ELSEVIER

Biochimica et Biophysica Acta 1240 (1995) 1–4

BBA Biochimica et Biophysica Acta

Rapid Report

Proton/peptide cotransporter (PEPT 2) from human kidney: Functional characterization and chromosomal localization

Sammanda Ramamoorthy [a], Wei Liu [a], Yen-Ying Ma [b], Teresa L. Yang-Feng [b], Vadivel Ganapathy [a], Frederick H. Leibach [a,*]

[a] *Department of Biochemistry and Molecular Biology, Medical College of Georgia, Augusta, GA 30912, USA*
[b] *Department of Genetics, Yale University School of Medicine, New Haven, CT 06550, USA*

Received 17 July 1995; accepted 18 July 1995

Abstract

We report here on the functional characterization of the H^+/peptide cotransporter PEPT 2 cloned from human kidney and on the chromosomal localization of the *PEPT* 2 gene. PEPT 2, when functionally expressed in HeLa cells, induces the transport of the neutral dipeptide glycylsarcosine. The induced transport activity is markedly influenced by extracellular pH. The optimum pH for the transport process is 6.0–7.0. Kinetic analysis has revealed that PEPT 2 is a high-affinity transporter, the Michaelis–Menten constant for glycylsarcosine being 74 ± 14 μM. The human intestinal H^+/peptide cotransporter PEPT 1 has 4-fold less affinity for the dipeptide under identical experimental conditions. Studies with other chemically diverse dipeptides have established that PEPT 2 possesses higher affinity than PEPT 1 not only for neutral peptides but also for peptides consisting of anionic and/or cationic amino acids. Somatic cell hybrid analysis and in situ hybridization have shown that the gene encoding PEPT 2 maps to human chromosome 3q13.3-q21.

Keywords: Proton/peptide cotransporter; PEPT 2; High affinity transporter; Chromosomal localization; (Human kidney)

The presence of an active mechanism for the reabsorption of small peptides in mammalian kidney has been well established [1–4]. This system is energized by a transmembrane electrochemical H^+ gradient [5–7]. Kinetic studies have indicated the existence of multiple peptide transport systems in the kidney [8–10]. Recent efforts in our laboratory have led to successful cloning of two structurally distinct H^+/peptide cotransporters, namely PEPT 1 [11,12] and PEPT 2 [13]. PEPT 1 was cloned from the intestine. This is a low-affinity transporter and is expressed not only in the intestine but also in the kidney. This is in agreement with previous studies from different laboratories [8–10] which have demonstrated the presence of a low-affinity peptide transporter in the kidney. PEPT 2 is expressed in the kidney but not in the intestine. Our initial studies have shown that PEPT 2 exhibits 50% homology in amino acid sequence to PEPT 1 [13] and that it catalyzes, like PEPT 1, H^+-coupled peptide transport. The present paper provides evidence that PEPT 2, in contrast to PEPT 1, is a high-affinity transporter. This corroborates earlier findings indicating the presence of a high-affinity peptide transport system in the kidney [8–10]. The present paper also reports on the chromosomal localization of the *PEPT* 2 gene.

PEPT 2 was functionally expressed in HeLa cells by using the vaccinia virus expression technique [14]. The functional characteristics of the expressed transporter were studied with respect to the pH-dependence and substrate affinity. The pH-dependence of PEPT 2 function was investigated by measuring the PEPT 2 cDNA-induced glycylsarcosine transport in HeLa cells at various extracellular pH. The uptake medium of different pH (5.0–9.0) was prepared by appropriately mixing two buffers, 25 mM Hepes/Tris (pH 9.0) and 25 mM Mes/Tris (pH 5.0), both containing 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, and 5 mM glucose. Kinetic parameters for PEPT 2-mediated glycylsarcosine transport were determined by studying the transport rate at varying concentrations of glycylsarcosine (10–500 μM). The experimental data were fit to a transport model describing a combination of a single mediated component and a diffusional component. HeLa cells do not have endogenous peptide transport activity [12,13]. The affinity of PEPT 2 for various other dipeptides was estimated, according to the method of Hajjar and Curran [15], from the potency of these peptides

* Corresponding author. Fax: +1 (706) 7216608.

SSDI 0005-2736(95)00178-6

to inhibit the PEPT 2-mediated glycylsarcosine transport, assuming competitive interaction between the inhibitory peptides and glycylsarcosine.

Chromosomal localization of the gene coding for PEPT 2 was accomplished by somatic cell hybrid analysis and by in situ hybridization of ^{3}H-labeled PEPT 2 cDNA to human metaphase chromosome spreads. A mapping panel consisting of mouse–human and Chinese hamster–human somatic hybrids (panel no. 1 from the National Institute of General Medical Sciences' Human Genetic Mutant Cell Repository) was used for somatic cell hybrid analysis. DNA was isolated from the hybrid cells, digested with *Eco*RI and the fragments were probed, after size fractionation, with ^{32}P-labeled PEPT 2 cDNA. In situ hybridization and emulsion autoradiography were performed as described previously [16,17] by using ^{3}H-labeled PEPT 2 cDNA as the probe. Chromosomes were G-banded using Wright's stain and G-banded chromosomes were analyzed for silver grain localization. The cDNA probe was labeled by nick translation in the presence of [α-^{32}P]dCTP (Southern hybridization) or [^{3}H]dCTP and [^{3}H]dTTP (in situ hybridization).

Fig. 1 describes the dependence of glycylsarcosine transport in PEPT 2 cDNA-transfected HeLa cells on extracellular pH. The transport was very low at pH 9.0, but it increased markedly when the pH was decreased. The pH-transport rate relationship was bell-shaped in the pH range of 5.5–9.0, with an optimum at pH 6.0–7.0.

Fig. 2 describes the kinetics of glycylsarcosine transport in PEPT 2 cDNA-transfected HeLa cells, measured at pH 6.0. The concentration of the dipeptide was varied over the range of 10–500 μM. The concentration-transport rate relationship was hyperbolic, indicating saturability of the PEPT 2 cDNA-induced transport process. The experimental data were found to fit best ($r = 0.99$) to a transport model describing a single carrier-mediated process plus a diffusional process. This analysis gave the following values for the kinetic parameters of the carrier-mediated process: K_t, 74 ± 14 μM and V_{max}, 504 ± 32 pmol/10^6 cells per 3 min.

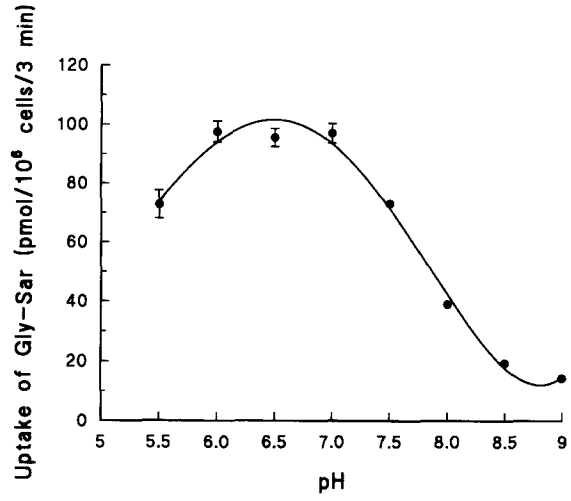

Fig. 1. Dependence of PEPT 2-mediated glycylsarcosine transport on extracellular pH. HeLa cells were transfected with PEPT 2 cDNA and 12 h following transfection transport of 30 μM glycylsarcosine was measured with a 3 min incubation at different extracellular pH. Values represent means ± S.E. for 3 determinations.

These results show that PEPT 2 codes for a high-affinity H^+/peptide cotransporter. The apparent affinity of PEPT 2 for glycylsarcosine is 4-fold greater than that of PEPT 1 under identical experimental conditions. Glycylsarcosine is a zwitterionic dipeptide, consisting of neutral amino acids. Therefore, it was of interest to know whether the relatively higher affinity of PEPT 2 compared to PEPT 1 is also true for other peptides consisting of anionic and/or cationic amino acids. We compared the ability of a number of anionic and cationic dipeptides to inhibit the transport of glycylsarcosine mediated by PEPT 1 and PEPT 2 following their expression in HeLa cells (Table 1). All dipeptides tested inhibited glycylsarcosine transport induced by PEPT 1 and PEPT 2. In the case of PEPT 1,

Table 1
Inhibition of PEPT 1- and PEPT 2-mediated glycylsarcosine transport by zwitterionic, anionic, and cationic dipeptides

Inhibitor	PEPT 1			PEPT 2		
	Gly-Sar transport		Estimated K_i (μM)	Gly-Sar transport		Estimated K_i (μM)
	pmol/10^6 cells per 3 min	%		pmol/10^6 cells per 3 min	%	
Control	429 ± 39	100	–	150 ± 8	100	–
Ala-Ala	196 ± 16	46	78	13 ± 1	9	6
Ala-Asp	198 ± 18	46	78	34 ± 4	23	18
Ala-Lys	240 ± 14	56	117	23 ± 4	15	11
Lys-Ala	352 ± 20	82	419	43 ± 5	28	23
Asp-Lys	231 ± 2	54	108	17 ± 3	12	8

HeLa cells were transfected with either PEPT 1 cDNA or PEPT 2 cDNA and the transport of glycylsarcosine was measured at pH 6.0 with a 3 min incubation. Concentration of Gly-Sar was 25 μM for PEPT 1 and 50 μM for PEPT 2. Concentration of inhibitory peptides was 100 μM. Gly-Sar transport measured in cells transfected with empty vector was subtracted from the transport measured in cDNA-transfected cells to determine PEPT 1- or PEPT 2-mediated transport. K_i values were estimated using a K_t of 290 μM for PEPT 1 and 74 μM for PEPT 2. Values represent means ± S.E. for 3 determinations.

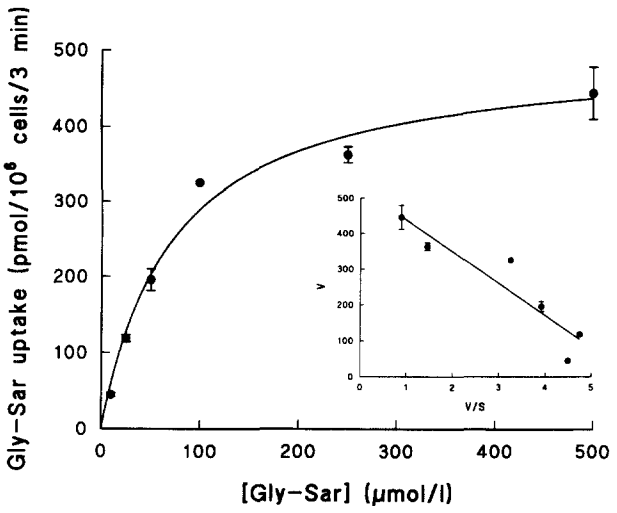

Fig. 2. Kinetics of glycylsarcosine transport induced by PEPT 2 cDNA in HeLa cells. Transport of glycylsarcosine in PEPT 2 cDNA-transfected HeLa cells was measured at pH 6.0 with a 3 min incubation over glycylsarcosine concentration range of 10–500 μM. Values represent means ± S.E. for 3 determinations. Inset: Eadie–Hofstee plot for carrier-mediated transport.

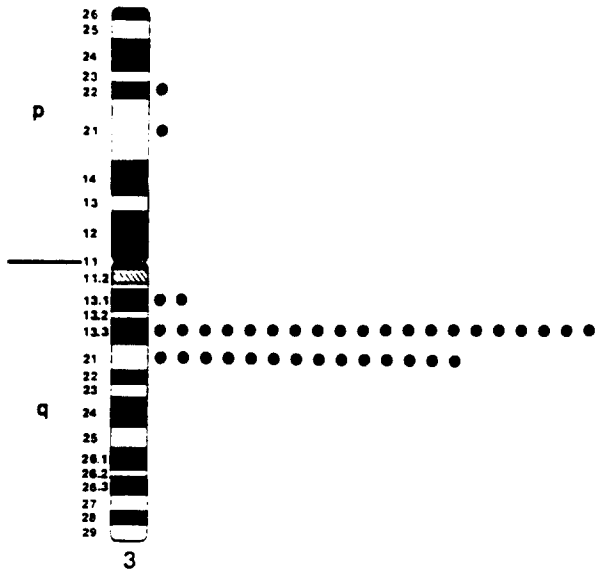

Fig. 3. Chromosomal location of the PEPT 2 gene. Position and relative abundance of silver grains on human chromosome 3 as determined by in situ hybridization using [^{3}H]PEPT 2 cDNA as a probe are indicated.

the transport was measured at a glycylsarcosine concentration of 25 μM. This concentration was less than one-tenth of the K_t value (290 μM). The inhibition caused by 100 μM of Ala-Asp, Ala-Lys, Lys-Ala and Asp-Lys under these conditions ranged between 20 and 55%. The inhibition by 100 μM of the neutral dipeptide Ala-Ala was also within this range. In the case of PEPT 2, the transport was measured at a glycylsarcosine concentration of 50 μM. This concentration was very close to the K_t value (74 μM). Under these conditions, the inhibition caused by 100 μM of Ala-Asp, Ala-Lys, Lys-Ala, Asp-Lys, and Ala-Ala was in the range of 70–90%. Using these inhibition data and the respective K_t values, we estimated the inhibition constants (K_i) for these peptides according to the method of Hajjar and Curran [15]. As shown in Table 1, the K_i values were in the range of 8–400 μM for PEPT 1. In contrast, the corresponding K_i values for PEPT 2 (range, 6–23 μM) were manifold lower than those for PEPT 1, demonstrating that PEPT 2 exhibits higher affinity than PEPT 1 not only for zwitterionic dipeptides but also for anionic and cationic dipeptides.

We have also determined the chromosomal location of the PEPT 2 gene. Southern blot hybridization of size-fractionated *Eco*RI fragments of DNA from mouse–human and Chinese hamster–human somatic cell hybrids using the PEPT 2 cDNA probe detected six human-specific fragments of 12.5, 6.4, 5.9, 5.5, 1.7, and 1.0 kb, four mouse-specific fragments of 11.0, 3.7, 2.4, and 1.3 kb, and three Chinese hamster-specific fragments of 13.5, and 2.9, and 2.0 kb. All human-specific fragments showed concordant segregation with human chromosome 3 (Table 2). Regional localization of the *PEPT* 2 gene on chromosome 3 was determined by in situ hybridization of the PEPT 2 cDNA to human metaphase chromosome spreads. Of 122 grains from 50 cells scored, 34 (28%) were found at chromosome 3q13.3-q21 (Fig. 3). No other chromosomal site was labeled above the background level.

In conclusion, the data presented here clearly establish that the cloned human PEPT 2 is a high-affinity H^+/peptide cotransporter in contrast to the recently cloned human PEPT 1 which is a low-affinity H^+/peptide cotransporter. The relatively higher affinity of PEPT 2 versus

Table 2
Correlation of human sequences detected by PEPT 2 cDNA probe with human chromosomes in rodent X human somatic cell hybrids

Hybridization/ chromosome	Human chromosome																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
Concordant +/+	3	4	9	5	4	7	6	7	0	2	1	5	5	8	7	0	9	6	4	5	4	4	0	2
−/−	8	7	8	5	4	4	4	4	8	6	4	4	7	4	5	7	3	7	5	4	5	5	8	7
Discordant +/−	6	5	0	2	4	2	2	1	9	3	7	3	3	1	1	8	0	3	4	3	4	4	6	6
−/+	1	2	0	4	5	5	4	3	1	2	4	5	0	4	2	2	6	2	2	5	4	3	1	1
Discordant clones	7	7	0	6	9	7	6	4	10	5	11	8	3	5	3	10	6	5	6	8	8	7	7	7
Informative clones [a]	18	18	17	16	17	18	16	15	18	13	16	17	15	17	15	17	18	18	15	17	17	16	15	16

[a] Data for chromosomes involved in rearrangement or present at a frequency of 0.1 or less were excluded.

PEPT 1 is seen for a variety of structurally diverse dipeptides. This marked difference in the affinity for peptide substrates is the first functional distinction to be reported between the cloned human PEPT 1 and human PEPT 2. PEPT 2 is expressed in the kidney but not in the small intestine. The high-affinity of PEPT 2 for peptides is physiologically important because the concentration of the peptide substrates in the renal tubular lumen is expected to be low. The high-affinity transport system is best suited for efficient reabsorption of peptides under these conditions. The low-affinity H^+/peptide cotransporter PEPT 1 is expressed in the intestine and also in the kidney. In the intestinal lumen, the concentration of peptides arising from the digestion of dietary proteins is expected to be very high and therefore the low-affinity/high-capacity transport system PEPT 1 is physiologically best suited for absorption of the peptides in the intestine. It is of interest that the kidney expresses both PEPT 1 and PEPT 2. The brush border membrane of the renal tubular cells is one of the richest sources of peptidases. It is likely that the concentration of small peptides is very low in the early parts of the proximal tubule, but the concentration increases along the length of the proximal tubule due to hydrolysis of larger peptides and proteins by these peptidases. We speculate that PEPT 2 and PEPT 1 may be expressed differentially in the proximal and distal regions of the proximal tubule, respectively, to function most effectively in the reabsorption of peptides under these conditions.

The studies reported here describing the functional distinction between PEPT 1 and PEPT 2 in terms of substrate affinity are also important for other reasons. In addition to endogenous peptide substrates, PEPT 1 and PEPT 2 also accept several peptidomimetic drugs as substrates [18–21]. It is likely that there are differences between PEPT 1 and PEPT 2 in the recognition of these drugs. This might indicate that significant quantitative as well as qualitative differences exist in the handling of these drugs between the intestine which expresses only PEPT 1 and the kidney which expresses primarily PEPT 2. In addition, the functional distinction between PEPT 1 and PEPT 2 in terms of substrate affinity may also be very useful in future studies directed at identifying the specific domains responsible for substrate binding in PEPT 1 and PEPT 2. The chromosomal localization studies are interesting because of the identification of distinct genes encoding PEPT 1 and PEPT 2. The *PEPT* 1 gene has been localized to human chromosome 13q33-q34 [13]. The present study has shown that the *PEPT* 2 gene maps to human chromosome 3q13.3-q21.

H^+-coupled peptide transport across the plasma membrane has not been described in tissues other than the intestine and the kidney. Nonetheless, RNA transcripts hybridizing to PEPT 1 cDNA are detectable by Northern blot in tissues such as placenta, liver, and pancreas [12]. Interestingly, PEPT 2 mRNA is not detectable by Northern blot in several of the human tissues tested, including kidney [13], suggesting low abundance of the transcript. However, evidence has been obtained for the presence of the PEPT 2 mRNA in the kidney by using RT-PCR [13], a method more sensitive than the Northern blot analysis. The intestine is negative for the PEPT 2 mRNA even when analyzed by this sensitive method. A similar approach has not been employed for the detection of the PEPT 2 mRNA in other tissues and therefore, it remains to be determined whether PEPT 2 is expressed in non-renal tissues.

Acknowledgements

The authors thank Bonnie Arms for expert secretarial assistance. This work was supported by National Institutes of Health Grant DK 28389 and DK 42069.

References

[1] Ganapathy, V. and Leibach, F.H. (1986) Am. J. Physiol. 251, F945–F953.
[2] Ganapathy, V., Miyamoto, Y. and Leibach, F.H. (1987) Adv. Biosci. 65, 91–98.
[3] Silbernagl, S. (1988) Physiol. Rev. 68, 911–1007.
[4] Ganapathy, V. and Leibach, F.H. (1991) Curr. Opin. Cell Biol. 3, 695–701.
[5] Ganapathy, V. and Leibach, F.H. (1983) J. Biol. Chem. 258, 14189–14192.
[6] Miyamoto, Y. Ganapathy, V. and Leibach, F.H. (1985) Biochem. Biophys. Res. Commun. 132, 946–953.
[7] Takuwa, N., Shimada, T., Matsumoto, H. and Hoshi, T. (1985) Biochim. Biophys. Acta 814, 186–190.
[8] Silbernagl, S., Ganapathy, V. and Leibach, F.H. (1987) Am. J. Physiol. 253, F448–F457.
[9] Daniel, H., Morse, E.L. and Adibi, S.A. (1991) J. Biol. Chem. 266, 19917–19924.
[10] Skopicki, H.A., Fisher, K., Zikos, D., Bloch, R., Flouret, G. and Peterson, D.R. (1991) Am. J. Physiol. 261, F670–F678.
[11] 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.
[12] Liang, R., Fei, Y.J., Prasad, P.D., Ramamoorthy, S., Han, H., Yang-Feng, T.L. Hediger, M.A., Ganapathy, V. and Leibach, F.H. (1995) J. Biol. Chem. 270, 6456–6463.
[13] 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.
[14] Blakely, R.D., Clark, J.A., Rudnick, G. and Amara, S.G. (1991) Analyt. Biochem. 194, 302–308.
[15] Hajjar, J.J. and Curran, P.F. (1970) J. Gen. Physiol. 56, 673–691.
[16] Harper, M.E. and Saunders, G.F. (1981) Chromosoma 83, 431–439.
[17] Yang-Feng, T.L., Floyd-Smith, G., Nemer, M., Drouin, J. and Francke, U. (1985) Am. J. Hum. Genet. 37, 1117–1128.
[18] Inui, K.I., Okano, T., Takano, M., Saito, H. and Hori, R. (1984) Biochim. Biophys. Acta 769, 449–454.
[19] Kramer, W., Girbig, F., Gutjahr, U., Kleemann, H.W., Leipe, I., Urbach, H. and Wagner, A. (1990) Biochim. Biophys. Acta 1027, 25–30.
[20] Daniel, H. and Adibi, S.A. (1993) J. Clin. Invest. 92, 2215–2223.
[21] Hori, R., Tomita, Y., Katsura, T., Yasuhara, M., Inui, K.I. and Takano, M. (1993) Biochem. Pharmacol. 45, 1763–1768.