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Purification, Molecular Cloning, and Immunohistochemical Localization of Dipeptidyl Peptidase II from the Rat Kidney and Its Identity with Quiescent Cell Proline Dipeptidase[1]

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We purified dipeptidyl peptidase II (DPP II) to homogeneity from rat kidney and determined its physicochemical properties, including its molecular weight, substrate specificity, and partial amino acid sequence. Furthermore, we screened a rat kidney cDNA library, isolated the DPP II cDNA and determined its structure. The cDNA was composed of 1,720 base pairs of nucleotides, and 500 amino acid residues were predicted from the coding region of cDNA. Human quiescent cell proline dipeptidase (QPP) cloned from T-cells is a 58-kDa glycoprotein existing as a homodimer formed with a leucine zipper motif. The levels of amino acid homology were 92.8% (rat DPP II *vs.* mouse QPP) and 78.9% (rat DPP II *vs.* human QPP), while those of nucleotide homology were 93.5% (rat DPP II *vs.* mouse QPP) and 79.4% (rat DPP II *vs.* human QPP). The predicted amino acid sequences of rat DPP II and human and mouse QPP possess eight cysteine residues and a leucine zipper motif at the same positions. The purified DPP II showed similar substrate specificity and optimal pH to those of QPP. Consequently, it was thought that DPP II is identical to QPP. Northern blot analysis with rat DPP II cDNA revealed prominent expression of DPP II mRNA in the kidney, and the order for expression was kidney ≥ testis ≥ heart > brain ≥ lung > spleen > skeletal muscle ≥ liver. In parallel with Northern blot analysis, the DPP II antigen was detected by immunohistochemical staining in the cytosol of epithelial cells in the kidney, testis, uterus, and cerebrum.

Key words: cDNA and identification, dipeptidyl peptidase II (DPP II), quiescent cell proline dipeptidase (QPP), rat kidney.

Dipeptidyl peptidases [EC 3.4.14.-] have been identified in various mammalian tissues and categorized into at least four distinct types (DPP I–IV) (*1*). Dipeptidyl peptidase II, also known as dipeptidyl aminopeptidase II (DPP II) [EC 3.4.14.2], preferentially releases dipeptide moieties (Xaa-Ala- or Xaa-Pro-) at acidic pH (pH 4.5–6.0) from N-termini of some oligopeptides or proteins (*2*, *3*). This enzyme is thought to be involved physiologically in the breakdown of some oligopeptides or their fragments, such as substance P (Arg-Pro-Lys-Pro), casomorphin (Tyr-Pro-Phe-Pro-Gly-Pro-Ile), and bradykinin fragment (Arg-Pro-Pro) and thyroliberin fragment (His-Pro-Val) (*4*).

DPP II was first identified in bovine anterior pituitary extracts by McDonald *et al.* (*2*) and has also been identified in a number of mammalian tissues, cells and body fluids such as muscle (*5*), kidney (*6–8*), ovary (*3*), brain (*4*), lung (*9*), macrophages (*10*), normal and malignant haemic cells (*11*, *12*), peritoneal mast cells (*13*), serum (*14*), urogenital system (*15*), and seminal plasma (*16*, *17*, *22*). As to its localization in cells, although Mentlein and Struckhoff (*4*) reported the presence of membrane-associated and soluble types of DPP II, which have similar characteristics, in the rat brain lysosomal fraction, DPP II is generally localized in lysosomes (*3*, *6*, *18*). Enzymes that can cleave proline-containing bonds include exopeptidases such as dipeptidyl peptidase II, dipeptidyl peptidase IV (CD26/DPP IV), prolylcarboxypeptidase (PCP, angiotensinase C), and quiescent cell proline dipeptidase (QPP).

Human QPP, which was recently isolated and cloned from human T cells (*19*, *20*), is a 58-kDa glycoprotein existing as a homodimer formed with a leucine zipper motif (*21*). As compared with the amino acid sequence of human QPP and the N-terminal amino acid sequences of porcine and human DPP IIs (*20*, *22*), we found that the N-terminal amino acid sequences of porcine seminal plasma DPP II (41

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Abbreviations: MCA, 4-methyl-coumaryl-7-amide; AFC, amino-4-trifluoromethylcoumarin; βNA, β-naphthylamide; MNA, 4-methoxy-2-naphthylamide; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; β-ME, β-mercaptoethanol; E-64, [*N*-(*N*-(L-3-trans-carboxyoxiran-2-carbonyl)-L-leucyl)agmatin; DFP, diisopropylphosphofluoridate; AEBSF, 4-(2-aminoethyl)-benzenesulfonyl fluoride; PMSF, phenylmethylsulfonyl fluoride; PCMBS, *p*-chloromercuri-benzene-sulfonic acid; NEM, *N*-ethylmaleimide.

amino acid residues) and rat kidney DPP II (50 amino acid residues) showed high homology (73.2 and 72%, respectively) with that of human QPP.

Here, we report the purification and physiochemical properties of DPP II from rat kidney and its localization as determined by Northern blot analysis and immunohistochemistry. We also describe the isolation and characterization of a cDNA coding for rat DPP II and show that the amino acid sequence predicted from the rat cDNA is identical to that of human QPP.

MATERIALS AND METHODS

Materials—Kidneys were obtained from Albino rats of the Wistar strain, which were kindly provided by Kiwa Laboratory Animals (Misato) and stored at −30°C until use. Fluorogenic peptide substrates such as Lys-Ala-, Gly-Pro-, Gly-Gly-, Gly-Arg-, Ala-Arg- and Arg-Arg-MCAs, Ala-, Pro-, Lys-, Gly-, Tyr-, Leu-, Phe- and Pyr-MCAs, and Suc-Gly-Pro-, Suc-Gly-Pro-Leu-Gly-Pro-, z-Phe-Arg- and z-Arg-Arg-MCAs were purchased from Peptide Institute (Osaka). Ala-Pro-AFC and Ala-Ala-βNA were purchased from Bachem Feinchemikalien AG (Bubendorf, Switzerland). Q-Sepharose, Superdex HR 200, Resource Q, and Hybond-C extra were purchased from Amersham (Buckinghamshire, UK). Phenyl Cellulofine and Zinc-chelate Cellulofine were purchased from Seikagaku. Matrex Gel Red A was purchased from Amicon (Lexinton, MA, USA). Lys-Ala-4-methoxy-2-naphthylamide and Fast Garnet GBC were also purchased from Sigma Chemical (St. Louis, MO, USA). A random primer DNA labeling kit was obtained from Takara Shuzo (Otsu). A human liver cDNA library, rat kidney cDNA library, rat multiple tissue Northern blot, and ExpressHyb™ Hybridization Solution were purchased from Clontech (Palo Alto, CA, USA). Histofine (SAB-Po kit) was purchased from Nichirei (Tokyo). The TA-cloning vector pCR2.1 was purchased from Invitrogen (Carlsbad, CA, USA).

All other chemicals were of analytical grade.

cDNA Cloning and Sequencing—To obtain a probe for cDNA cloning, nested amplification was performed using primers specific to human QPP. The primary amplification mixture contained 1× polymerase chain reaction buffer, 0.2 μM dNTPs, 0.2 μM primer QF1 (5′-GCAGCGTCTGGACCACTTCAAC-3′) and 0.2 μM primer QR1 (5′-GTGGGGATACTTCATCCTCAGG-3′), 0.5 μl of Pyrobest DNA polymerase and human liver λgt11 cDNA library amplified according to the manufacturer's instructions (Clontech, Palo Alto, CA, USA) as a template. A prominent band of 350 base pairs was excised, extracted from the gel, and TA-cloned into pCR2.1. Several clones obtained were then sequenced with an ABI PRISM cycle sequencing kit using a PE Applied Biosystems DNA sequencer PRISM 310 (Foster City, CA, USA). The nucleotide sequence of 360 base pairs was confirmed to correspond to a fragment of human QPP cDNA.

A 360-base-pair cDNA labeled using a random primer DNA labeling kit was used as a probe for the screening of the rat kidney cDNA library. Several positive clones were isolated and sequenced as described above using a PE Applied Biosystems DNA sequencer PRISM 310.

Assay of Enzyme Activity—Enzyme activity was assayed by fluorometrically measuring (excitation, 380 nm; emission, 440 nm for MCA, excitation, 400 nm; emission, 505 nm for AFC, and excitation, 330 nm; emission, 415 nm for βNA) the liberation of MCA, AFC and βNA at 37°C in a mixture containing 10 μl of 10 mM substrate, 100 μl of 0.5 M sodium phosphate buffer, pH 6.0, 1–20 μl of enzyme solution, and Milli Q water (18 mΩ) in a total volume of 1 ml. After incubation for 30 min, 2 ml of 0.2 N acetic acid was added to the mixture to terminate the reaction. One unit of activity was defined as the amount of enzyme that is capable of hydrolyzing 1 μmol of substrate per minute. Protein was measured by absorbance at 280 nm in a 1-cm light path, and 1 mg of protein was defined as the concentration required to yield an absorbance of 1.0 ($E^{0.1\%}_{280nm}$ = 1.0).

Polyacrylamide Gel Electrophoresis—Samples were resolved by electrophoresis on 12.5% polyacrylamide slab gels in 25 mM Tris, 192 mM glycine, pH 8.3, and 0.1% SDS by the method of Laemmli (*23*). Proteins in the gel were stained with Coomassie Brilliant Blue R-250. To detect DPP II activity, 12.5% polyacrylamide gels in the absence of SDS were also incubated at 37°C for 15 min in 100 mM sodium phosphate buffer, pH 6.0, containing 1 mM Lys-Ala-MNA, and then the gel was stained with 0.15% Fast Garnet GBC in 2.1 M sodium acetate buffer, pH 4.2 (*24*).

Determination of Molecular Weight—The apparent molecular weight (M_r) of DPP II was determined by gel filtration chromatography on a Superdex HR200 column (1.0 × 30 cm) equilibrated with 20 mM sodium phosphate-buffered saline, pH 6.0, at a flow rate of 0.15 ml/min in an FPLC system. The molecular weight of the purified DPP II was calculated by comparison with the log M_r for the standard proteins, catalase (M_r 232,000), albumin (M_r 67,000), ovalbumin (M_r 43,000), chymotrypsinogen A (M_r 25,000), and ribonuclease (M_r 13,700).

The molecular weight of the enzyme was determined by Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) mass spectrometry using a Voyager™-RP, PerSeptive Biosystems according to the manufacturer's instructions. Sinapinic acid (3,5-dimethoxy-4-hydroxy cinnamic acid) was used as the matrix. The standard was acquired using bovine serum albumin (M_r 66,431) and its divalent substance (M_r 33,216).

N-Terminal Amino Acid Sequencing—Purified DPP II (30 μg; ~550 pmol) was bound onto a Prospin membrane (Applied Biosystem, Foster City, CA, USA), then sequenced by automated Edman degradation using an Applied Biosystems Model 473 Protein Sequencer equipped with an online phenylthiohydantoin analyzer (Applied Biosystems 120A analyzer).

Bromocyan Digestion—Purified DPP II (25 μg: ~450 pmol) was digested with bromocyan (10 mg/ml) in the presence of 70% formic acid and 20 mM β-ME for 24 h at room temperature. After digestion, the reaction mixture was frozen and dried. The digest was neutralized with 1 M Tris-HCl, then applied onto 15% SDS-PAGE. After electrophoresis, the fragments on the gel were blotted onto a PVDF membrane. Each fragment transferred to the membrane was cut out and sequenced using an Applied Biosystems Model 473 Protein Sequencer.

Northen Blot Analysis—Rat multiple tissue Northern blot was used to detect the distribution of rat DPP II transcripts. Rat multiple tissue Northern blot contains 2 μg of poly A^+ RNA from the heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis. Rat DPP II cDNA was ^{32}P-

labeled and used as a probe. After pre-hybridization, the filter was hybridized in a ExpressHyb™ Hybridization Solution. The blot was washed to a final stringency of 0.1× SSC/0.1% SDS at 68°C and exposed for 4 h to X-ray firm.

Antibody Preparation and Western Immunoblotting—Anti-porcine DPP II rabbit IgG was purified by ammonium sulfate fractionation (0–35% saturation) and DEAE-Sephacel column chromatography (*25*) for immunohistochemistry. Anti-porcine DPP II IgG was further purified by use of Protein G. The affinity-purified anti-porcine DPP II IgG was also used for Western immunoblotting.

Immunohistochemistry—Most of the adult rat tissues, including liver, kidney, lung, trachea, heart, aorta, spleen, pancreas, muscle, cerebellum, cerebrum, thymus, thyroid and adreal glands, testis, epididymis, seminal vesicle, prostate, uterus, ovary and placenta, were obtained within 5 min of anesthetic death by diethyl ether. The tissue specimens were fixed in 10% neutral buffered formalin to be embedded in paraffin, and 5-μm-thick paraffin sections were cut. Immunohistochemical staining of these tissue sections was carried out as described previously (*26*).

Purification of DPP II from Rat Kidney—All purification steps were conducted at 4°C unless otherwise specified. At each step, the activity of DPP II was measured in 50 mM sodium phosphate buffer, pH 6.0, using Lys-Ala-MCA.

Step 1. Homogenation: Normal rat kidney tissue (~ 400 g) was chopped into small pieces and homogenized with 4 volumes of cold 0.9% NaCl for 5 min using a Warring blender. The homogenate was centrifuged at 12,000 ×*g* for 45 min, and the supernatant was centrifuged further at 105,000 ×*g* for 60 min.

Step 2. Ammonium sulfate fractionation: The supernatant obtained in step 1 was brought to 70% saturation with solid ammonium sulfate. After stirring for an additional 60 min, the solution was centrifuged at 10,000 ×*g* for 45 min. The precipitate obtained was dissolved in a minimum volume of 20 mM sodium phosphate buffer, pH 8.0, and the solution was dialyzed overnight against the same buffer.

Step 3. Q-Sepharose column chromatography: The dialyzate was applied at a flow rate of 200 ml/h to a column of Q-Sepharose (5.0 × 20 cm) pre-equilibrated with 20 mM sodium phosphate buffer, pH 8.0. The column was washed extensively with the same equilibration buffer, then a linear gradient was formed with 1,500 ml of the same buffer and 1,500 ml of 20 mM sodium phosphate buffer, pH 8.0, containing 0.5 M NaCl (Fig. 1). Fractions containing DPP II activity were collected and concentrated by the addition of solid ammonium sulfate (70% saturation). After stirring the solution for 60 min, the precipitate was sepasated by centrifugation at 10,000 ×*g* for 45 min. The precipitate was resuspended in a minimum volume of 20 mM sodium phosphate buffer, pH 7.5, and the solution was dialyzed overnight against the same buffer.

Step 4. Matrex Gel Red A column chromatography: The dialyzate was applied to a Matrex Gel Red A column (2.5 × 50 cm) pre-equilibrated with 20 mM sodium phosphate, pH 7.5, and the pass-through fraction containing DPP II activity was collected. The collected sample was concentrated by ammonium sulfate fractionation (70% saturation). The precipitate was dissolved in a minimum volume of 20 mM sodium phosphate-buffered saline, pH 8.0, then dialyzed overnight against the same buffer.

Step 5. Zinc-Chelate Cellulofine column chromatography: The dialyzate was applied at a flow rate of 15 ml/h to a Zinc-Chelate Cellulofine column (1.5 × 8 cm) pre-equilibrated with 20 mM sodium phosphate-buffered saline, pH 8.0. The pass-through fraction containing DPP II activity was collected, and ammonium sulfate was added to a final concentration of 2.0 M.

Step 6. Phenyl-Cellulofine column chromatography: The fraction containing the enzyme was applied at a flow rate of 15 ml/h to a Phenyl-Cellulofine column (1.5 × 8 cm) pre-equilibrated with 20 mM sodium phosphate buffer, pH 8.0, containing 2.0 M ammonium sulfate. Non-absorbed proteins were washed out with the same buffer, then a decreasing salt gradient elution was performed with 150 ml of the above buffer and 150 ml of 20 mM sodium phosphate buffer, pH 8.0. Fractions containing DPP II activity were collected and concentrated by ammonium sulfate fractionation (70% saturation). The precipitate was dissolved in a minimum volume of 20 mM sodium phosphate-buffered saline, pH 8.0 and dialyzed overnight against the same buffer.

Step 7. Superdex 200 HR column chromatography in FPLC: The dialyzate was applied at a flow rate of 0.15 ml/min to a Superdex 200 HR column (1.0 × 30 cm) pre-equilibrated with 20 mM sodium phosphate-buffered saline, pH 8.0. The fractions containing DPP II activity were collected

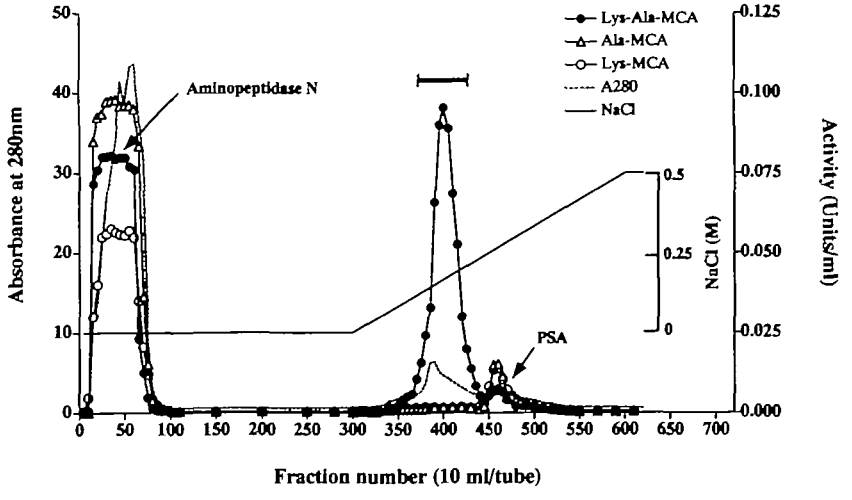

Fig. 1. **Purification of dipeptidyl peptidase II (DPP II) from rat kidneys on Q-Sepharose column chromatography.** Fractions containing DPP II activity were collected. Bar indicates the fractions pooled. Absorbance at 280 nm, (– – – –); DPP II (–●– toward Lys-Ala-MCA); aminopeptidase N (CD13), and puromycin-sensitive alanyl aminopeptidase (PSA) (–○– toward Lys-MCA, –△– toward Ala-MCA, and –●– toward Lys-Ala-MCA).

and dialyzed overnight against 20 mM sodium phosphate buffer, pH 8.0.

Step 8. Resource Q column chromatography in FPLC: The dialyzate was applied at a flow rate of 0.15 ml/min to a Resource Q column (bed volume: 1.0 ml) pre-equilibrated with 20 mM sodium phosphate buffer, pH 8.0. The column was washed with the same equilibration buffer, then a linear gradient was formed with 10 ml of the same buffer and 10 ml of 20 mM sodium phosphate buffer, pH 8.0, containing 0.5 M NaCl. Fractions containing DPP II activity were collected and dialyzed overnight against 20 mM sodium phosphate buffer, pH 8.0. After dialysis, the sample solution was used in subsequent experiments.

RESULTS

Enzyme Purification—Dipeptidyl peptidase II (DPP II) was purified from approximately 400 g of rat kidney by Q-Sepharose, Matrex Gel Red A, Zinc-Chelate Cellulofine, Phenyl Cellulofine, Superdex 200 HR, and Resource Q column chromatographies. As shown in Fig. 1, three peaks possessing hydrolyzing activity towards Lys-Ala-MCA were observed on Q-Sepharose column chromatography. The pass-through fraction (first peak) and third peak also cleaved both Ala-MCA and Lys-MCA for aminopeptidase N (CD13) and puromycin-sensitive alanyl aminopeptidase, but the second peak did not (*27*, *28*). Puromycin inhibited the enzyme activities in the pass-through fraction and third peak. IC_{50}-values of puromycin on both enzyme activities were calculated to be 200 and 0.8 μM, respectively (*28*). It is thought that the pass-through fraction contained aminopeptidase N and the third peak contained puromycin-sensitive alanyl aminopeptidase. Accordingly, we used the second peak for further purification of DPP II. Table I shows typical results. Rat kidney DPP II was purified approximately 130-fold with a 10.5% yield over Q-Sepharose column chromatography, and the purified enzyme at the final step migrated as a single band on non-denatured PAGE, and SDS-PAGE in the presence or absence of β-ME (Fig. 2, A and B).

Properties of the Purified Enzyme—The molecular weight (M_r) of purified DPP II was 52,000 or 54,000 in the absence or presence of β-ME on SDS-PAGE, and 54,053.8 according to TOF-MS (figure not shown). It was further estimated to be 105,000 by non-denatured PAGE (Fig. 2, A and B) and 105,000 by gel filtration chromatography with Superdex HR 200 in the FPLC system (figure not shown). The activity of DPP II toward Lys-Ala-MNA coincided with the band of the protein on non-denatured PAGE (Fig. 2A).

Substrate Specificity and Kinetic Properties of the Purified Enzyme—DPP II exhibited preferentially high activity toward the synthetic substrates Gly-Pro-MCA and Lys-Ala-MCA. It did not hydrolyze Gly-Arg-, Gly-Gly-, Ala-Arg-, and Arg-Arg-MCAs. It also did not hydrolyze substrates of other aminopepidases, such as Ala-, Pro-, Lys-, Gly-, Tyr-, Leu-, Phe-, Met-, Arg-, and Pyr-MCAs, or the substrates of endopeptidases, such as Boc-Val-Leu-Lys-, Boc-Phe-Ser-Arg-, Boc-Ala-Gly-Pro-Arg-, Suc-Ala-Ala-Ala-, Suc-Ala-Pro-Ala-,

TABLE I. **Purification of dipeptidyl peptidase II from rat kidney.**

Step	Total absorbance (280 nm)	Total activity (Units)	Specific activity (Units/mg*)	Yield (%)	Purification (-fold)
Q-Sepharose	2,230	17.8	0.0080	100	1
Matrex Gel Red A	614.3	17.5	0.0285	98.7	3.6
Zinc-Chelate Cellulofine	117.0	8.7	0.0741	49.0	9.3
Phenyl-Cellulofine	16.5	4.3	0.263	24.8	32.9
Superdex 200 (FPLC)	4.5	3.2	0.70	17.7	87.5
Resource Q (FPLC)	1.7	1.8	1.05	10.5	131.3

*Protein concentration was measured by absorbance at 280 nm in a 1-cm light path, and 1 mg of protein was defined as the concentration required to yield an absorbance ($E^{1\%}_{280nm}$ = 1.0).

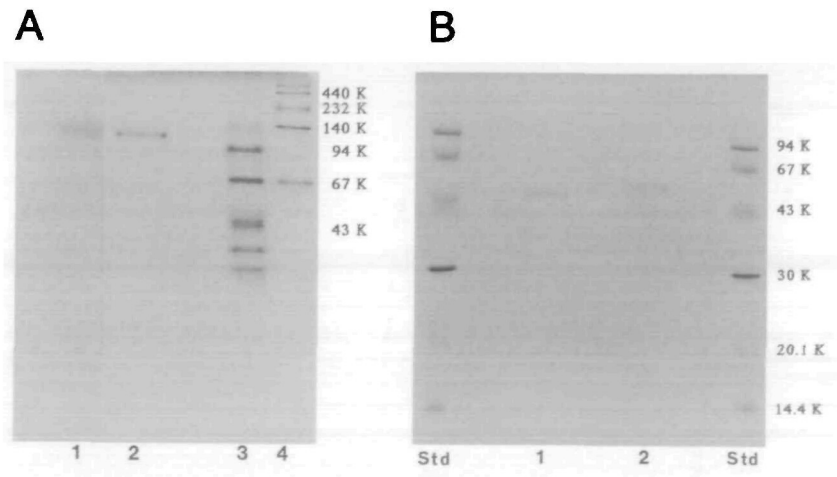

Fig. 2. **Polyacrylamide gel electrophoresis of DPP II from rat kidney.** (A) The purified enzyme was resolved by electrophoresis on a 12.5% polyacrylamide gel in the absence of SDS [lanes 1 (3 μg) and lane 2 (3 μg)]. The gels were stained for DPP II activity using Lys-Ala-4-methoxy-2-naphthylamide (lane 1) in combination with Fast Garnet GBC as described in "MATERIALS AND METHODS." Protein in lane 2 was stained with Coomassie Brilliant Blue R-250. Lane 3 included the following standard proteins: phosphorylase *b* (M_r 94,000), bovine serum albumin (M_r 67,000), ovalbumin (M_r 43,000), carbonic anhydrase (M_r 30,000), soybean trypsin inhibitor (M_r 20,100), and α-lactalbumin (M_r 14,400). Lane 4 included the following standard proteins: thyroglobulin (M_r 669,000), ferritin (M_r 440,000), catalase (M_r 232,000), lactate dehydrogenase (M_r 140,000), and bovine serum albumin (M_r 67,000). (B) The purified enzyme was resolved by electrophoresis on a 12.5% polyacrylamide gel in the presence of SDS, and then the gel was stained with Coomassie Brilliant Blue R-250. Lane 1 contained 3 μg of the purified enzyme in the absence of β-ME, and lane 2 contained 3 μg of the purified protein in the presence of β-ME. Std included the same standard proteins as lane 3 of (A).

Suc-Gly-Pro-, Suc-Leu-Leu-Val-Tyr-, Suc-Ala-Ala-Pro-Phe-, Suc-Gly-Pro-Leu-Gly-Pro-, z-Phe-Arg-, z-Arg-Arg-, and z-Gly-Ala-Gln-MCAs. However, it did exhibit a high activity toward Ala-Pro-AFC and a weak activity toward Ala-Ala-βNA (Table II).

Effects of Inhibitors, Metal Ions, and Other Reagents on the Purified Enzyme—DPP II activity was potently inhibited by DFP and PCMBS (at pH 6.0) and was moderately inhibited by PMSF and AEBSF (Table III). However, it was resistant to the other protease inhibitors, benzamidine, α-PMSF, NEM, E-64, iodoacetamide, iodoacetic acid, antipain, chymostatin, leupeptin, pepstatin, elastatinal, Diprotin A, Actinonin, and phosphoramidon. Furthermore, some aminopeptidase inhibitors, including amastatin, arphamenine A, bestatin, and leuhistin, did not inhibit DPP II activity. Among metal ions, Hg^{2+} completely inhibited the enzyme activity and Cu^{2+} moderately inhibited it (64% of the control). Furthermore, 0.4 M KCl and 50 mM Tris (pH 5.5) inhibited the enzyme activity up to 32 and 33% of the control, respectively (data not shown).

Optimal pH and Enzyme Stability—The hydrolytic activity of DPP II for Lys-Ala-MCA was assayed in the pH range of 2.5 to 11.5. The activity towards this substrate was maximal at pH 5.5. Furthermore, to examine the effect of pH on stability, DPP II was incubated at various pH values for 24 h at 4°C. The enzyme was stable over a broad range of pH (3.5 to 10.0). In 20 mM sodium phosphate-buffered saline, pH 8.0, it was stable for at least several months at −30°C. It was also resistant to several repeated cycles of freezing and thawing.

To examine the effect of temperature on its stability, DPP II was incubated at various temperatures for 30 min and was found to be stable up to 50°C.

Determination of the Amino Acid Sequence of N-Terminal and BrCN Fragments—The first 47 amino acids of the N-terminus of DPP II purified from rat kidney were Leu-Asp-Pro-Asp-Phe-Arg-Glu-Asn-Tyr-Phe-Glu-Gln-Tyr-Met-Asp-His-Phe-Asn-Phe-Glu-Ser-Phe-Ser-Asn-Lys-Thr-Phe-Gly-Gln-Arg-Phe-Leu-Val-Ser-Asp-Lys-Phe-Trp-Lys-Met-Gly-Glu-Gly-Pro-Ile-Phe-Phe-Tyr-Thr-. Only leucine was detected as the N-terminal amino acid. The amino acid sequences of five fragments obtained by BrCN-digestion were Lys-Tyr-Pro-, Asp-Tyr-Pro-Tyr-Pro-Thr-Asn-Phe-Leu-Gly-Pro-Leu-Pro-Ala-Asn-Pro-Val-Lys-Val-Gly-Cys-Glu-Arg-Leu-, Gly-Leu-Arg-Ala-Leu-Ala-Gly-Leu-Val-Tyr-Asn-Ser-Ser-Gly-, Glu-Pro-Cys-Phe-Asp-Ilu-Tyr-Gln-, and Phe-Pro-Glu-Ile-Pro-Phe-Ser-Asp-Glu-Leu-Arg-Gln-Gln-Tyr-Cys-Leu-Asp-Thr-Trp-Gly-Val-Trp-Pro-Arg-Pro-Asp-Trp-Leu-Gln-Thr-Ser-Phe-. All amino acid sequences of the five fragments and of the N-terminal end were found in the deduced amino acid sequence from the rat kidney DPP II cDNA structure (Fig. 4).

cDNA Cloning and Its Structural Analysis—A λgt 11 cDNA library constructed from rat kidney was screened with a product of nested amplification (360-bp DNA fragment of human QPP) described in "MATERIALS AND METHODS." Two positive clones with the same length were obtained from a total of 2×10^6 plaques and were subjected to nucleotide sequence analysis. The two clones had completely identical sequences, and we named them λRDII. The sequence strategy is outlined in Fig. 3. The nucleotide sequence and deduced amino acid sequence are shown in Fig. 4. The cDNA insert in λRDII (1,720 base pairs) was composed of 32 base pairs of a 5′ noncoding sequence, 1,500 base pairs coding for pre DPP II, a stop codon, 170 base pairs of a 3′ noncoding sequence, and 15 base pairs of a poly(A) tail. The polyadenylation or processing sequence of AATAAA was present at 13 base pairs upstream from the poly(A) tail.

The total number of amino acid residues of DPP II predicted from the coding region is 500. The amino acid composition of pre DPP II was as follows: Asp_{30}, Asn_{21}, Thr_{24},

TABLE II. **Substrate specificity of dipeptidyl peptidase II from rat kidney.**

Substrate	Activity (%)	Specific activity (units/mg)
Lys-Ala-MCA	100	1.05
Gly-Pro-MCA	200	2.11
Gly-Gly-MCA	0	—
Gly-Arg-MCA	0	—
Ala-Arg-MCA	0	—
Arg-Arg-MCA	0	—
Ala-Pro-AFC	—	2.85
Ala-Ala-βNA	—	0.30

Each assay was carried out at 37°C in 0.5 M phosphate buffer, pH 6.0, in the presence of 0.5 μg of the enzyme and 100 μM substrate. Data are mean values from triplicate experiments.

TABLE III. **Effects of various protease inhibitors on rat kidney DPP II activity.**

Inhibitors	Concentration (μM)	Residual activity (%)
Control	None	100
Benzamidine	1,000	90
EDTA	2,000	100
DFP	1,000	0
	500	3
	100	25
PMSF	1,000	55
AEBSF	1,000	40
PCMBS	1,000	23
NEM	1,000	97
E-64	100	94
Diprotin A	100	93
Actinonin	100	100
Hg^{2+}	1,000	0
Cu^{2+}	1,000	60

0.5 μg of the enzyme was pre-incubated with each inhibitor for 15 min at room temperature in 50 mM sodium phosphate buffer (pH 6.0), and assays were started by adding 100 μM Lys-Ala-MCA. The reaction mixtures were incubated for an additional 30 min at 37°C. Data are mean values from triplicate experiments.

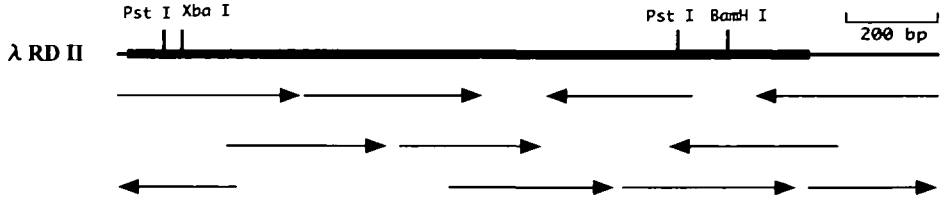

Fig. 3. **Restriction map and sequencing strategy for cDNA insert in λRDII.** Sequences were determined using an original primer designed from the previous sequence. The extent of sequencing is shown by the length of each arrow.

Ser_{34}, Glu_{20}, Gln_{31}, Pro_{29}, Gly_{43}, Ala_{48}, Val_{28}, Met_{12}, Ile_{18}, Leu_{51}, Tyr_{18}, Phe_{31}, Lys_{13}, His_{8}, Arg_{20}, Trp_{11}, and $1/2\text{-}Cys_{10}$. This corresponds to a molecular weight of 55,113.49. Consensus sequences for the active-site serine residue of serine-type proteases and the same leucine zipper motif as observed in human QPP were also found (Fig. 4).

Furthermore, we compared the nucleotide and amino acid sequences of rat DPP II with those of mouse and human QPPs derived from the Blast and FASTA-PIR sequence databases. As shown in Fig. 5, the primary structure of rat DPP II showed significant similarity to those of mouse and human QPPs (Fig. 5). The levels of amino acid homology were 92.8% (rat DPP II *vs.* mouse QPP), 78.9% (rat DPP II *vs.* human QPP), and 80.8% (mouse *vs.* human QPPs), while those of nucleotide homology were 93.5% (rat

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                                        CATGACTGCCAGCGGCCCGCTCAGAATCAGGC   -32
ATGGGTCTCCATCCTTGTTCTCCTGTGGACCATGGGGTCCCCTCCTGGGTCCTGGTCTTG       60
 M  G  L  H  P  C  S  P  V  D  H  G  V  P  S  W  V  L  V  L        20

TTGCTGACACTGGGTCTGTGCAGCCTGCAGGCTACAGCCGACAGCGTTCTAGACCCTGAC     120
 L  L  T  L  G  L  C  S  L  Q  A  T  A  D  S  V  L  D  P  D        40

TTTCGTGAGAATTACTTTGAGCAGTACATGGACCATTTCAACTTTGAGAGTTTCAGCAAC     180
 F  R  E  N  Y  F  E  Q  Y  M  D  H  F  N  F  E  S  F  S  N        60

AAGACCTTTGGCCAGCGGTTCCTGGTGTCAGATAAGTTCTGGAAGATGGGCGAGGGGCCC     240
 K  T  F  G  Q  R  F  L  V  S  D  K  F  W  K  M  G  E  G  P        80

ATTTTCTTCTACACAGGGAATGAGGGGGATATCTGGTCCCTCGCTAACAACTCTGGCTTC     300
 I  F  F  Y  T  G  N  E  G  D  I  W  S  L  A  N  N  S  G  F       100

ATAGTGGAACTGGCAGCCCAGCAGGAGGCCCTGCTGGTCTTTGCTGAGCATCGGTATTAT     360
 I  V  E  L  A  A  Q  Q  E  A  L  L  V  F  A  E  H  R  Y  Y       120

GGGAAATCGCTTCCATTCGGTGTGCAGTCCACACAGCGGGGATACACACAGCTGCTGACT     420
 G  K  S  L  P  F  G  V  Q  S  T  Q  R  G  Y  T  Q  L  L  T       140

GTGGAGCAGGCGCTGGCTGACTTCGCCGTGCTGCTCCAGGCCCTGCGGCACAATCTTGGG     480
 V  E  Q  A  L  A  D  F  A  V  L  L  Q  A  L  R  H  N  L  G       160

GTCCAGGATGCCCCCACCATAGCCTTTGGAGGGAGTTATGGGGGAATGCTGAGCGCCTAC     540
 V  Q  D  A  P  T  I  A  F  G  G  S  Y  G  G  M  L  S  A  Y       180

ATGAGGATGAAGTACCCCCACCTGGTGGCTGGGGCACTGGCGGCCAGCGCTCCTGTTATA     600
 M  R  M  K  Y  P  H  L  V  A  G  A  L  A  A  S  A  P  V  I       200

GCTGTCGCAGGCCTTGGCAACCCCGACCAATTCTTCCGAGATGTCACAGCGGACTTCTAT     660
 A  V  A  G  L  G  N  P  D  Q  F  F  R  D  V  T  A  D  F  Y       220

GGCCAGAGTCCCAAGTGTGCCCAGGCTGTGCGGGATGCTTTCCAGCAAATCAAAGACTTG     720
 G  Q  S  P  K  C  A  Q  A  V  R  D  A  F  Q  Q  I  K  D  L       240

TTCCTCCAGGGAGCCTATGACACCATCAGCCAGAATTTTGGGACCTGCCAATCACTTTCC     780
 F  L  Q  G  A  Y  D  T  I  S  Q  N  F  G  T  C  Q  S  L  S       260

AGCCCAAAGGACCTGACTCAGCTCTTTGGGTTTGCCCGGAATGCATTTACTGTTCTCGCC     840
 S  P  K  D  L  T  Q  L  F  G  F  A  R  N  A  F  T  V  L  A       280

ATGATGGATTACCCGTACCCTACTAACTTTCTAGGGCCCCTTCCTGCCAACCCTGTCAAG     900
 M  M  D  Y  P  Y  P  T  N  F  L  G  P  L  P  A  N  P  V  K       300

GTGGGCTGTGAACGGTTGTTGAGTGAGGGCCAGAGGATCATGGGTCTACGGGCACTGGCA     960
 V  G  C  E  R  L  L  S  E  G  Q  R  I  M  G  L  R  A  L  A       320

GGGCTGGTCTACAACTCCTCTGGCATGGAACCCTGTTTTGACATCTACCAGATGTACCAA    1020
 G  L  V  Y  N  S  S  G  M  E  P  C  F  D  I  Y  Q  M  Y  Q       340

AGCTGTGCTGACCCCACTGGCTGTGGCACTGGCTCCAATGCCAGGGCCTGGGACTACCAG    1080
 S  C  A  D  P  T  G  C  G  T  G  S  N  A  R  A  W  D  Y  Q       360

GCCTGTACGGAGATCAATCTGACCTTTGACAGCAACAACGTGACAGACATGTTCCCTGAA    1140
 A  C  T  E  I  N  L  T  F  D  S  N  N  V  T  D  M  F  P  E       380

ATCCCTTTCTCTGATGAACTCCGCCAGCAATACTGCCTGGATACATGGGGCGTGTGGCCT    1200
 I  P  F  S  D  E  L  R  Q  Q  Y  C  L  D  T  W  G  V  W  P       400

CGGCCAGACTGGCTGCAGACCAGCTTCTGGGGTGGCGACCTTAAAGCAGCCAGCAACATC    1260
 R  P  D  W  L  Q  T  S  F  W  G  G  D  L  K  A  A  S  N  I       420

ATTTTCTCCAACGGTGACCTAGACCCCTGGGCAGGGGGTGGGATCCAGCGCAACCTGAGT    1320
 I  F  S  N  G  D  L  D  P  W  A  G  G  G  I  Q  R  N  L  S       440

ACATCAATCATTGCTGTCACCATCCAAGGGGGAGCACATCACCTAGACCTCAGAGCATCT    1380
 T  S  I  I  A  V  T  I  Q  G  G  A  H  H  L  D  L  R  A  S       460

AACTCAGAAGACCCCCCGTCTGTCGTGGAGGTGCGTAAGCTGGAAGCTACCCTCATTCGC    1440
 N  S  E  D  P  P  S  V  V  E  V  R  K  L  E  A  T  L  I  R       480

GAGTGGGTAGCAGCAGCCAGACTAAAGCAACCAGCAGAGGCACAGTGGCCTGGGCCTAAG    1500
 E  W  V  A  A  A  R  L  K  Q  P  A  E  A  Q  W  P  G  P  K       500

TAGCAGCCTTCCAGCCGAGATGCAGCAAACCCAGAGACCCTGTTCTATGTGAGCCGCTGT    1560
GTGACAGCTCGCCTTACGTCTGATACTATCCAGACTCATGGTGCTTTGTCTGAGCTGGAC    1620
CTCAGCAAGGAGCTGGTGGGAGATGGAGAGACAGAATAAACACGTGCTATGTCAAAAAAA    1680
AAAAAAAA
```

Fig. 4. **Nucleotide sequence and deduced amino acid sequence of the DPP II cDNA.** The single-letter code is used for amino acids. The sequences of derived peptides by BrCN digestion are underlined with dotted lines. The consensus sequence for the active-site serine residue and associated two amino acids (aspartic acid and histidine) of serine-type pepetidase are enclosed in boxes. The presumed leucine zipper motif is underlined with a solid single line. Presumed *N*-glycosilation sites are underlined with wavy lines. Polyadenylation or processing sequence of AATAAA is shown by a heavy bar.

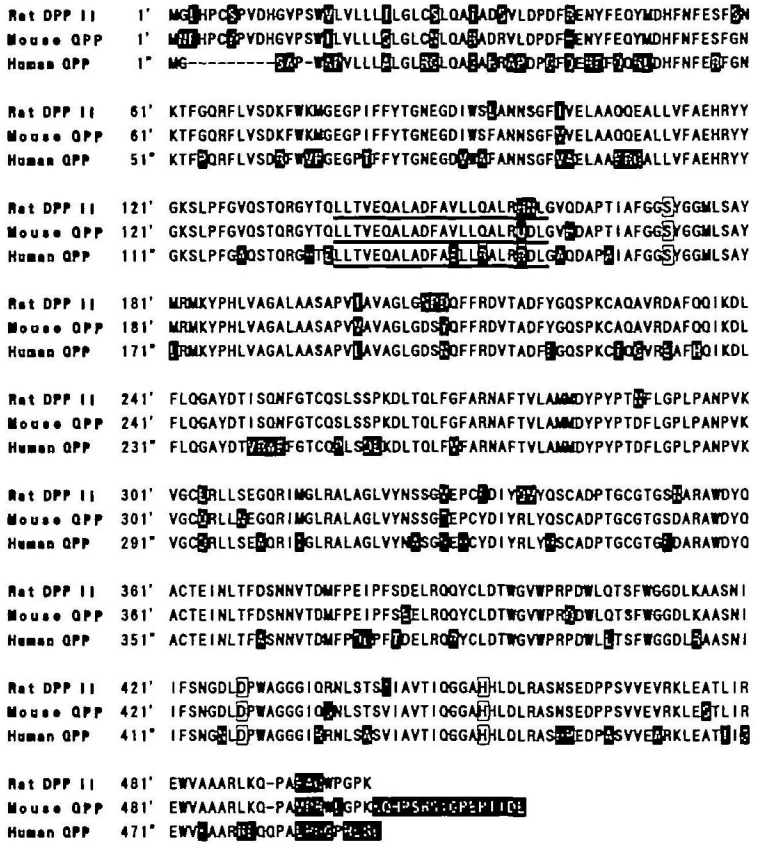

Fig. 5. **Amino acid sequences of rat DPP II, mouse and human QPP.** *Dashes indicate gaps introduced into* the sequences. Uncommon residues among DPP II, mouse QPP and human QPP are shown in filled boxes. The consensus sequence for the active-site serine residue and associated two amino acids (aspartic acid and histidine) of serine-type pepetidase are enclosed in boxes. Presumed leucine zipper motifs are shown by solid bars.

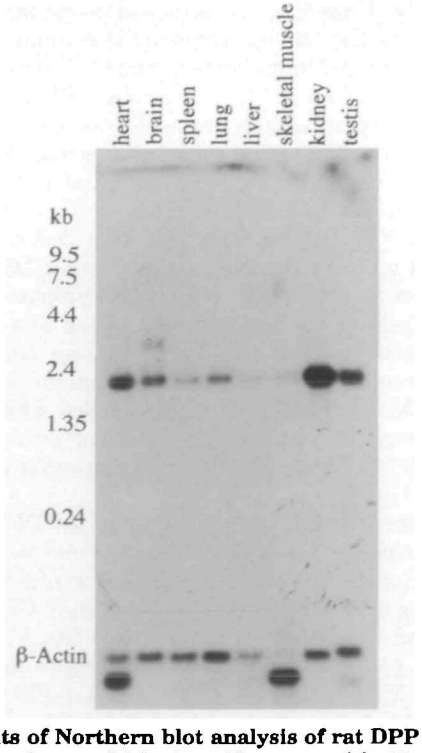

Fig. 6. **Results of Northern blot analysis of rat DPP II mRNA in rat organs.** A rat multiple tissue blot was used for Northern blot analysis. Each lane contains approximately 2 μg of purified poly A^+RNA. Tissue-specific β-actins as controls are shown at the bottom.

DPP II *vs.* mouse QPP), 79.4% (rat DPP II *vs.* human QPP), and 79.7% (mouse *vs.* human QPPs). In these amino acid sequences, the positions of eight cysteine residues coincide exactly (Fig. 5).

Northern Blot Analysis—Northern blot analysis with rat DPP II cDNA also revealed a widespred distribution of DPP II expression in rat organs. Among the various organs, DPP II was prominently expressed in the kidney. DPP II expression was relatively strong in the testis and heart, moderate in the brain and lung, and weak in the liver, skeletal muscle and spleen.

Furthermore, another band of approximately 3,000 base pairs appeared in the brain, and the density of this band was almost equivalent to that of DPP II mRNA in the brain (Fig. 6).

Immunohistochemistry—Anti-porcine DPP II rabbit IgG reacted to rat DPP II by Western blotting (data not shown). Immunohistochemical staining with this antibody revealed a widespread distribution of DPP II in rat organs. DPP II was intensely stained in epithelial cells of distal and collecting tubules in the kidneys (Fig. 7), in Leydig cells in the testis, in columnar epithelium, lamina propria, and glandular epithelium in the uterus and chorioid epithelium of the cerebrum. The tissue distribution of rat DPP II is summarized in Table IV.

DISCUSSION

DPP II is thought to play an important role in the degradation of some oligopeptides or their fragments such as substance P, casomorphin, and thyroliberin fragment (*4*), and

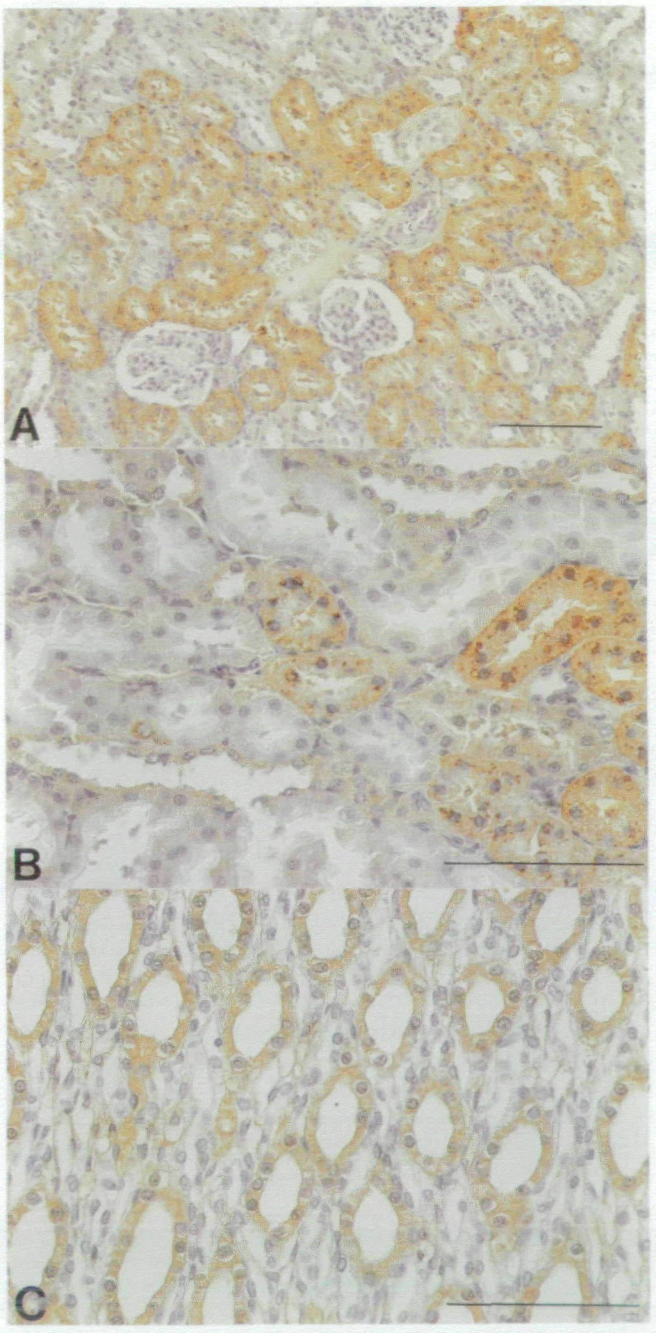

Fig. 7. **Immunohistochemical localization of DPP II in the rat kidney.** (A) cortex, (B) outer stripe, and (C) inner medulla. Epithelial cells of the distal and collecting tubules in the kidney were intensively stained, but the glomerulus and Bowman's capsule were not stained. Original magnifications: A × 200, B and C × 400. Bars = 100 μm.

it is also known to exist in a number of mammalian tissues, cells and body fluids (*3–17*, *22*). However, little is known about the structural features and expression of DPP II.

In the present study, we purified DPP II to homogeneity from the rat kidney using several column chromatographies. The molecular weight of the purified enzyme was calculated to be 105,000 on non-denatured PAGE and 54,000 on SDS-PAGE in the presence of β-ME, and 54,053.8 in TOF-MS. The activity of DPP II toward Lys-Ala-4-methoxy-2-naphthylamide was coincident with the band of the protein on non-denatured PAGE (Fig. 2A). These findings suggest that native DPP II is composed of two identical subunits (*4*, *8*). The isoelectric point (pI) of the enzyme was 4.55 according to chromatofocusing using the Phast System. This pI value was significantly lower than those (5.3–6.0) from human kidney (*7*), rat brain (*4*), and porcine seminal plasma (*22*).

As shown in Table II, the purified DPP II preferentially hydrolyzed Lys-Ala-, Gly-Pro-MCAs, and Ala-Pro-AFC. It did not hydrolyze substrates of other dipeptidyl peptidases, substrates with an N-blocked Gly-Pro sequence, substrates of aminopeptidases or substrates of endopeptidases (Table II). Thus, DPP II preferentially released Xaa-Ala- or Xaa-Pro- from the N-termini of peptides as previously reported (*2*, *3*). In particular, it strongly cleaved Ala-Pro-AFC as a substrate of QPP (*20*). This also suggests that DPP II cleaves the Xaa-Pro motif to a greater degree than it does the Xaa-Ala motif.

Rat kidney DPP II was strongly inhibited by DFP and moderately inhibited by AEBSF and PMSF. These findings strongly indicate that DPP II is a serine-type exopeptidase. In fact, the deduced amino acid sequence of its cDNA contained a typical active site (-Gly^{170}-Gly-Ser^{172}-Tyr-Gly^{174}-) of a serine protease (Fig. 4). The enzyme activity was also affected by PCMBS and Hg^{2+} (Table III). This finding also indicates that an SH residue is involved in the expression or regulation of the activity of DPP II. We determined other physicochemical properties, including optimal pH, pH stability and heat stability. These properties were similar to those previously reported (*4*, *7*, *8*, *22*).

As mentioned in the introduction, we presumed that both DPP II and QPP are closely related members of a superfamily. Therefore, we screened a rat kidney cDNA library using the 360-bp fragment of human QPP cDNA obtained by nested amplification, and we isolated one clone, λRD II, coding for DPP II. The cDNA (λRD-II) is 1,720-bp long with an open reading frame of 1,500 base pairs encoding a protein of 500 amino acid residues with M_r 55,113.49 (Fig. 4). As shown in Fig. 4, a potential initiation codon (ATG) began at nucleotide number 1. The nucleotide sequence (GGCATGG) surrounding this codon is in good agreement with the consensus sequence (A/GXXATGG) for the start of translation in eukaryotes proposed by Kozak (*29*). It is reasonable to consider that the first amino acid residue (Met) starts at the first initiation codon. Furthermore, we confirmed that the deduced amino acid sequence of this cDNA coding for DPP II coincided with the amino acid sequences of N-terminal and bromocyan-digested fragments (Fig. 4). There are six *N*-glycosylation sites on the molecule (Fig. 4).

The deduced amino acid sequence of rat DPP II showed clear homology to those of human and mouse QPPs (78.9 and 92.8%, respectively). These three enzymes had the consensus sequence for the active site (-Gly^{170}-Gly-Ser^{172}-Tyr-Gly^{174}-) and its associated amino acids (Asp^{428} and His^{452}) for serine proteases and the leucine zipper motif (Leu^{138}-Leu-Tyr^{140}-Val-Glu-Gln-Ala-Leu^{145}-Ala-Asp-Phe-Ala-Val^{150}-Leu-Leu-Gln-Ala-Leu^{156}-Arg-His-Asn-Leu^{159}) (Fig. 5). The eight cysteine residues on the rat DPP II molecule that are important for tertiary structure formation also occupied identical positions to those of human and mouse QPPs (Fig.

TABLE IV. **Distribution of dipeptidyl peptidase II (DPP II) in rat tissues.**

Tissue	Immunostaining for DPP II	Tissue	Immunostaining for DPP II	Tissue	Immunostaining for DPP II
Esophgus		bronchial epithelium	+	tall collumnar cells	+ or +/−
stratified squamous epithelium	+	cilia cells	+	connective tissue	−
lamina muscularis mucosa	+	Kidney		Testis	
stiated muscle	+	glomerulus	+/−	basement cells	− or +−
Stomach		Bowmanns capsule	+/−	spermatogonia	− or +/−
surface epithelial cells	+	Proximal tubules	+	spematozoa	+ or +
parietal cells	+	distal tubules	++	Sertoli cells	− or +
mucous neck cells	+/−	collecting tubules	++	Leydig cells	++
chief cells	+/−	Urinary bladder		Epididymis	
cornification	+	transitional cells	+	efferent ductules	+
lamina muscularis mucosa	+	Heart		ductus epididymis	+
Small intestine		myocytes	+	stereocilia	+
columnar epithelium	+	coronary artery	+−	Uterus	
goblet cells	+/−	Aorta	+/−	cervical epithelium	+
intestinal glands	+/−	Striated muscle (diaphragm)		columnar epithelium	++
lamina muscularis mucosa	+/−	muscle fiber	+/−	lamina propria	++
Large intestine		Smooth muscle		glandular epithelium	++
epithelium	+	Spleen		circlar layer	+ or +/−
goblet cells	+/−	red pulp	+	logitudinal layer	+ or +/−
lamina muscularis mucosa	+/−	white pulp	+/−	Ovary	
Liver		Thymus		follicles	NE
hepatocytes	+ or ++	epithelioid cells	+/−	corpus luteum	NE
Kupffer cells	+/−	Hasalls bodies	+/−	corpus albicus	NE
sinusoidal cells	+	septa	+/−	Cerebrum	
bile duct	+	Adrenal gland		pyramidal cells	+/−
Pancreas		cortex	+	neuroglia cells	+/−
acinar cells	+/−	zona glomerulosa	+	axon	+−
ducts	+−	zona fasciculata	+	remified dendrites	+/−
islet of Langerhans	+	zona reticularis	+	chorioid epithelium	++
Trachea		medulla	+	Cerebellum	
epithelium	+	medullary cells	+	neuroglia cells	+
lamina propria	+	interstitial connective tissue		small granule cells	+−
muscle layer	+/−	Thyroid gland		urkinje cells	− or +/−
cartilaginous cells	+/−	Prostate		remified dendrites	+−
Lung and Bronchus		epithelium	+ or +/−	myelinated fibers	+−
alveolus	+/−	connective tissue	−		
pleura	+−	Seminal vesicle			

++: strong reactivity in all cells, +: positive staining in most cells, ++/− or +/−: positive staining in some cells, −+: weak staining in some cells, −: no staining in any cells, NE: not examined.

5). In terms of substrate specificity and optimal pH, rat DPP II also resembles porcine DPP II (*22*) and QPP. The N-terminal amino acid sequence (the first 41 amino acid residues) of porcine DPP II showed significant homology to that of rat DPP II (75.7%) and human QPP (73.2%) (figure not shown). On the basis of these structural features and biochemical properties, we confirmed that DPP II is identical to QPP.

Underwood *et al.* (*20*) reported that the arrangement of catalytic amino acid residues (serine, aspartic acid, and histidine) and the homology (42% amino acid, identity over their entire open reading frame) of both QPP and PCP suggest that these two enzymes can be categorized into the serine-type peptidases clan SC, family S28. Accordingly, rat DPP II is also categorized into the same clan and family.

Although DPP II has been purified from many tissues (*3–17*, *22*), little is known about its immunohistochemical localization. As shown in Fig. 7, the enzyme was strongly stained in lysosomes in many tissues, including the kidney, brain and uterus. Its tissue localization is summarized in Table IV. The data suggest that DPP II is expressed in many tissues and is one of the house-keeping proteins. This speculation is supported by the fact that DPP II has been purified from many tissues (see Introduction). Accordingly, it may play important roles in peptide degradation, protein catabolism, or protein processing in many tissues. Furthermore, the level of mRNA expression of DPP II was highest in the kidney (Fig. 6). The data suggest the possibility that DPP II is related to degradation of peptides affecting functions of the distal tuble and collecting duct, including electrolyte channels regulated by aldosterone and arginine vasopressin, prostaglandin E2 produced in the medulla and so on. It is still not clear whether DPP II degrades intracellular products or exotic peptides taken into the epithelial cells of distal tuble and collecting duct by endocytosis. Recently, Chiravuri *et al.* (*19*) reported that QPP is expressed in resting and activated peripheral blood mononuclear cells (PBMCs) and Jurkat cells and that blocking of QPP by highly specific post-proline aminopeptidase inhibitors L-valinyl-L-boroproline (VbP) leads to cell death (apoptosis) only in resting PBMCs. They speculated that QPP had many intracellular substrates for survival of resting PBMCs. Thus, DPP II may not be involved in degradation of exogenous regulatory peptides but, rather, may play important roles in peptide degradation and some protein catabolisms in resting cells.

Further studies, including studies using knockout mice, are needed to determine the real function of DPP II in cells and its physico-pathological significance.

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REFERENCES

1. McDonald, J.K. and Barrett, A.J. (1986) *Mammalian Proteases: A Glossary and Bibliography,* Vol. 2, pp. 111–144, Academic, London
2. McDonald, J.K., Leibach, F.H., Grindeland R.E., and Ellis, S. (1968) Purification of dipeptidyl peptidase II (dipeptidyl arylamidase II) of the anterior pituitary glands. *J. Biol. Chem.* **243**, 4143–4150
3. Eisenhauer, D.A. and McDonald, J.K. (1986) A novel dipeptidyl peptidase II from the porcine ovary. Purification and characterization of a lysosomal serine protease showing enhanced specificity for prolyl bonds. *J. Biol. Chem.* **261**, 8859–8865
4. Mentlein, R. and Struckhoff. (1989) Purification of two dipeptidyl aminopeptidases II from rat brain and their action on proline-containing neuropeptides. *J. Neurochem.* **52**, 1284–1293
5. Stauber, W.T. and Ong, S-H. (1981) Fluorescence determination of dipeptidyl peptidase II in skeletal, cardiac, and vascular smooth muscles. *J. Histochem. Cytochem.* **29**, 672–677
6. Kugler, P. (1982) Quantitative histochemistry of the lysosomal dipeptidyl arylamidase II in the proximal tubule of the rat kidney. *Histochemistry* **76**, 557–566
7. Mantle, D. (1991) Characterization of dipeptidyl and tripeptidyl aminopeidases in human kidney soluble fraction. *Clin. Chem. Acta* **196**, 135–142
8. Fukasawa, K., Fukasawa, M.K., Hiraoka, B.Y., and Harada, M. (1983)Purification of dipeptidyl peptidase II from rat kidney. *Biochim. Biophys. Acta* **745**, 6–11
9. Randell, S.H. and Sannes, P.L. (1985) Cytochemical localization and biochemical evaluation of a lysosomal serine protease in lung: Dipeptidyl peptidase II in the normal rat. *J. Histochem. Cytochem.* **33**, 677–686
10. Sannes, P.L. (1983) Subcellular localization of dipeptidyl peptidases II and IV in rat alveolar macrophages. *J. Histochem. Cytochem.* **31**, 684–690
11. Khalaf, M.R., Bevan, P.C., and Hayhoe, F.G.J. (1986) Comparative cytochemical study of dipeptidyl aminopeptidase DAP II and IV in normal and malignant hemic cells. *J. Clin. Pathol.* **39**, 891–896
12. Lojda, Z. (1981) Proteinases in pathology: Usefulness of histochemical methods. *J. Histochem. Cytochem.* **29**, 481–493
13. Struckhoff, G. and Heymann, E. (1986) Rat peritoneal mast cells release dipeptidyl peptidase II. *Biochem. J.* **236**, 215–219
14. Kojima, K., Mihama, R., Saki, T., Togari, A., Matsui, T., Shinpo, K., Fujita, K., Fukasawa, K., Harada, M., and Nagatsu, Y. (1987) Serum activities of dipeptidyl- aminopeptidase II and dipeptidyl-aminopeptidase IV in tumor-bearing animals and in cancer patients. *Biochem. Med. Metab. Biol.* **37**, 35–41
15. Gossrau, R. and Lojda, Z. (1980) Study on dipeptidylpeptidase II (DPP II). *Histochemistry* **70**, 53–76
16. Agrawal, Y. and Vanha-Perttula, T. (1986) Dipeptidyl peptidases in bovine reproductive organs and secretions. *Int. J. Androl.* **9**, 435–452
17. Ohkubo, I., Huang, K., Ochiai, Y., Takagaki, M., and Kani, K. (1994) Dipeptidyl peptidase IV from porcine seminal plasma: Purification, characterization, and N-terminal amino acid sequence. *J. Biochem.* **116**, 1182–1186
18. Andersen, K.J. and McDonald, K.J. (1989) Lysosomal heterogeneity of dipeptidyl peptidase II active on collagen-related peptides. *Renal Physiol. Biochem.* **12**, 32–40
19. Chiravuri, M., Schmitz, T., Yardley, K., Underwood, R., Dayal, Y., and Huber, B.T. (1999) A novel apoptotic pathway in quiscent lymphocytes identified by inhibition of a post-proline cleaving aminodipeptidase: a candidate target protease, quiescent cell proline dipeptidase. *J. Immunol.* **163**, 3092–3099
20. Underwood, R., Chiravuri, M., Lee, H., Schmitz, T., Kabcenell, A.K., Yardley, K., and Huber, B.T. (1999) Sequence, purification, and cloning of an intracellular serine protease, quiescent cell proline dipeptidase. *J. Biol.Chem.* **274**, 34053–34058
21. Chiravuri, M., Lee, H., Mathieu, S.L., and Huber, B.T. (2000) Homodimerization via a leucine zipper motif is required for enzymatic activity of quiescent cell proline dipeptidase. *J. Biol. Chem.* **275**, 26994–26999
22. Huang, K., Takagaki, M., Kani, K., and Ohkubo, I. (1996) Dipeptidyl peptidase II from porcine seminal plasma: purification, characterization and its homology to granzymes, cytotoxic cell proteinases (CCP1-4) *Biochim. Biophys. Acta* **1290**, 149–156
23. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685
24. Ogata, S., Misumi, Y., and Ikehara, Y. (1989) Primary structure of rat liverdipeptidyl peptidase IV deduced from its cDNA and identification of the NH_2-terminal signal sequence as the membrane-anchoring domain. *J. Biol. Chem.* **264**, 3596–3601
25. Ohkubo, I., Niwa, M., and Sasaki, M. (1988) Purification and characterization of human plasma Zn-α2-glycoprotein. *Prep. Biochem.* **18**, 413–430
26. Nishi, K., Fukunaga, T., Yamamoto, Y., Kane, M., Tanegashima, A., Rand, S., and Brinkmann, B. (1992) ABH-related antigens in the human male genital tract, a histochemical examination. *Int. J. Leg. Med.* **105**, 75–80
27. Huang, K., Takahara, S., Kinouchi, T., Tateyama, M., Ishida, T., Ueyama, H., Nishi, K., and Ohkubo, I. (1997) Alanyl aminopeptidase from human seminal plasma: purification, characterization, and immunohistochemical localization in the male genital tract. *J. Biochem.* **122**, 779–787
28. Yamamoto, Y., Li, Y-H., Huang, K., Ohkubo, I. and Nishi, K. (1998) Isolation and characterization of an alanyl aminopeptidase from rat liver cytosol as a puromycin-sensitive enkephalin-degrading aminopeptidase. *Biol. Chem.* **379**, 711–719
29. Kozak, M. (1987) An analysis of 5′-noncoding sequences from 699 vertebrate messenger RNAs. *Nucleic Acids Res.* **15**, 8125–8148