed on the cell surface (*33*, *34*). This indicates that the newly synthesized DPPXs are more slowly transported from the ER to the Golgi complex. The longer retention of DPPXs in the ER may be in part due to the overexpression of the molecules under the conditions used.

It is of interest to note that a significant amount of DPPX was synthesized as an unglycosylated form in the transfected COS-1 cells. A similar result was obtained for DPPIV when it was synthesized in the transfected cells (*28*, *33*). The unglycosylated form of DPPIV was not detected when it was endogenously synthesized in primary cultured rat hepatocytes (*33*). These results suggested that the unglycosylated form of DPPX might be produced only in the transfected cells. All the DPPX nascent peptides, when overproduced by the introduced cDNA, may not be properly translocated across the ER membrane, resulting in unglycosylation of the products remaining in the cytoplasm. The unglycosylated DPPX thus overproduced appears to be an abnormal form that is rapidly degraded within the cells, as observed in other cases (*28*, *33*, *35*).

Western immunoblotting analysis revealed that DPPX-S, but not DPPX-L, was detectable only in brain tissues, including cerebrum, cerebellum and brain stem. This is based on the findings that the immunoreactive antigen has the same molecular mass (115 kDa) as that determined for DPPX-S synthesized in the transfected cells and that the protein is not recognized by anti-N antibodies specific for DPPX-L. On the other hand, Northern blot analysis demonstrated that the DPPX mRNA is also detectable only in brain tissues. Because only DPPX-S is detectable as the translation product, it is reasonable to conclude that the major 4.5-kb mRNA detected here corresponds to that for DPPX-S. However, the mRNA for DPPX-L may exist at a much lower level at least in brain tissues, because the isolation of the DPPX-L cDNA clone was based on the presence of its mRNA. In fact, using a more sensitive method that combines the PCR and Southern blot analysis, Wada *et al.* (*16*) demonstrated that DPPX-L mRNA was exclusively expressed in brain. Furthermore, by *in situ* hybridization of mRNA in brain, the DPPX-S mRNA was detected at a high level in the hipocampal complex and some other regions, while the DPPX-L mRNA was detectable only in limited regions of brain with the highest level of expression in the medial habenula (*16*). Taken together, these observations suggest that (i) the two mRNAs are probably produced by an alternative splicing of RNA derived from the same gene; (ii) the DPPX-S mRNA is a major transcript preferentially expressed in brain; (iii) the DPPX-L mRNA is a minor one expressed in a specific region of brain where the mRNA is spliced differently from that for DPPX-S.

DPPX and DPPIV are clearly different gene products: the human DPPX gene is assigned to chromosome 7 (*36*), while the DPPIV gene is mapped to chromosome 2 (*37*). Nevertheless, the active site motif Gly-X-Ser-X-Gly and other active residues Asp and His for serine proteases are conserved in the corresponding positions in DPPX, although the active Ser is replaced by Asp. Substitution of Ser for the Asp residue in DPPX to produce the same catalytic triad as in DPPIV, however, failed to generate an enzymatically active form. This may be due to a conformational difference between DPPX and DPPIV. The three residues, even if present in the same positions in the primary structure, may not form the same conformationally active triad structure as in DPPIV (*2*, *8*).

The membrane topology of DPPX, having the majority of the molecule extracellularly, is quite similar to that of DPPIV. It is well documented that DPPIV is involved in many physiological processes, such as cell-matrix adhesion of hepatocytes (*11*) and cancer cells (*38*); transmembrane signaling in immune cells serving as a cell surface marker, CD26 (*9*, *12*); accumulation of adenosine deaminase on the T cell surface, which may contribute to the pathophysiology of human severe combined immunodeficiency (*10*); and neoplastic transformation of melanocytes (*39*). The involvement of DPPIV in these events does not always require the catalytic site of the peptidase. DPPX also shows about 30% amino acid sequence identity with fibroblast activation protein (FAP) α (*40*). FAP α is a type II integral membrane protein with a short cytoplasmic domain, and is a surface antigen selectively expressed in reactive stromal fibroblasts of epithelial cancers, granulation tissue of healing wounds, and malignant cells of bone and soft tissue sarcomas. The significant similarity in the primary structure and membrane topology to DPPIV and FAP α suggests the possibility that DPPX is also involved in an extracellular interaction process required for brain functions.

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