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Short sequence-paper

Molecular cloning and tissue distribution of rat peptide transporter PEPT2 [1]

Hideyuki Saito [a], Tomohiro Terada [a], Masahiro Okuda [a], Sei Sasaki [b], Ken-ichi Inui [a,*]

[a] *Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan*
[b] *Second Department of Internal Medicine, School of Medicine, Tokyo Medical and Dental University, Tokyo 113, Japan*

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Abstract

A cDNA encoding rat H^+-coupled peptide transporter PEPT2 was isolated. The cDNA encoded a protein of 729 amino acids with 48% amino acid identity to the rat PEPT1. The mRNA expression of rat PEPT2 was predominant in the kidney. When expressed in *Xenopus* oocytes, rat PEPT2 stimulated the uptake of bestatin, a dipeptide-like drug.

Keywords: Peptide transport; Proton-coupled transporter; cDNA sequence; Bestatin; Renal tubular reabsorption

Peptide transporter functionally characterized in the small intestine and the renal proximal tubule has been considered to play a key role in the maintenance of protein nutrition. In addition to efficient absorption of small peptides in these tissues, the peptide transporter has been demonstrated to mediate both the intestinal absorption [1–5] and the renal tubular reabsorption [6,7] of oral active β-lactam antibiotics [1–6] and bestatin [7], a dipeptide-like anticancer drug, implying that this transporter plays a pharmacologically important role in chemotherapy. Studies using brush-border membrane vesicles isolated from these tissues revealed that the transporter is an electrogenic H^+-coupled transport system specific for di- and tripeptides [8–10]. However, unlike the peptide transport in the intestine, it has been reported that there are at least two distinct peptide transporters in the brush-border membranes of the renal proximal tubules [11–13]. Recently, cDNA encoding an oligopeptide transporter (PEPT1) expressed in the rabbit small intestine was isolated by *Xenopus* oocyte expression system [14]. Using PCR technique based on the amino acid sequence of rabbit PEPT1, we also isolated cDNA encoding a rat H^+/peptide cotransporter (PEPT1) mediating absorption of β-lactam antibiotics in the small intestine and the kidney proximal tubule [15]. We report here the isolation of a full cDNA of the rat H^+-coupled peptide transporter PEPT2, its functional expression and tissue distribution.

Total RNA was extracted from several tissues of adult male rats using the guanidine/isothiocyanate method [16]. Poly(A)$^+$RNA was purified by oligo(dT)-cellulose (Collaborative Research, Bedford, MA) affinity column chromatography [17]. Degenerate PCR primers were designed and synthesized chemically based on the amino acid sequence of rabbit PEPT1: sense strand with *Sal*I site, 5′-CCGTCGACTT(T/C)TT(T/C)AT(T/C)GT-NGTNAA-3′ (corresponding to amino acid sequence 17–22); antisense strand with *Not*I site, 5′-CCGCGGCCG-CAC(A/G)CA(A/G/T)GG(T/C)TT(A/G)ATNCC-3′ (corresponding to amino acid sequence 138–143). Reverse transcription (RT)-coupled PCR was performed as described previously [15]. The purified PCR product was cut with *Sal*I and *Not*I on both ends, ligated into *Sal*I- and *Not*I-cut pSPORT1 (GIBCO BRL, Life Technologies, Gaithersburg, MD), and transformed into *Escherichia coli* DH5α competent cells (GIBCO). Both strands of the subcloned cDNA inserts were sequenced by the chain-termination method with a Sequenase version 2.0 DNA sequencing kit (United States Biochemical, Cleveland, OH). An oligo(dT)-primed directional rat kidney cDNA library was constructed in the *Sal*I-*Not*I site of λgt22A (Superscript cDNA synthesis kit, GIBCO) and screened

Abbreviations: RT, reverse transcription; PCR, polymerase chain reaction.

* Corresponding author. Fax: +81 75 7514207.

[1] The nucleotide sequence data of rat PEPT2 cDNA have been submitted to the DDBJ, EMBL and GenBank nucleotide sequence databases with the following accession number D63149.

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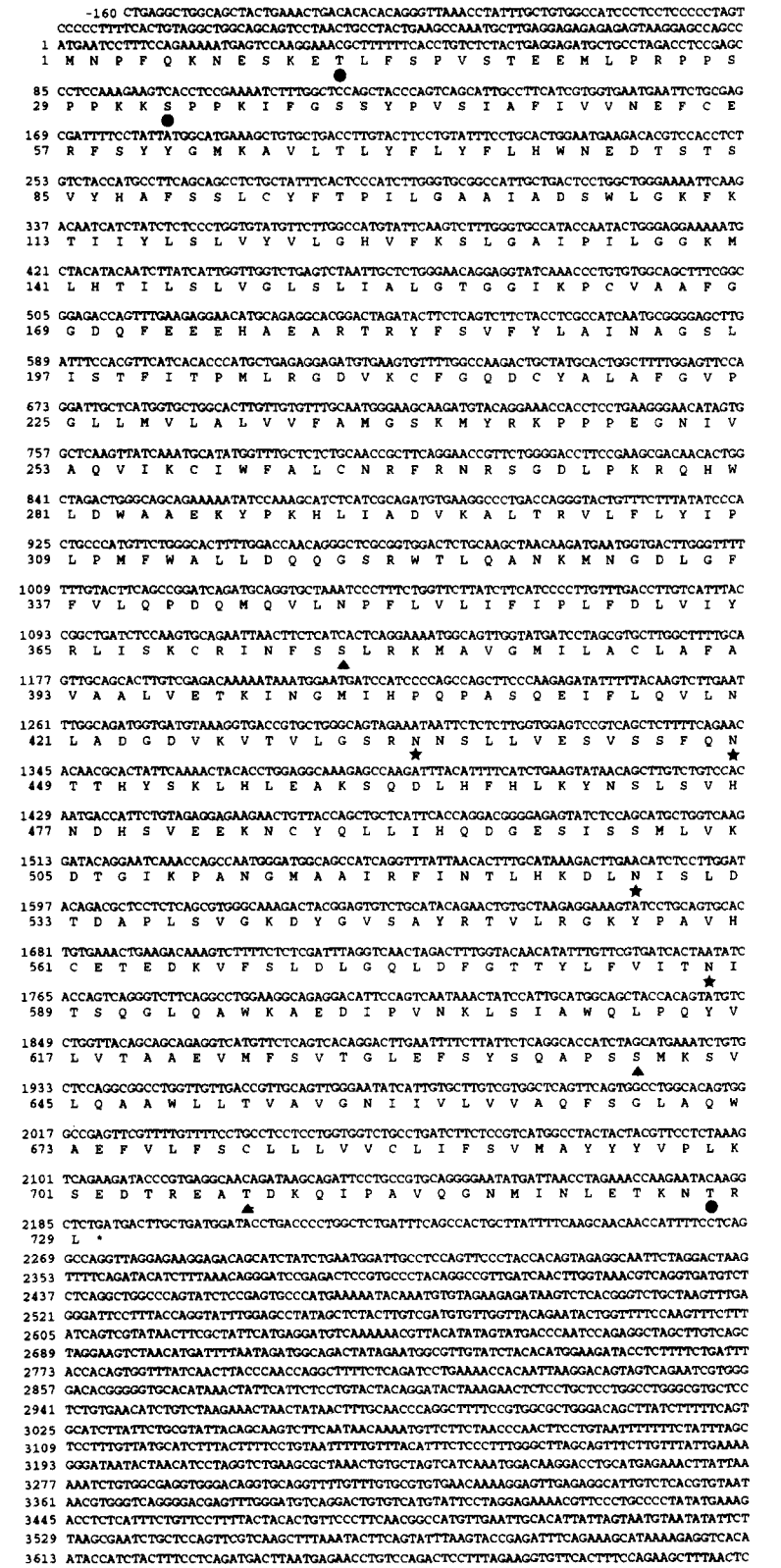

Fig. 1. Nucleotide and deduced amino acid sequence of rat PEPT2 cDNA. Potential N-linked glycosylation sites are indicated by asterisks. Potential protein kinase C phosphorylation sites (▲) and protein kinase A phosphorylation sites (●) are indicated. The polyadenylation signal is boxed.

[15,18]. Positive clones were subcloned into *Sal*I- and *Not*I-cut pSPORT1. The complete sequence was determined on both strands using synthetic oligonucleotide primers. 5 μg of poly(A)$^+$RNA from tissues were resolved by electrophoresis in 1% agarose gels containing formaldehyde and transferred on nylon membranes. Quality of RNA was assessed by ethidium bromide staining. After transfer, blots were hybridized (50% formamide, 5 × SSPE, 5 × Denhardt's solution, 0.2% SDS, 10 μg/ml salmon sperm DNA at 42°C) with a whole rat PEPT2 cDNA labeled with [α-^{32}P]dCTP. 10 ng of capped complementary RNA (cRNA) transcribed in vitro from *Not*I-linearized rat PEPT1 or PEPT2 cDNA by use of T7 RNA polymerase were injected into *Xenopus* oocytes. Injected oocytes were maintained in modified Barth's medium at 18°C for 2 days. Functional expression of rat PEPT1 and PEPT2 was evaluated by measuring uptake of [^{3}H]bestatin (12.7 GBq/mmol) (Nippon Kayaku, Tokyo, Japan) in groups of oocytes injected with 50 nl of water or cRNA as described previously [15].

Two distinct PCR products were isolated by RT-PCR of the rat kidney cortex mRNA using degenerate primers designed on the amino acid sequence of rabbit PEPT1. One product showed ≈ 80% nucleotide identity and another showed ≈ 60% nucleotide identity to the rabbit PEPT1. Using these PCR products as a probe, a rat kidney λgt22A cDNA library was screened, and 3-kb and 4-kb clones, designated rat PEPT1 [15] and rat PEPT2, respectively, were obtained. Sequencing of the insert revealed that the rat PEPT2 cDNA had 3938 bp, which encodes for a 729-amino acid protein of relative molecular mass of 81 kDa with a poly(A)$^+$ tail (Fig. 1). The open reading frame

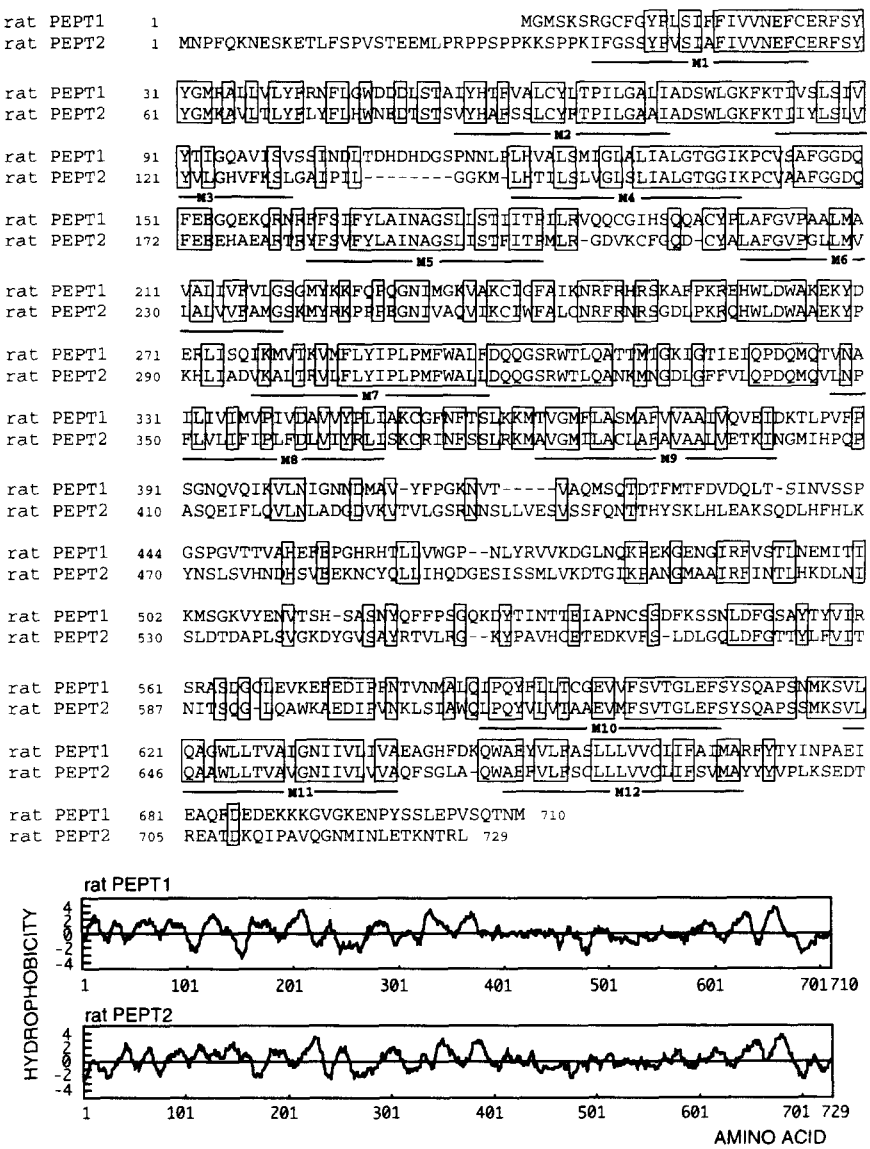

Fig. 2. Comparison of deduced amino acid sequences (upper) and hydrophobicity plots (bottom) of the rat PEPT1 and PEPT2. Amino acid identities in the sequences are boxed. Putative membrane-spanning α-helices are underlined.

was assigned by a stop codon and a Kozak consensus initiation sequence [19]. Overall amino acid identity was 48% with the rat PEPT1 (Fig. 2). The Kyte–Doolittle hydropathy analysis [20] of rat PEPT2 indicated that the transporter has twelve putative membrane-spanning α-helices and four potential N-linked glycosylation sites (N-X-S/T) at a predicted large extracellular loop between α-helices 9 and 10, exhibiting a high degree of topological similarity to the rabbit and rat PEPT1 (Fig. 2). Three potential protein kinase A phosphorylation sites [21] at serine position 33 and threonine positions 12 and 727, and three potential protein kinase C phosphorylation sites [21] at serine positions 376 and 640 and at threonine position 708 were identified in the predicted intracellular domains. The rat PEPT2 showed 83% amino acid identity to the recently-cloned human PEPT2 consisted of 729 amino acids [22]. The protein kinase A phosphorylation sites at threonine positions 12 and 727 were conserved in the sequence of human PEPT2. Among three phosphorylation sites for protein kinase C, both the serine at 376 and 640 were identified in the human PEPT2.

The tissue distribution of rat PEPT2 mRNA transcripts was examined by Northern blot analysis of poly(A)$^+$ RNA extracted from each of several rat tissues, using the full-length rat PEPT2 cDNA as a probe. A $\approx$ 4-kb mRNA corresponding to the full-length rat PEPT2 cDNA was expressed predominantly in the kidney, especially in the medulla (Fig. 3). It has been reported that the human PEPT2 mRNA expression in the kidney was failed to detect by Northern blot analysis, but was specifically detected in the kidney by RT-PCR using specific primers for the human PEPT2 cDNA [22,23]. In rats, a $\approx$ 4-kb band corresponding to the rat PEPT2 mRNA was also detected in the brain, lung and spleen, but was undetectable in the heart, liver and small intestine. We found that rat PEPT1 mRNA is expressed in both the small intestine and kidney [15], indicating a marked difference in tissue distribution between rat PEPT1 and PEPT2.

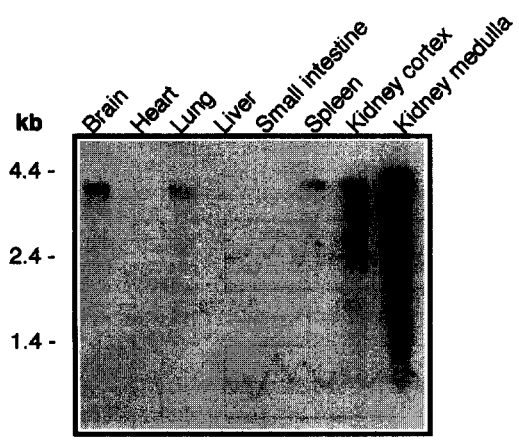

Fig. 3. Northern blot analysis of rat PEPT2 mRNA in rat tissues. 5 μg of poly(A)$^+$ RNA from the indicated tissues was run in lane and hybridized using the full-length rat PEPT2 cDNA under high stringency.

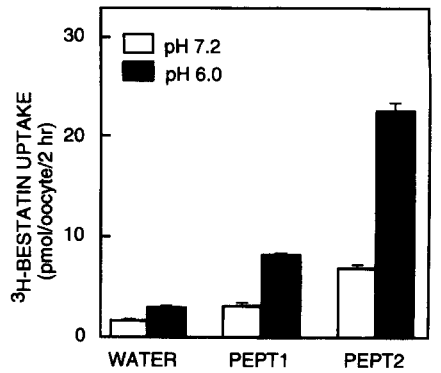

Fig. 4. Uptake of [^{3}H]bestatin by oocytes injected with water or in vitro transcribed rat PEPT1 or PEPT2 cRNA. Uptake by oocytes was assayed for 2 h at 25°C in the presence of 30 μM [^{3}H]bestatin 2 days after injection of 50 nl of water or cRNA (10 ng). Each column represents the mean $\pm$ S.E. of four experiments. Four oocytes were used for each uptake experiment.

When expressed in *Xenopus* oocytes, both rat PEPT1 and PEPT2 stimulated the uptake of [^{3}H]bestatin, a nonmetabolizable dipeptide-like drug, remarkably in the presence of an inward H^+ gradient (Fig. 4). The H^+-gradient dependent uptake of [^{3}H]bestatin into oocytes injected with rat PEPT2 cRNA was much greater than that with rat PEPT1 cRNA, indicating that rat PEPT2 has higher transport activity for bestatin than rat PEPT1. More recently, it was reported that there are differences between the human PEPT1 and PEPT2 in the recognition of β-lactam antibiotics [23], and that the PEPT2 possesses higher affinity than PEPT1 for diverse dipeptides [24].

We have found that the rat PEPT1 is expressed in the brush-border membranes of both the small intestine and kidney cortex by Western blot analysis using specific antibody against C-terminal peptide of rat PEPT1 protein [15]. In the kidney, small peptides generated by brush-border peptidases and filtered through glomerulus are efficiently reabsorbed via the peptide transport systems. Moreover, a variety of oral β-lactam antibiotics and bestatin can also be recognized by the peptide transporter and reabsorbed from the tubular fluid, and thereby the transporter contributing renal handling of these drugs [6,7]. The present finding that the rat PEPT2 is predominantly expressed in the kidney medulla suggests that the transporter as well as the PEPT1 plays a key role in both the maintenance of protein nutrition and therapeutic efficacy of peptide-like drugs. Therefore, further studies on functional characterization and intrarenal localization of the PEPT1 and PEPT2 will enable comparison of their physiological and pharmacological significance in the kidney. Alternatively, in the brain, lung and spleen which appeared to express the PEPT2 mRNA, the transporter may participate in unrecognized physiological function(s) related to active transport of small peptides across plasma membranes of these tissues. It has been reported that di- and tripeptides can be transported across the epithelium of lung [25], suggesting the expression of peptide transport system.

In conclusion, a cDNA encoding rat H^+-coupled peptide transporter PEPT2 was isolated. Northern blot analysis revealed that the PEPT2 mRNA is expressed predominantly in the kidney, especially in medulla compared to cortex, and also in the brain, lung and spleen, but not in the small intestine.

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References

[1] Okano, T., Inui, K., Maegawa, H., Takano, M. and Hori, R. (1986) J. Biol. Chem. 261, 14130–14134.
[2] Okano, T., Inui, K., Takano, M. and Hori, R. (1986) Biochem. Pharmacol. 35, 1781–1786.
[3] Tsuji, A., Terasaki, T., Tamai, I. and Hirooka, H. (1987) J. Pharmacol. Exp. Ther. 241, 594–601.
[4] Inui, K., Okano, T., Maegawa, H., Kato, M., Takano, M. and Hori, R. (1988) J. Pharmacol. Exp. Ther. 247, 235–241.
[5] Muranushi, N., Yoshikawa, T., Yoshida, M., Oguma, T., Hirano, K. and Yamada, H. (1989) Pharm. Res. 6, 308–312.
[6] Inui, K., Okano, T., Takano, M., Saito, H. and Hori, R. (1984) Biochim. Biophys. Acta 769, 449–454.
[7] Hori, R., Tomita, Y., Katsura, T., Yasuhara, M., Inui, K. and Takano, M. (1993) Biochem. Pharmacol. 45, 1763–1768.
[8] Ganapathy, V. and Leibach, F.H. (1983) J. Biol. Chem. 258, 14189–14192.
[9] Hoshi, T. (1985) Jpn. J. Physiol. 35, 179–191.
[10] Takuwa, N., Shimada, T., Matsumoto, H. and Hoshi, T. (1985) Biochim. Biophys. Acta 814, 186–190.
[11] Silbernagl, S., Ganapathy, V. and Leibach, F.H. (1987) Am. J. Physiol. 253, F448–F457.
[12] Miyamoto, Y., Coone, J.L., Ganapathy, V. and Leibach, F.H. (1988) Biochem. J. 249, 247–253.
[13] Daniel, H., Morse, E.L. and Adibi, S.A. (1991) J. Biol. Chem. 266, 19917–19924.
[14] Fei, Y.-J., Kanai, Y., Nussberger, S., Ganapathy, V., Leibach, F.H., Romero, M.F., Singh, S.K., Boron, W.F. and Hediger, M.A. (1994) Nature 368, 563–566.
[15] Saito, H., Okuda, M., Terada, T., Sasaki, S. and Inui, K. (1995) J. Pharmacol. Exp. Ther. 275, 1631–1637.
[16] Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294–5299.
[17] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) in: Molecular Cloning. A laboratory manual, 2nd Edn. pp. 7.26–7.29, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
[18] Uchida, S., Sasaki, S., Furukawa, T., Hiraoka, M., Imai, T., Hirata, Y. and Marumo, F. (1993) J. Biol. Chem. 268, 3821–3824.
[19] Kozak, M. (1987) Nucleic Acids Res. 15, 8125–8148.
[20] Kyte, J. and Doolittle, R.F. (1982) J. Mol. Biol. 157, 105–132.
[21] Kennelly, P.J. and Krebs, E.G. (1991) J. Biol. Chem. 266, 15555–15558.
[22] Liu, W., Liang, R., Ramamoorthy, S., Fei, Y.-J., Ganapathy, M.E., Hediger, M.A., Ganapathy, V. and Leibach, F.H. (1995) Biochim. Biophys. Acta 1235, 461–466.
[23] Ganapathy, M.E., Brandsch, M., Prasad, P.D., Ganapathy, V. and Leibach, F.H. (1995) J. Biol. Chem. 270, 25672–25677.
[24] Ramamoorthy, S., Liu, W., Ma, Y.-Y., Yang-Feng, T.L., Ganapathy, V. and Leibach, F.H. (1995) Biochim. Biophys. Acta 1240, 1–4.
[25] Helliwell, P.A., Meredith, D., Boyd, C.A.R., Bronk, J.R., Lister, N. and Bailey, P.D. (1994) Biochim. Biophys. Acta 1190, 430–434.