Mini-Review

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Substrates of the human oligopeptide transporter hPEPT2

Dongxin Zhao[1,*], Kui Lu[1,2,*]

[1] *School of Chemistry and Chemical Engineering, Henan University of Technology, Zhengzhou, China;*
[2] *Department of Materials and Chemical Engineering, Henan Institute of Engineering, Zhengzhou, China.*

Summary **Oligopeptide transporters serve important functions in nutrition and pharmacology. In particular, these transporters help maintain the homeostasis of peptides. The peptide-transporter PEPT2 is a high-affinity and low-capacity type oligopeptide transporter from the proton-coupled oligopeptide transporter family. PEPT2 has recently received attention because of its potential application in targeted drug delivery. PEPT2 is widely distributed in kidney, central nervous system, and lung of organisms. In general, all dipeptides, tripeptides, and peptide-like drugs such as β-lactam antibiotics and angiotensin-converting enzyme inhibitors could be mediated and transported as a substrate of PEPT2. The design of many extant drugs and prodrugs is based on the substrate structure of PEPT2 to accelerate absorption via peptide transporters. Thus, this paper summarizes the substrate features of PEPT2 to promote the rational design of drugs and prodrugs that target peptide transporters.**

Keywords: PEPT2, peptide, drug, substrate structure, regulation

1. Introduction

Proton-coupled oligopeptide transporters (POTs) are membrane proteins that can translocate various dipeptides, tripeptides, and peptide-like drugs across the biological membrane (*1,2*). The POT family, also called the solute carrier 15 family (SLC15), comprises four peptide transporters in mammals: PEPT1 (SLC15A1), PEPT2 (SLC15A2), PHT1 (SLC15A4), and PHT2 (SLC15A3). The peptide-transporter PEPT2 is widely expressed in various tissues, with predominant expression in the kidney, brain, lung, eye, prostate, astrocytes, spleen, uterus, and mammary gland (*3-6*). Given its 15 times higher affinity than PEPT1 to the same substrates, PEPT2 was characterized as a high-affinity, low-capacity transporter (*7,8*). PEPT2 can sequence-independently transport more than 400 dipeptides and 8,000 tripeptides, which are comprised of 20 essential L-α-amino acids and most D-enantiomers. Aside from peptides, drugs such as numerous aminocephalosporins, selected angiotensin-converting enzyme inhibitors, peptidase inhibitors, and various novel prodrugs can also be recognized by PEPT2 (*9-11*). Therefore, PEPT2 influences the uptake, efflux, and pharmacological effects of chemical substances, such as peptides and drugs (*12*).

The primary structure of PEPT2 has been deduced by molecular cloning studies in human and animals. As shown in Figure 1, human PEPT2 (hPEPT2) contains 12 transmembrane domains (TMDs) and an extracellular loop between TMDs 9 and 10, with the N- and C-termini facing the cytosol (*13,14*). The conserved Arg57, His121, Tyr56, Tyr64, and Tyr167 were essential for transport activity and substrate binding (*14*). And the putative substrate-binding domain in PEPT2 lays in the region TMDs 7, 8, and 9. The phenotypic characteristics of PEPT2 are determined by TMDs 1 to 9. The region between the centers of the TMDs 2 and 3 significantly contributes to the characteristic pH-dependency of transport. In addition, the large extracellular loop between TMDs 9 and 10 might not be responsible for substrate binding (*7,10,15*). And the amino acids were critical for functional divergence located in the hydrophobic region between predicted

**Address correspondence to:*
Dr. Dongxin Zhao, School of Chemistry and Chemical Engineering, Henan University of Technology, Locus Street, Hi-Tech Industrial Development Zone, Zhengzhou 450001, Henan, China.
E-mail: zhaodx798@163.com

Dr. Kui Lu, School of Material and Chemical Engineering, Henan Institute of Engineering, Zhengzhou 450007, Henan, China.
E-mail: luckyluke@126.com

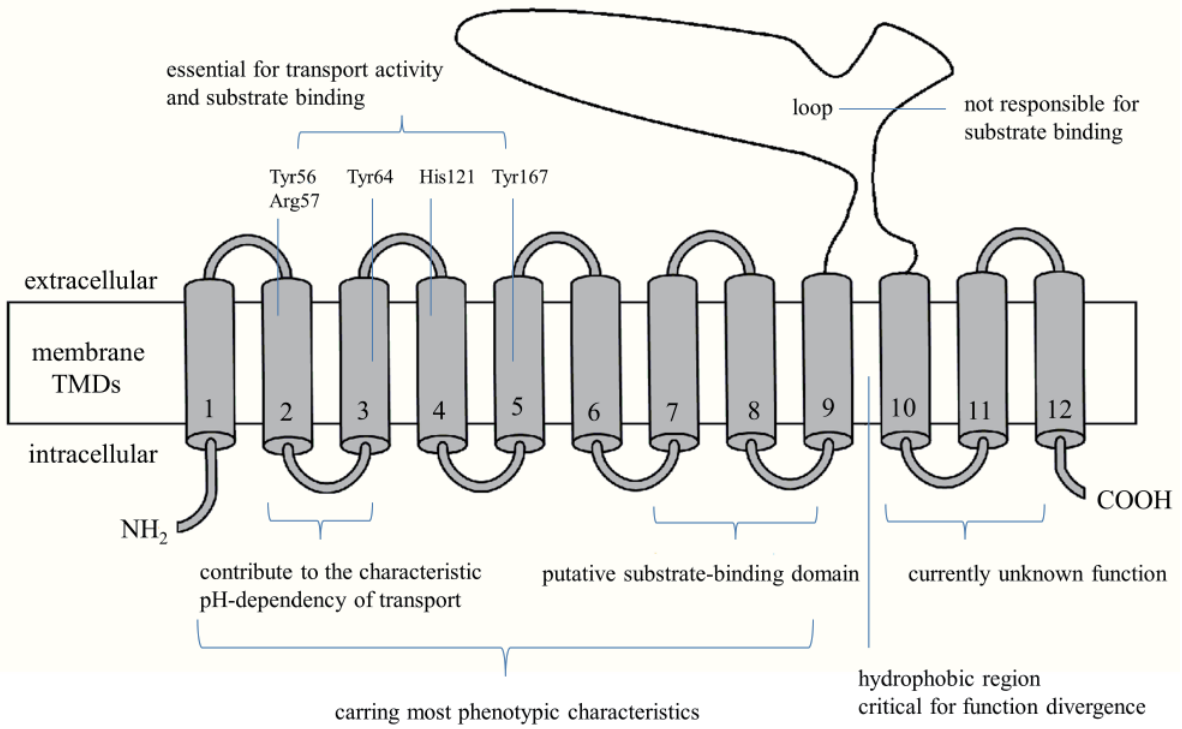

Figure 1. Functionally relevant domains of PEPT2 revealed by chimeric and mutant proteins.

TMDs 9 and 10 (*16*).

Functional studies on PEPT2 principally focused on the kidney and choroid plexus. In the kidney, PEPT2 is mainly found in the S3 segment of the tubule, but the renal PEPT2 is almost entirely responsible for the reabsorption of peptides and peptidomimetics (*10,11,16*). In the choroid plexus, PEPT2 is the only transporter responsible for the exposure of peptide and peptidomimetics in the cerebrospinal fluid (*17*). A few studies also detected PEPT2 in other organs. For example, PEPT2 might have an important role in the metabolism of the central nervous system (*18*), the delivery of peptides and peptidomimetics in human lung, and the reuptake of small peptides that accumulate from the hydrolysis of milk proteins in the mammary gland (*19*). Otherwise, PEPT2 alterations in various tissues could lead to the functional loss of transport activity. However, experiments demonstrated that PEPT2-null animals were healthy and fertile. In addition, neither clinical chemistry data obtained from plasma and urine samples nor general physiological measures indicated any significant metabolic perturbation (*4,20,21*). Therefore, the exact physiological role of PEPT2 warrants further studies.

Previous research determined the transport capability of PEPT2 through a systematic investigation of its structural influence on the uptake or transport of dipeptides and tripeptides, β-lactam antibiotics, and peptidase inhibitors in different organs (*4,22*). However, the function of PEPT2 remains unclear, and the structural features of its substrates are complex and incertain. In addition, a standard molecular structure model of the substrates has yet to be established. Accordingly, this review summarizes reports about the substrate structure of PEPT2 to understand the basic characteristics of PEPT2.

2. Substrate structure

As a high-affinity, low-capacity transporter, PEPT2 can transport almost all dipeptides, tripeptides and peptide-like drugs which differ in physicochemical characteristics, molecular mass, charge, and polarity. However, PEPT2 also displays specificity through its various substrate affinities depending on the particular substrate structure. This phenomenon is important when designing pharmacologically active compounds for delivery *via* PEPT2 because the substrate-binding sites provide freedom to accommodate molecules with unusual structures and pharmacological activities (*23*).

The molecular basis of the natural peptide and drug substrates of PEPT2 must be explored to understand the basic structural features of substrates (*24*). Rational and nonrational approaches have been applied to explain individual substrate specificities of PEPT2 and design compounds optimized for absorption by PEPT2. The essential structural elements of substrates will be discussed in detail below.

2.1. *α-Amino group*

A free α-amino may be essential for the substrate to be recognized by PEPT2. Many substrates have a free

α-amino group that shows high affinity to PEPT2. The α-amino group interacts with histidine residues of PEPT2 that may be involved in substrate recognition by peptide transporters (*25*). By contrast, reports also suggested that free α-amino may be unessential for recognition. For example, a study on the transport of quinapril, captopril, and enalapril showed that a free α-amino group is not an absolute requirement for substrate recognition by PEPT2 in the kidney (*26,27*). However, the substitution of this amino group by hydroxy or mercapto groups leads to loss of affinity. Thus, the presence of a free α-amino group is important but not mandatory for recognition by PEPT2.

2.2. *Peptide bond*

Peptide bond is an unessential group for recognition by PEPT2. The peptide bond of aminolevulinic acid (ALA) was replaced by a ketomethylene, which can serve as a substrate of PEPT2 (*27*). With a positively and a negatively charged head group separated by at least four methylene groups, omega-amino fatty acids can be transported by PEPT2 (*27*). Various compounds without peptide bond(s) also can be accepted as PEPT2 substrates. Such compounds include 4-aminophenylacetic acid, δ-amino levulinic acid, ω-amino fatty acid, aminoacid aryl amide, zidovudine, L-valyl ester of acyclovir, and valacyclovir (*28-31*), which strongly challenge the obligatory need for a peptide bond. However, only dipeptides and tripeptides with the trans-configuration of peptide bonds can be transported.

2.3. *N-terminal, C-terminal and carbonyl*

Groups at the ends of substrates may play important roles in recognition. The hydrophobicity of the N-terminal region of aminopenicillins increases affinity to PEPT2 (*27*). Anserine with an N-terminal β-amino acid displays a high affinity to PEPT2 (*32*). In addition, the hydroxyl group at the N-terminal phenyl ring of a few antibiotics may be involved in the interaction with PEPT2 (*25*). The terminal carboxylic group in substrates is not required for transport and can be replaced by an electrogenic group to form amino acid aryl amides (*10*). The presence of acidic amino acids in the amino terminus may result in greater reduction in affinity than the presence of the same amino acids in the C-terminus. However, the reverse effect has been observed for basic residues (*33*). Cephalosporins and penicillins with an N-terminal amino group and a hydroxyl group at the N-terminal phenyl ring promote high affinity. However, insufficient information is available regarding the influence of the C-terminal part (*34*).

The substrate affinity of the amino fatty acid substantially increases when an additional carbonyl group is incorporated into the backbone, as realized in delta-ALA (*27*). A comparison of the chemical structures of various substrates shows that the α- or β-amino carbonyl function is the common structure that exhibits a high affinity to PEPT2 (*35*). The affinity and transport currents of compounds can increase by more than 30-fold after introducing a single carbonyl group into the backbone (*22*).

These results demonstrate that a free amino-terminus, a correctly positioned backbone carbonyl group, and a carboxylic group positioned in a suitable distance from the intramolecular carbonyl function and the amino terminal head group are the major features for substrate recognition and transport by PEPT2. The higher the hydrophobicity of the N-terminal amino acid, the higher is the affinity to PEPT2.

2.4. *Side chain*

Side chains can considerably affect the recognition and affinity of substrates. In normal dipeptides and tripeptides, the substrate binding site in PEPT2 can accept various side chains of amino acids. However, side chains are accommodated in asymmetric binding pockets. The presence of a large aromatic hydrophobic group in the side chain of the N-terminal amino acid of dipeptides could evidently enhance the binding affinity of several derivatives to PEPT2 (*36*). The terminal carboxy group requires a distinct sterical location, and binding pockets that accommodate side chains show strong hydrophobicity-dependent stereoselectivity but are asymmetric (*22*). Dipeptides that contain hydrophobic side chains, particularly those of C-terminal residues, possess high affinities. These data suggest that the presence of hydrophobic side chains is an important factor that determines substrate affinity (*10, 33*).

2.5. *Stereoselectivity*

The probability of transport is decided by the 3D structure of the substrate. Statistics indicate that substrates are always transported in a stereoselective manner with a preference for L-α amino acids. However, the presence of D-amino acids results in the lack of uptake and transport of substrate. Peptides that solely contain D-amino acids do not bind with the substrate-binding domain as free amino acids, and peptides with four or more amino acids do not serve as substrates of PEPT2 (*5*). Dipeptides that contain proline are poor substrates because of the conformational difference to normal L-α-amino acids (*37*). As a typical L-amino acid alkyl ester, L-valine methyl ester could be recognized and transported by rat PEPT2 (rPEPT2) and shows high affinity (*29,38*). PEPT2 favors dipeptides with an amino acid in LL-configuration over those in DL-configuration. The stereoselectivity of the carrier protein is pronounced for dipeptides in LD-configuration. And DD-dipeptides

are unrecognized by PEPT2 (*39*). Similar to dipeptides, tripeptides with a D-configured N-terminal amino acid show lower affinities to PEPT2 than tripeptides in LLL-configuration. Tripeptides with LDL-configuration are low-affinity substrates of PEPT2, and DDD-configured tripeptides are unrecognized (*37,40*). These data show that L-α amino acids play important roles in the structure of substrates recognized by PEPT2.

2.6. *Basic structural characteristics of the substrates*

PEPT2 can transport many dipeptides, tripeptides, and drugs, but minimal differences in affinity exist among these compounds. Dipeptides with glycine and proline in the N-terminal position show lower affinities than other dipeptides. Tripeptides that contain hydrophobic amino acid residues show the highest affinity to PEPT2. The presence of charged or uncharged amino acids among other tripeptides also influences affinity to the H^+/peptide symporter PEPT2 (*37*).

Biegel *et al*. developed a comprehensive 3D quantitative structure activity relationship model based on 83 compounds (32 dipeptides and dipeptide derivatives, 27 tripeptides, and 24 β-lactam antibiotics) (*41*). The analyses reveal that a free N-terminal amino group, a high electron density around the carboxylic group in dipeptides or around the carbonyl group of the second amino acid in tripeptides, high electron densities at the first and third side chains, and the presence of hydrophobic side chains can significantly increase affinity to PEPT2.

PepT2 displays broad substrate selectivity and interacts with numerous drugs, including those without similar chemical structures such as peptides (*42*). Some drugs or prodrugs such as β-lactam antibiotic cephalexin, penicillins (*43*), 5-ALA (*44*), and valacyclovir (*29*) not only interact with PEPT2 but can also be transported.

A precise pharmacophore model is currently unavailable because of the lack of information regarding the 3D structure of the transporter proteins. However, preferred configurations and conformational features of PEPT2 substrates (Figure 2) should include the following:

1) A peptide backbone of two to three amino acid residues (*45*);

2) Dipeptides must be in zwitterionic forms, and the intramolecular distance between oppositely charged NH_2 and COOH head groups is > 500 and < 630 picometers (*46-48*);

3) A correctly positioned backbone carbonyl group (*28,41,48*);

4) A free amino group in α or β position;

5) Stereoselectivity with L-amino acids and trans-conformers being preferred (*37,39,40*);

6) Chiral centers at α-carbons and backbone torsion angles ψ, φ, and ω (*23,48*);

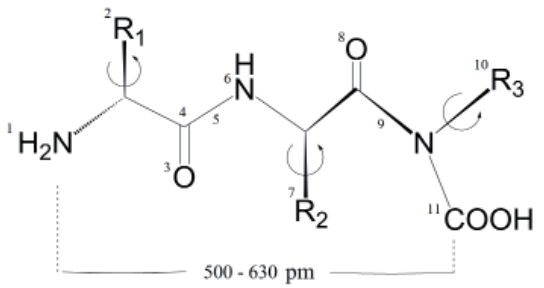

Figure 2. Molecular structures of selected pharmacologically compounds that may serve as substrates of PEPT2. 1. unessential, preferentially a free -NH_3^+; **2**. unessential, hydrophobic side chains can increase affinity but stereoselectivity; **3**. planar length-defined backbone from the N-terminal carbon to R2, with an incorporated backbone carbonyl function for hydrogen bonding; **4**. unessential, can be modified; **5**. unessential, only dipeptides and tripeptides with the trans-configuration can be transported; **6**. unessential, can be modified; **7**. unessential, hydrophobic side chains can increase affinity and stereoselectivity; **8**. unessential, more can increase affinity; **9**. non-required, can be modified; **10**. unessential, preferentially hydrophobic residues to increase affinity and stereoselectivity; **11**. unessential, can be replaced by stereoselective group.

7) Tripeptides with an uncharged amino acid residue in position 3.

8)Affinity of substrates could be improved by the presence of hydrophobic side chains or a C-terminal acid group.

In general, these data suggest that current models must be refined by trial and error until the 3D structure of transporter proteins is established. Furthermore, predictions of structure-affinity relationships and the substrate structure of transporter proteins on the basis of these models might promote the rational design of drugs and prodrugs targeting peptide transporters, and play major roles in treating various systemic diseases (*8*).

3. Regulation

Several reports focused on the function and substrate affinities of PEPT2. Meanwhile, minimal but important data are currently available on the regulation of PEPT2. For example, Mitsuoka *et al*. hypothesized that the growth of cancer cells could be suppressed by the inhibition of oligopeptide transporters under nutrient deficiency in vitro (*49*). Søndergaard and Bravo *et al*. reported that epidermal growth factor (EGF) has a strong inhibitory effect on rPEPT2 transport capacity (*50,51*). Otherwise, the mRNA and protein levels of PEPT2, amino acid homeostasis and drug pharmacokinetics could also be regulated by changes in thyroid function (*52,53*).

Takahashi *et al*. reported that 5/6 nephrectomized rats display unregulated mRNA and protein levels of PEPT2 at 2 weeks after surgery and downregulated mRNA level at 16 weeks after surgery. The up-regulation of PEPT2 expression promotes the reabsorption of small peptides and peptide-like drugs

across the brush-border membranes during chronic renal failure (*54*). Similarly, Tramonti *et al*. reported that a reduction in renal mass increases the expression of peptide transporters (influx) and P-glycoprotein (efflux) located at the brush border of renal tubular epithelial cells (*55*).

Sugiura *et al*. reported that the PDZ domain protein PDZK1 can affect the subcelluar localization and activity of PEPT2, thereby altering the membrane transport of various substrate compounds (*56*). Noshiro *et al*. also demonstrated that the capability of PDZK1 to couple PEPT2 to the Na^+/H^+ exchanger NHE3 may provide the necessary lumen-to-cell proton gradient (*57,58*). Boehmer *et al*. demonstrated that the serum and glucocorticoid-inducible kinase SGK1 and the Na^+/H^+ exchange regulating factor NHERF2 activated PEPT2 by stabilizing the transporter at the cell surface (*59*). Wenzel *et al*. reported that a reduction in cytosolic Ca^{2+} levels decreases the mRNA and protein levels of PEPT2, and kinase C changed the kinetic property of pig PEPT2 in a renal cell line (*60*).

And a few compounds also can inhibit the transport activity of PEPT2. These compounds include oral hypoglycemic agent nateglinide (*61*), the ACE inhibitor quinapril (*28*), cephalosporin (*62*), L-4,4′-biphenylalanine-L-proline (*36*), and amastatin (*63*). The shortage of information about regulation limits the development of drugs and prodrugs that target PEPT2. Therefore, the relevant factors that influence regulation warrant further research.

4. Summary and Perspective

Although the number of peptide transporters is fewer than that of amino acid transporters, numerous investigations have shown that PEPT2 plays an essential role in the absorption and reclamation of small peptides produced from the digestion of dietary proteins (*64*). The extensive substrate specificities of PEPT2 allow it to be exploited therapeutically for the delivery of peptides and peptidomimetic drugs in microbes and human (*23*). The design of many extant peptides and prodrugs is based on the substrates structure of the oligopeptide transporter to accelerate absorption *via* specific carrier proteins (*65*). Clinically relevant interactions between drugs and peptide transporter-mediated drugs are beginning to become an important aspect in therapy and toxicology (*66*).

Though PEPT2 appears to be a good target for the delivery of drugs because of the high affinity for peptides and drugs in tissues such as the kidney and lung, the structure of PEPT2 and related structure-function relationships are still unclear. Previous research demonstrated that PEPT2-null animals can still survive, therefore, the exact role of PEPT2 warrants further investigations.

In conclusion, PEPT2 not only plays important physiological and nutritional roles but also demonstrates pharmacokinetic and pharmacological significance. Further molecular clarification of the drug recognition mechanisms of PEPT2 will provide useful information for drug design and delivery systems to improve the efficiency of drug therapy.

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