N-terminal Halves of Rat H^+/Peptide Transporters Are Responsible for Their Substrate Recognition[1]

Tomohiro Terada,[2] Hideyuki Saito,[2] Kyoko Sawada,[2] Yukiya Hashimoto,[2] and Ken-ichi Inui[2,3]

Received June 1, 1999; accepted September 25, 1999

Purpose. Peptide transporters PEPT1 and PEPT2 differ substantially in their substrate affinity and recognition. The aim of this study is to define the structural domains which influence the functional characteristics of both transporters

Methods. Two kinds of chimeric peptide transporters (PEPT-N1C2 and PEPT-N2C1) were constructed, and their functional characteristics were compared with those of wild-type transporters in stable transfectants.

Results. PEPT-N1C2, the N-terminal half of rat PEPT1 and the C-terminal half of rat PEPT2, and the reciprocal chimera PEPT-N2C1 were functionally expressed in LLC-PK_1 cells. The pH-profiles of [^{14}C] glycylsarcosine uptake by PEPT-N1C2 and PEPT-N2C1 were close to those of PEPT1 and PEPT2, respectively. Substrate recognition for PEPT-N1C2 and PEPT-N2C1 was also similar to that of PEPT1 and PEPT2, respectively. However, substrate affinities for PEPT-N1C2 were higher than those for PEPT1, although those for PEPT-N2C1 and PEPT2 were comparable.

Conclusions. These results indicate that functional regions which are associated with the extracellular pH changes and are responsible for substrate recognition of PEPT1 and PEPT2 may be located in the N-terminal halves of the proteins. In addition, it is suggested that the domain to affect the substrate affinity exists in the C-terminal as well as in the N-terminal half of rat PEPT2.

KEY WORDS: peptide transporters; chimeras; intestinal absorption; renal reabsorption; β-lactam antibiotics.

INTRODUCTION

The reabsorption of oligopeptides (1) and oral β-lactam antibiotics (2,3) from the glomerular filtrate along the nephron in the kidney is mediated by two apparently distinct peptide transport systems localized at brush-border membranes. Using purified renal brush-border membrane vesicles, these peptide transport systems were found to be electrogenic and H^+-gradient-driven cotransporters (4). These two H^+-coupled peptide transporters, PEPT1 (5–8) and PEPT2 (9–11), have been cloned from various species. Both transporters have similar primary and secondary structures with about 50% amino acid identity. PEPT1 is expressed mainly in the small intestine and at low levels in the kidney (7,12), whereas PEPT2 appears in various tissues such as kidney, brain and lung (11). In addition to tissue distribution, PEPT1 and PEPT2 differ in substrate affinity and recognition. PEPT2 shows higher affinity for chemically diverse dipeptides than PEPT1 (13). The recognition characteristics of β-lactam antibiotics by PEPT1 and PEPT2 are also distinct; for example, human PEPT1 and PEPT2 preferred cyclacillin (aminopenicillin) and cefadroxil (aminocepharospolin), respectively (14). Previously, using LLC-PK_1 cells stably transfected with either rat PEPT1 or PEPT2 cDNA, we compared the two transporters in their recognition of various β-lactam antibiotics (15). Our results demonstrated that PEPT2 showed higher affinity for amino β-lactam antibiotics than PEPT1, whereas PEPT1 preferred β-lactam antibiotics without an α-amino group.

In this way, a large body of information describing the transport characteristics of PEPT1 and PEPT2 has become available, but the structural domains responsible for these functional differences have not been elucidated. In the present study, we constructed two chimeric peptide transporters derived from rat PEPT1 and PEPT2, and compared their transport characteristics with those of PEPT1 and PEPT2.

MATERIALS AND METHODS

Materials

Cefadroxil (Bristol Meyers Co., Tokyo, Japan), cefixime (Fujisawa Pharmaceutical Co., Osaka, Japan), ceftibuten and cephalexin (Shionogi and Co., Osaka, Japan), cephradine (Sankyo Co., Tokyo, Japan), and cyclacillin (Takeda Chemical Industries, Osaka, Japan) were gifts from the respective suppliers. Glycylsarcosine and ampicillin were obtained from Sigma Chemical Co. (St. Louis, MO). [^{14}C]Glycylsarcosine (1.89 GBq/mmol) was obtained from Daiichi Pure Chemicals Co., Ltd. (Ibaraki, Japan). All other chemicals used were of the highest purity available.

Construction of PEPT Chimeric cDNAs

For construction of PEPT chimeric cDNAs, we used the plasmid pSPORT1 (GIBCO, Life Technologies) into which was inserted rat PEPT1 or PEPT2 cDNA at *Sal*I and *Not*I sites (7,11). The restriction site of *Nde*I is unique in the sequence of rat PEPT2 cDNA and does not exist in rat PEPT1 cDNA. The *Nde*I site in rat PEPT1 cDNA was created by polymerase chain reaction (PCR) with synthetic oligonucleotides containing the *Nde*I site. The rat PEPT1 cDNA served as a template for PCR using the following primers: T7 promoter primer and primer 1 (5′-AAG**CATATG**CACTTGGCCACTTTGCCCA TG-3′, *Nde*I in boldface and mutated nucleotides underlined), which generated PCR fragment 1; and M13 primer and primer 2 (5′-GTG**CATATG**CTTTGCCATCAAAAACAGGTT-3′, *Nde*I in boldface and mutated nucleotides underlined), which generated PCR fragment 2. Primer 1 encoded antisense nucleotides 722-696 and primer 2 encoded sense nucleotides 717–743 of rat PEPT1 cDNA (7). PCR fragment 1 was digested with *Sal*I and *Nde*I and inserted between the corresponding *Sal*I and *Nde*I restriction sites of rat PEPT2 cDNA (PEPT-N1C2 cDNA). PCR fragment 2 was digested with *Nde*I and *Not*I and inserted between the corresponding restriction sites of rat PEPT2 cDNA (PEPT-N2C1 cDNA). The constructed chimeric cDNAs were confirmed by sequence analysis with Sequenase version 2.0 (United States Biochemical, Cleveland, OH).

[1] Presented in part at the 31st Annual Meeting of the American Society of Nephrology, J. Am. Soc. Nephrol. **9**:58A (1998).

[2] Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Kyoto 606-8507, Japan.

[3] To whom correspondence should be addressed. (e-mail: inui@kuhp.kyoto-u.ac.jp)

0724-8741/00/0100-0015$18.00/0

Cell Culture and Transfection

Parental LLC-PK_1 cells obtained from the American Type Culture Collection (ATCC CRL-1392) were cultured in complete medium consisting of Dulbecco's modified Eagle's medium (GIBCO), supplemented with 10% fetal bovine serum (Whittaker Bioproducts Inc., Walkersville, MD) without antibiotics in an atmosphere of 5% CO_2-95% air at 37°C. PEPT-N1C2 and PEPT-N2C1 cDNAs were subcloned into the *Sal* I- and *Not* I-cut mammalian expression vector pBK-CMV (Stratagene, La Jolla, CA) and transfected into LLC-PK_1 cells by $CaPO_4$-coprecipitation method as described previously (16). G418 (1 mg/ml)-resistant cells were picked up, and cells expressing the chimeras were selected by measuring [^{14}C]glycylsarcosine uptake. Both transfectants were maintained in complete medium with G418 (1 mg/ml). In the uptake studies, the cells were cultured in the complete medium without G418.

Isolation of Total RNA and Reverse Transcription-Polymerase Chain Reaction

Total RNA was isolated from each transfectant grown to confluence on 35-mm plastic dishes using a RNeasy Mini Kit (QIAGEN GmbH, Germany). Reverse transcription was performed using a cDNA synthesis kit (GIBCO). The synthesized cDNAs were used for subsequent PCR with the following sets of primers: 1) sense primer, bases 50-70 of rat PEPT1 cDNA, sequence, 5′-ATGGGGATGTCCAAGTCTCGG-3′, and antisense primer, bases 2157-2134 of rat PEPT2 cDNA, sequence, 5′-CATATTCCCCTGCACGGCAGGAAT-3′; 2) sense primer, bases 245-265 of rat PEPT2 cDNA, sequence, 5′-CCTCCAAAGAAGTCACCTCCG-3′, and antisense primer, bases 2177-2153 of rat PEPT1 cDNA, sequence, 5′-GTTTGTCTGTGAGACAGGTTCCAA-3′. The junction sites of chimeric cDNAs were included between the sense and antisense primers. PCR (30 cycles) was performed under the following conditions: 94°C for 1 min, 68°C for 1 min, 72°C for 2.5 min. Aliquots of the PCR products were electrophoresed in 0.7% agarose gels and stained with ethidium bromide.

Uptake Studies by Cell Monolayers

Uptake of [^{14}C]glycylsarcosine was measured in cells grown on 35-or 60-mm plastic dishes as described (15). The protein contents of cell monolayers solubilized in 1N NaOH were determined by the method of Bradford (17), using a Bio-Rad protein assay kit with bovine γ-globulin as the standard.

RESULTS

Chimeric peptide transporters were generated by recombination at the intracellular loops between the 6th and the 7th putative transmembrane domains of rat PEPT1 and PEPT2 (Fig. 1). The chimera in which the N- and C-terminal halves were from rat PEPT1 and PEPT2, respectively, was designated as PEPT-N1C2, and the reciprocal chimera was named PEPT-N2C1. To analyze the transport characteristics of PEPT-N1C2 and PEPT-N2C1, each cDNA was stably transfected into LLC-PK_1 cells. As illustrated in Fig. 2A, both transfectants were confirmed to express the respective chimeric mRNA by reverse transcription-PCR. We designated PEPT-N1C2- and PEPT-N2C1-expressing cells as LLC-N1C2 and LLC-N2C1 cells,

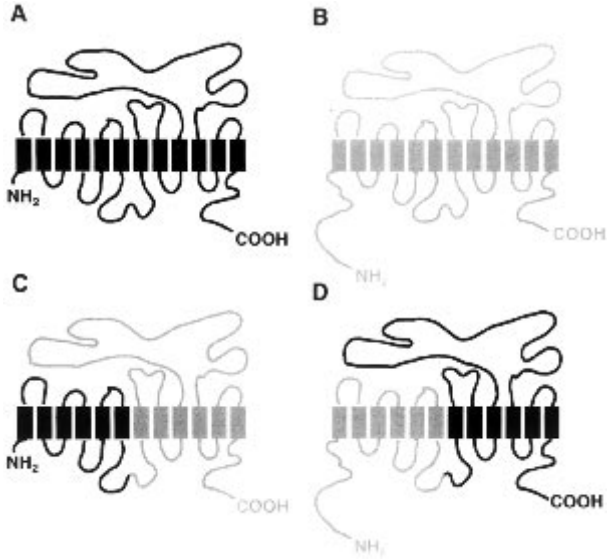

Fig. 1. Schematic representation of PEPT1 (black) (A), PEPT2 (gray) (B) and constructed chimeras. All the proteins are represented as possessing twelve putative membrane-spanning domains with their COOH and NH_2 termini within the cytoplasm. The chimera with transmembrane domains 1–6 of PEPT1 and 7–12 of PEPT2 was designated as PEPT-N1C2 (C), and the reciprocal as PEPT-N2C1 (D).

respectively. Using these two transfectants, we first examined the time course of [^{14}C]glycylsarcosine uptake at pH 6.0 (Fig. 2B). Based on the data, for subsequent experiments we selected incubation times of 15 and 60 min for LLC-N1C2 and LLC-N2C1 cells, respectively (Fig. 2B).

Figure 3 shows the effects of the medium pH on [^{14}C]glycylsarcosine uptake by chimeric and wild-type peptide transporters. In LLC-N1C2 cells and PEPT1-expressing cells, [^{14}C]glycylsarcosine uptake was maximal at pH 5.5, whereas maximal uptake was seen at pH 6.5-7.0 in LLC-N2C1 cells and PEPT2-expressing cells.

Figure 4 illustrates the concentration dependence of [^{14}C]glycylsarcosine uptake at pH 6.0 by both chimera-expressing cells. Specific uptake was calculated by subtracting the nonspecific uptake estimated in the presence of excess unlabeled dipeptide from the total uptake, and kinetic parameters were calculated according to the Michaelis-Menten equation. The apparent *Km* values of glycylsarcosine uptake were 250 μM for LLC-N1C2 cells and 150 μM for LLC-N2C1 cells, respectively.

To determine the substrate specificity, we examined the abilities of various β-lactam antibiotics to inhibit [^{14}C]glycylsarcosine uptake at pH 6.0 by LLC-N1C2 and LLC-N2C1 cells. The typical inhibition curves of three β-lactam antibiotics are shown in Fig. 5. The order of inhibition of [^{14}C]glycylsarcosine uptake by LLC-N1C2 cells was cefadroxil (aminocephalosporin) = cyclacillin (aminopenicillin) > ceftibuten (anionic cephalosporin without an α-amino group). Similarly, the [^{14}C]glycylsarcosine uptake by LLC-N2C1 cells was suppressed by cefadroxil > cyclacillin > ceftibuten.

The apparent inhibition constant (*Ki*) values of various β-lactam antibiotics were estimated from the competition curves by nonlinear least square regression analysis as described (15). The estimated *Ki* values of these antibiotics for PEPT-N1C2

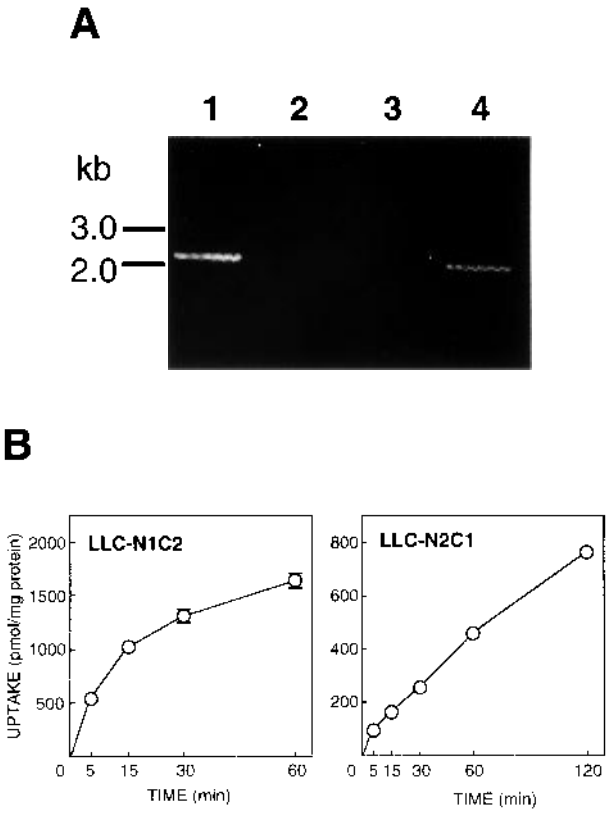

Fig. 2. *A,* Expression of PEPT-N1C2 and PEPT-N2C1 mRNA in transfectants. Total RNA isolated from LLC-N1C2 and LLC-N2C1 cells was reverse transcribed. The synthesized PEPT-N1C2 cDNA *(lanes 1 and 2)* and PEPT-N2C1 cDNA *(lanes 3 and 4)* were amplified with sets of primers as follows: *lanes 1 and 3*, sense and antisense primers were used for PEPT1- and PEPT2-specific sequences, respectively; *lanes 2 and 4*, sense and antisense primers were used for PEPT2- and PEPT1-specific sequences, respectively. *B,* Time course of [^{14}C]glycylsarcosine uptake by LLC-N1C2 (left panel) and LLC-N2C1 cells (right panel). Both cells were incubated for the specified periods at 37°C with incubation medium containing [^{14}C]glycylsarcosine (20 μM, 37 kBq/ml) at pH 6.0. Each point represents the mean ± S.E. of three experiments (left panel) and the mean of two experiments (right panel). When the error bars are not shown, they are smaller than the symbol.

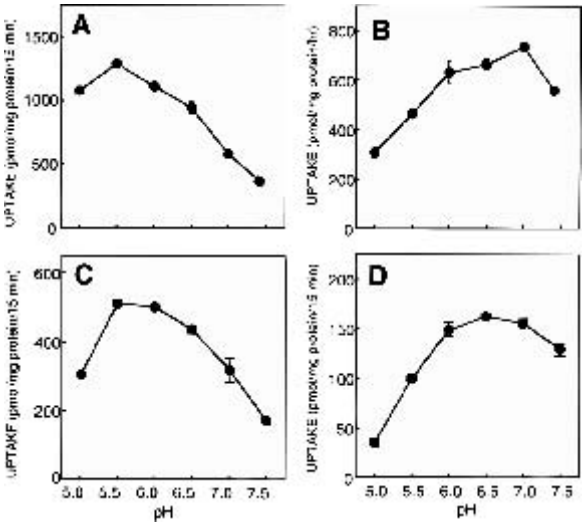

Fig. 3. pH-dependence of [^{14}C]glycylsarcosine uptake by LLC-N1C2 cells (A), LLC-N2C1 cells (B), PEPT1-expressing cells (C) and PEPT2-expressing cells (D). Data of (C) and (D) were taken from our previous paper (15). LLC-N1C2 and LLC-N2C1 cells were incubated for 15 and 60 min at 37°C with incubation medium at various pHs containing [^{14}C]glycylsarcosine (20 μM, 37 kBq/ml), respectively. Each point represents the mean ± S.E. of three experiments. When the error bars are not shown, they are smaller than the symbol.

and PEPT-N2C1 are summarized in Table I with the *Ki* values of those for PEPT1 and PEPT2 taken from our previous paper (15). [^{14}C]Glycylsarcosine uptake by both chimera-expressing transfectants was inhibited by cefadroxil > cyclacillin > cephradine > cephalexin > ampicillin. These amino-β-lactam antibiotics showed relatively strong inhibition of [^{14}C]glycylsarcosine uptake *via* PEPT-N2C1, compared to that *via* PEPT-N1C2. In contrast, ceftibuten and cefixime had a tendency of stronger inhibition in PEPT-N1C2 than in PEPT-N2C1.

DISCUSSION

To date, little information has been reported regarding the structural domains responsible for substrate recognition and affinity by the peptide transporters PEPT1 and PEPT2. To define the structural domains which influence the functional characteristics of PEPT1 and PEPT2, we constructed and expressed chimeric peptide transporters PEPT-N1C2 and PEPT-N2C1. For ease of interpretation of the data, simple chimeras which possessed each half of the transmembrane domains of rat PEPT1 and PEPT2 were prepared. Namely, PEPT-N1C2 consisted of the N-terminal half (transmembrane domains 1–6) of rat PEPT1 and C-terminal half (transmembrane domains 7–12) of rat PEPT2, and PEPT-N2C1 was the reciprocal chimera.

In the present study, we compared the apparent *Km* and *Ki* values of various substrates between PEPT-N1C2 and PEPT-N2C1. Our results indicated that glycylsarcosine and amino β-lactam antibiotics had higher affinity for PEPT-N2C1 than for PEPT-N1C2. On the other hand, ceftibuten and cefixime preferred PEPT-N1C2 to PEPT-N2C1. Ceftibuten and cefixime, neither of which possess an α-amino group, showed low affinity for PEPT2, in contrast to most other substrates which showed higher affinity for PEPT2 than PEPT1 (13,15). Therefore, the substrate recognition characteristics of PEPT-N1C2 and PEPT-N2C1 were similar to those of PEPT1 and PEPT2, respectively. In addition, the pH-profiles of glycylsarcosine uptake by PEPT-N1C2 and PEPT-N2C1 were also close to those of PEPT1 and PEPT2, respectively (15). The pH-profiles of glycylsarcosine uptake by both peptide transporters should be closely related to the protonation of amino acid residue which can serve as the H^+-binding site. Therefore, the present findings suggest that the H^+-binding site as well as substrate recognition site are located in the N-terminal halves, *i.e.*, 1-6 transmembrane domains, of PEPT1 and PEPT2.

We reported previously that histidines 57 and 121, which are located at the predicted transmembrane domains 2 and 4 of rat PEPT1, are involved in substrate binding and/or are responsible for the intrinsic activity of the transporter (18). Histidines 87 and 142 of human PEPT2, which are located in similar topological positions to histidines 57 and 121 of PEPT1,

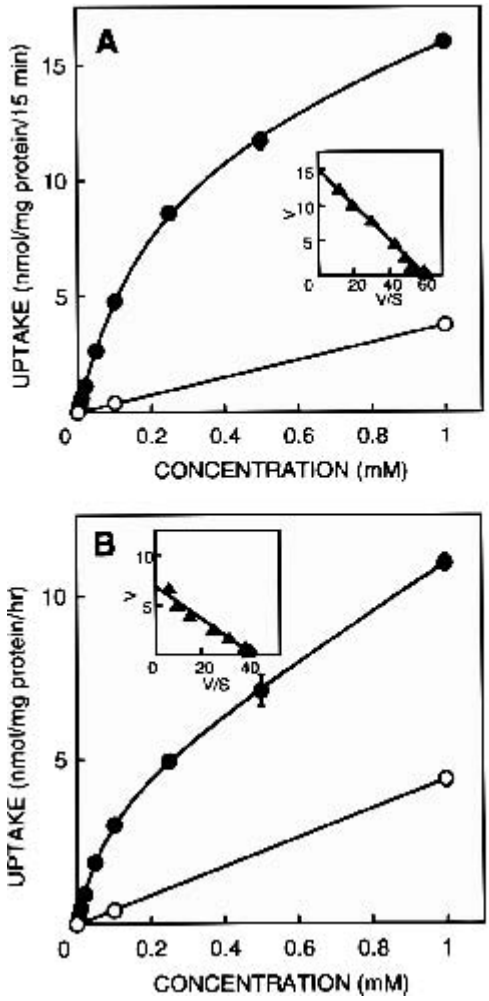

Fig. 4. Concentration-dependence of [^{14}C]glycylsarcosine uptake by LLC-N1C2 (A) and LLC-N2C1 cells (B). [^{14}C]Glycylsarcosine uptake (pH 6.0) by LLC-N1C2 and LLC-N2C1 cells was measured at various concentrations for 15 and 60 min, respectively, at 37°C in the absence (●) or presence (◯) of 10 mM unlabeled glycylsarcosine. Each point represents the mean ± S.E. of three experiments. When the error bars are not shown, they are smaller than the symbol. *Inset*: Eadie-Hofstee plot of the uptake after correction for the nonsaturable component.

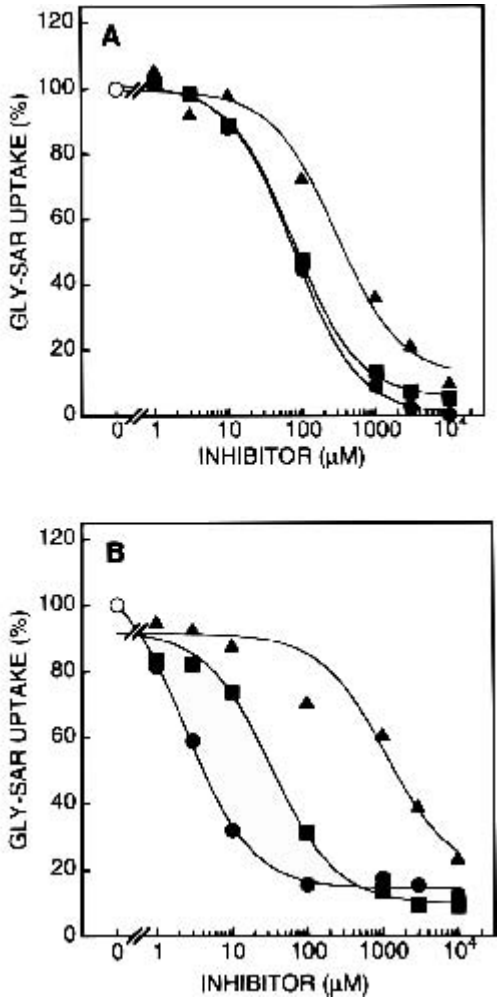

Fig. 5. Inhibition of [^{14}C]glycylsarcosine uptake by β-lactam antibiotics in LLC-N1C2 (A) and LLC-N2C1 cells (B). LLC-N1C2 and LLC-N2C1 cells were incubated for 15 and 60 min, respectively, at 37°C with incubation medium containing [^{14}C]glycylsarcosine (20 μM, 37 kBq/ml) at pH 6.0 in the absence (◯) or presence of increasing concentrations of cyclacillin (■), cefadroxil (●) or ceftibuten (▲). Each point represents the mean of two experiments.

were also reported to play a significant function in the maintenance of transport activity (19). Using PEPT1- and PEPT2-expressing cells, we investigated the functional roles of histidine residues, and suggested that either of these residues might be the binding site of an α-amino group of the substrates (20). Yeung *et al.* (21) demonstrated that tyrosine 167 in transmembrane domain 5 of human PEPT1 played an essential role in dipeptide uptake. These previous results, that is, those essential amino acid residues are located in the N-terminal halves, are compatible with the present findings.

Our previous studies have shown that the *Km* values of glycylsarcosine for PEPT1 and PEPT2 were 1.1 and 0.11 mM, respectively (15). By comparing the *Km* and *Ki* values of glycylsarcosine and amino β-lactam antibiotics for PEPT-N1C2 with those for PEPT1, it was found that PEPT-N1C2 had higher affinity for substrates than PEPT1; *i.e.*, the affinity constants of PEPT-N1C2 were in the μM range, whereas those of PEPT1 were in the mM range. Therefore, it seems reasonable that the domain which affects the substrate affinity also exists in the C-terminal half of rat PEPT2. In contrast, substrate affinities for PEPT-N2C1 and PEPT2 were comparable, indicating that the C-terminal half of rat PEPT1 did not play such roles. Although these results suggested that the C-terminal half of rat PEPT2 did not have an apparent effect on the substrate affinity of rat PEPT2 itself, both the N- and C-terminal halves of rat PEPT2 may be involved in the determination of the substrate affinity. Using functional chimeras prepared from the Na^+/dicarboxylate cotransporter and the Na^+/sulfate cotransporter, Pajor *et al.* (22) found that the substrate recognition site of these transporters was located in the carboxy-terminal portion of the proteins, but residues that affect substrate affinity were located in the amino terminus. They concluded that the domains responsible for substrate recognition and substrate affinity are different, concurring with our present observations.

Table I. Inhibition Constants of β-Lactam Antibiotics for PEPT1, PEPT2, PEPT-N1C2 and PEPT-N2C1

Drug	*Ki* (μM)			
	PEPT1	PEPT2	PEPT-N1C2	PEPT-N2C1
Ampicillin	48,000	670	700	650
Cyclacillin	170	27	72	29
Cephalexin	4,500	49	160	72
Cefadroxil	2,200	3	60	3
Cephradine	8,500	47	150	51
Ceftibuten	600	1,300	270	970
Cefixime	6,900	12,000	3,500	4,100

Note: Ki values for PEPT1 and PEPT2 were taken from our previous paper (15). Each value for PEPT-N1C2 and PEPT-N2C1 represents the mean of two experiments. LLC-N1C2 and LLC-N2C1 cells were incubated for 15 and 60 min, respectively, at 37°C with incubation medium containing [^{14}C]glycylsarcosine (20 μM, 37 kBq/ml) at pH 6.0 in the absence or presence of increasing concentrations of each competitor. The apparent inhibition constant (*Ki*) values were estimated from the competition curves by nonlinear least square regression analysis as described (15).

Previously, Döring *et al.* (23) showed that phenotypic characteristics of rabbit PEPT2 were determined by transmembrane domains 1–9 based on the study of one chimeric peptide transporter. Our present results showed that much narrower domains were important for phenotypic characteristics of both PEPT1 and PEPT2. Independently, using chimeras between human PEPT1 and rat PEPT2, Fei *et al.* (24) demonstrated that transmembrane domains 7–10 played a critical role in the substrate affinity, and suggested that the putative substrate binding site was in transmembrane domains 7, 8 and 9 consistent with the findings of Döring *et al.* (23). However, Fei *et al.* (24) only compared *Km* values of glycylsarcosine for wild-type transporters with those for chimeric transporters in the electrophysiologic studies. It is reported that different domains are responsible for substrate recognition and substrate affinity (19), and that multiple domains could affect the substrate affinity (25,26). Therefore, the domain influencing the substrate affinity is not necessarily the substrate binding site. In addition, if the substrate binding site was located in transmembrane domains 7–9, then two essential histidine residues located in transmembrane domains 2 and 4 were not involved in the substrate binding. Although Fei *et al.* (24) believed these histidine residues to be located sterically close to transmembrane domains 7–9, there is no evidence of this. Moreover, because they constructed chimeras between human PEPT1 and rat PEPT2, their findings might be due to species differences. Given the problems in the study of Fei *et al.* (24), it is reasonable to conclude that transmembrane domains 7–9 of rat PEPT2 are the critical regions for the substrate affinity, rather than the substrate binding site.

In conclusion, our study clearly indicated that the N-terminal halves, namely transmembrane domains 1–6, in PEPT1 and PEPT2 contained the primary domains which are associated with the extracellular pH changes and are responsible for substrate recognition. In addition, it was suggested that the residues which influence the substrate affinity were located in the C-terminal half of rat PEPT2 as well as in the N-terminal half of rat PEPT2.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research (B) and a Grant-in-Aid for Scientific Research on Priority Areas of "Bio-molecular Design for Biotargeting" (No. 296) from the Ministry of Education, Science, Sports, and Culture of Japan, and by grants from the Uehara Memorial Foundation and from the Yamada Science Foundation.

REFERENCES

1. H. Daniel, E. L. Morse, and S. A. Adibi. The high and low affinity transport systems for dipeptides in kidney brush border membrane respond differently to alterations in pH gradient and membrane potential. *J. Biol. Chem.* **266**:19917–19924 (1991).
2. H. Daniel and S. A. Adibi. Transport of β-lactam antibiotics in kidney brush border membrane. Determinants of their affinity for the oligopeptide/H^+ symporter. *J. Clin. Invest.* **92**:2215–2223 (1993).
3. I. Naasani, K. Sato, K. Iseki, M. Sugawara, M. Kobayashi, and M. Miyazaki. Comparison of the transport characteristics of ceftibuten in rat renal and intestinal brush-border membranes. *Biochim. Biophys. Acta* **1231**:163–168 (1995).
4. V. Ganapathy and F. H. Leibach. Role of pH gradient and membrane potential in dipeptide transport in intestinal and renal brush-border membrane vesicles from the rabbit. Studies with L-carnosine and glycyl-L-proline. *J. Biol. Chem.* **258**:14189–14192 (1983).
5. Y.-J. Fei, Y. Kanai, S. Nussberger, V. Ganapathy, F. H. Leibach, M. F. Romero, S. K. Singh, W. F. Boron, and M. A. Hediger. Expression cloning of a mammalian proton-coupled oligopeptide transporter. *Nature (Lond.)* **368**:563–566 (1994).
6. R. Liang, Y.-J. Fei, P. D. Prasad, S. Ramamoorthy, H. Han, T. L. Yang-Feng, M. A. Hediger, V. Ganapathy, and F. H. Leibach. Human intestinal H^+/peptide cotransporter. Cloning, functional expression, and chromosomal localization. *J. Biol. Chem.* **270**:6456–6463 (1995).
7. H. Saito, M. Okuda, T. Terada, S. Sasaki, and K. Inui. Cloning and characterization of a rat H^+/peptide cotransporter mediating absorption of β-lactam antibiotics in the intestine and kidney. *J. Pharmacol. Exp. Ther.* **275**:1631–1637 (1995).
8. K. Miyamoto, T. Shiraga, K. Morita, H. Yamamoto, H. Haga, Y. Taketani, I. Tamai, Y. Sai, A. Tsuji, and E. Takeda. Sequence, tissue distribution and developmental changes in rat inetestinal oligopeptide transporter. *Biochim. Biophys. Acta* **1305**:34–38 (1996).
9. M. Boll, M. Herget, M. Wagener, W. M. Weber, D. Markovich, J. Biber, W. Clauss, H. Murer, and H. Daniel. Expression cloning and functional characterization of the kidney cortex high-affinity proton-coupled peptide transporter. *Proc. Natl. Acad. Sci. U.S.A.* **93**:284–289 (1996).
10. W. Liu, R. Liang, S. Ramamoorthy, Y.-J. Fei, M. E. Ganapathy, M. A. Hediger, V. Ganapathy, and F. H. Leibach. Molecular cloning of PEPT2, a new member of the H^+/peptide cotransporter family, from human kidney. *Biochim. Biophys. Acta* **1235**:461–466 (1995).
11. H. Saito, T. Terada, M. Okuda, S. Sasaki, and K. Inui. Molecular cloning and tissue distribution of rat peptide transporter PEPT2. *Biochim. Biophys. Acta* **1280**:173–177 (1996).
12. H. Ogihara, H. Saito, B.-C. Shin, T. Terada, S. Takenoshita, Y. Nagamachi, K. Inui, and K. Takata. Immuno-localization of H^+/peptide cotransporter in rat digestive tract. *Biochem. Biophys. Res. Commun.* **220**:848–852 (1996).
13. S. Ramamoorthy, W. Liu, Y.-Y. Ma, T. L. Yang-Feng, V. Ganapathy, and F. H. Leibach. Proton/peptide cotransporter (PEPT2) from human kidney: Functional characterization and chromosomal localization. *Biochim. Biophys. Acta* **1240**:1–4 (1995).
14. M. E. Ganapathy, M. Brandsch, P. D. Prasad, V. Ganapathy, and F. H. Leibach. Differential recognition of β-lactam antibiotics by intestinal and renal peptide transporters, PEPT1 and PEPT2. *J. Biol. Chem.* **270**:25672–25677 (1995).

15. T. Terada, H. Saito, M. Mukai, and K. Inui. Recognition of β-lactam antibiotics by rat peptide transporters, PEPT1 and PEPT2, in LLC-PK_1 cells. *Am. J. Physiol.* **273**, F706–F711 (1997).
16. T. Terada, H. Saito, M. Mukai, and K. Inui. Characterization of stably transfected kidney epithelial cell line expressing rat H^+/peptide cotransporter PEPTI: Localization of PEPT1 and transport of β-lactam antibiotics. *J. Pharmacol. Exp. Ther.* **281**:1415–1421 (1997).
17. M. M. Bradford. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254 (1976).
18. T. Terada, H. Saito, M. Mukai, and K. Inui. Identification of the histidine residues involved in substrate recognition by a rat H^+/peptide cotransporter, PEPT1. *FEBS Lett.* **394**:196–200 (1996).
19. Y.-J. Fei, W. Liu, P. D. Prasad, R. Kekuda, T. G. Oblak, V. Ganapathy, and F. H. Leibach. Identification of the histidyl residue obligatory for the catalytic activity of the human H^+/peptide cotransporters PEPT1 and PEPT2. *Biochemistry* **36**:452–460 (1997).
20. T. Terada, H. Saito, and K. Inui. Interaction of β-lactam antibiotics with histidine residue of rat H^+/peptide cotransporters, PEPT1 and PEPT2. *J. Biol. Chem.* **273**:5582–5585 (1998).
21. A. K. Yeung, S. K. Basu, S. K. Wu, C. Chu, C. T. Okamoto, S. F. Hamm-Alvarez, H. von Grafenstein, W.-C. Shen, K.-J. Kim, M. B. Bolger, I. S. Haworth, D. K. Ann, and V. H. L. Lee. Molecular identification of a role for tyrosine 167 in the function of the human intestinal proton-coupled dipeptide transporter (hPepT1). *Biochem. Biophys. Res. Commun.* **250**:103–107 (1998).
22. A. M. Pajor, N. Sun, L. Bai, D. Markovich, and P. Sule. The substrate recognition domain in the Na^+/dicarboxylate and Na^+/sulfate cotransporters is located in the carboxy-terminal portion of the protein. *Biochim. Biophys. Acta* **1370**:98–106 (1998).
23. F. Döring, D. Dorn, U. Bachfischer, S. Amasheh, M. Herget, and H. Daniel. Functional analysis of a chimeric mammalian peptide transporter derived from the intestinal and renal isoforms. *J. Physiol. (Lond)* **497**:773–779 (1996).
24. Y.-J. Fei, J.-C. Liu, T. Fujita, R. Liang, V. Ganapathy, and F. H. Leibach. Identification of a potential substrate binding domain in the mammalian peptide transporters PEPT1 and PEPT2 using PEPT1-PEPT2 and PEPT2-PEPT1 chimeras. *Biochem. Biophys. Res. Commun.* **246**:39–44 (1998).
25. K. J. Buck and S. G. Amara. Chimeric dopamine-norepinephrine transporters delineate structural domains influencing selectivity for catecholamines and 1-methyl-4-phenylpyridinium. *Proc. Natl. Acad. Sci. U.S.A.* **91**:12584–12588 (1994).
26. D. Peter, T. Vu, and R. H. Edwards. Chimeric vesicular monoamine transporters identify structural domains that influence substrate affinity and sensitivity to tetrabenazine. *J. Biol. Chem.* **271**:2979–2986 (1996).