Amino Acids (2006) 31: 137–156
DOI 10.1007/s00726-006-0331-0

Amino Acids
Printed in Austria

The renal type H^+/peptide symporter PEPT2: structure-affinity relationships

Review Article

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Received December 14, 2005
Accepted January 4, 2006
Published online August 2, 2006;

Summary. The H^+/peptide cotransporter PEPT2 is expressed in a variety of organs including kidney, lung, brain, mammary gland, and eye. PEPT2 substrates are di- and tripeptides as well as peptidomimetics, such as β-lactam antibiotics. Due to the presence of PEPT2 at the bronchial epithelium, the aerosolic administration of peptide-like drugs might play a major role in future treatment of various pulmonary and systemic diseases. Moreover, PEPT2 has a significant influence on the in vivo disposition and half-life time of peptide-like drugs within the body, particularly in kidney and brain. PEPT2 is known to have similar but not identical structural requirements for substrate recognition and transport compared to PEPT1, its intestinal counterpart. In this review we compiled available affinity constants of 352 compounds, measured at different mammalian tissues and expression systems and compare the data whenever possible with those of PEPT1.

Keywords: PEPT1 – PEPT2 – Peptide transport – Dipeptides – Tripeptides – β-Lactam antibiotics – Peptidomimetics – Drugs – Drug-delivery

Abbreviations: Abu, aminobutyric acid; Ac, acetyl; Adc, aminododecanoic acid; Ahp, aminoheptanoic acid; Ahx, aminohexanoic acid; Aib, aminoisobutyric acid; Aoc, Aminooctanoic acid; Apt, aminopentanoic acid; 5(*S*) Aobh, 5(*S*)-Amino-4-oxo-benzenehexanoic acid; 5(*S*), 4(*R*) Ahbh, 5(*S*)-Amino-4(*R*)-hydroxy-benzenehexanoic acid; 5(*R*), 4(*S*) Ahbh, 5(*R*)-Amino-4(*S*)-hydroxy-benzenehexanoic acid; 5(*S*), 4(*R*) Ahph, 5(*S*)-Amino-4(*R*)-hydroxy-6-phenyl-2-hexanoic acid; 5(*S*), 4(*S*) Ahph, 5(*S*)-Amino-4(*S*)-hydroxy-6-phenyl-2-hexanoic acid; Boc, tert. butyloxycarbonyl; Bz, benzoyl; Bz(NO_2), 4-nitrobenzoyl; BzBz, 4-benzoyl-benzoyl; Bzl, benzyl; Dab, 2,4-diaminobutyric acid; Hpr, hydroxyproline; Nle, norleucine; OBzl, benzyl ester; OMe, methyl ester; Orn, ornithine; Pac, 2-phenylacetyl; Pac(NO_2), 4-nitrophenyl-acetyl; Pbu, 4-phenylbutyryl; Pbu(NO_2), 4-nitro-4-phenylbutyryl; Phx, 6-phenylhexanoyl; Pip, pipecolic acid; Ppr, 3-phenyl-propionyl; Sar, sarcosine; Z, benzyloxycarbonyl; Z(NO_2), 4-nitrobenzyloxycarbonyl

Introduction

The H^+/peptide cotransporters PEPT1 and PEPT2 play a key rol\e in the maintenance of mammalian protein nutrition (Ganapathy et al., 1994; Daniel, 1996; Ganapathy and Leibach, 1996; Adibi, 1997; Daniel and Herget, 1997; Daniel and Rubio-Aliaga, 2003; Daniel and Kottra, 2004). Proteins are degraded by peptidases to small peptides and amino acids of different molecular size, charge and solubility. Transport of short chain peptides across biological membranes has been found in many organisms including man, bacteria, yeast, molds, and germinating seeds of higher plants. Since H^+/peptide symporters transport not only di- and tripeptides, but also peptidomimetic drugs across epithelial barriers, it is a matter of pharmaceutical relevance to understand structure-activity relationships of their substrates. Besides di- and tripeptides, the peptide carriers transport β-lactam antibiotics, valacyclovir, certain angiotensin-converting enzyme (ACE) inhibitors, the antineoplastic agent bestatin, and other peptidomimetics (Döring et al., 1998b; Ganapathy et al., 1998; Bretschneider et al., 1999; Terada et al., 2000; Zhu et al., 2000; Shu et al., 2001; Luckner and Brandsch, 2005). Amino acids and tetrapeptides are not recognized (Ganapathy et al., 1981; Daniel et al., 1992).

The first evidence for transport of intact peptides across the intestinal epithelium was observed around 1960

(Newey and Smyth, 1959, 1962). Benoit and Watten reported the renal reabsorption of dipeptides containing hydroxyproline, which were derived from the degradation of collagen in nephrons of the kidney (Benoit and Watten, 1968). In 1976, Nutzenadel and Scriver (1976) measured the L-carnosine (β-alanyl-L-histidine) uptake in rat kidney cortex. The active transport of dipeptides against a concentration gradient was first shown by Adibi and co-workers in 1977 using non-hydrolysable peptides (Adibi et al., 1977). It was generally assumed at the time, that the peptide transport is Na^+-dependent, however, Leibach's group was able to demonstrate in 1981 the co-transport of peptides with protons (Ganapathy et al., 1981). They also reported about the net transfer of a positive charge during the transport process and proposed the model of active H^+/peptide symport at the apical membrane of mammalian epithelial cells (Ganapathy et al., 1981; Ganapathy and Leibach, 1982, 1983). Inui's group was first to prove carrier-mediated transport of amino-cephalosporins at renal brush border membranes and that cephalexin is actively transported by a system responsible for dipeptide uptake (Inui et al., 1983, 1984). Silbernagl and co-workers demonstrated in 1987 in vitro and in vivo that two different transport systems, later named PEPT1 and PEPT2, are expressed in kidney epithelium (Silbernagl et al., 1987). In 1990, Tiruppathi and co-workers provided the first evidence that tripeptides are transported intact across renal brush-border membranes (Tiruppathi et al., 1990).

The intestinal peptide transporter PEPT1 was first cloned in 1994 by Fei and co-workers from rabbit (Fei et al., 1994). Liu and co-workers could first identify the cDNA of human PEPT2 (Liu et al., 1995). Both cDNAs were cloned by expression cloning in *Xenopus laevis* oocytes. PEPT1 and PEPT2 belong to the solute carrier (SLC) family of the proton-dependent oligopeptide transporters (SLC15A1 and SLC15A2) as do the peptide/histidine transporters PTR3 (SLC15A3) and PTR4 (SLC15A4) (for review see Herrera-Ruiz and Knipp, 2003; Daniel and Kottra, 2004).

PEPT1 is mainly responsible for the uptake of small peptides from diet, whereas the general function of PEPT2 is the removal of small peptides (e.g. hydrolysis products of the action of certain peptidases) from extracellular fluids. PEPT1 is expressed in the apical membrane of epithelial cells of intestine, kidney, pancreas, extrahepatic bile duct, and liver (Ogihara et al., 1996; Bockman et al., 1997; Thamotharan et al., 1997; Shen et al., 1999; Zhou et al., 2000; Knütter et al., 2002; Daniel and Kottra, 2004). PEPT2 is expressed in a variety of organs including kidney, lung, brain, mammary gland, pituitary gland, testis, prostate, ovary, uterus, and eye (Berger and Hediger, 1999; Dieck et al., 1999; Shen et al., 1999; Groneberg et al., 2001a, b, 2002; Ocheltree et al., 2003; Fujita et al., 2004; Lu and Klaassen, 2005). Recently, PEPT2 was also found in the mammalian enteric nervous system (Rühl et al., 2005). In the kidney, PEPT2 is responsible for the reabsorption of di- and tripeptides as well as peptidomimetic drugs from glomerular filtrate (Daniel and Rubio-Aliaga, 2003). Its function in brain is to remove hydrolysis products of neuropeptides from the cerebrospinal fluid. The carrier might contribute to glutathione metabolism by providing cysteinylglycine derived from extracellular glutathion for glial glutathione resynthesis in astrocytes. PEPT2 acts as an efflux transporter in choroid plexus and might be involved in the regulation of neuropeptide levels in the brain (Dringen et al., 1998; Berger and Hediger, 1999; Dieck et al., 1999; Groneberg et al., 2001a; Teuscher et al., 2001; Shu et al., 2002; Hu et al., 2005; Ocheltree et al., 2005; Shen et al., 2005). Its presence in lung might be the key for the development of novel therapeutic strategies to deliver drugs via aerosolic administration for the treatment of infectious and neoplastic diseases (Groneberg et al., 2001b, 2004). In PEPT2 knockout mice, functional deficiencies were shown by the almost abolished uptake of Gly-Sar into cells of choroid plexus and by impaired renal absorption of dipeptides. However, the Pept2(−/−) mice showed no obvious phenotypic abnormalities (Rubio-Aliaga et al., 2003; Shen et al., 2003).

PEPT1 and PEPT2 are tertiary active transport systems. The driving force of active peptide transport is provided by an inwardly directed H^+ gradient and an inside-negative membrane potential. The H^+ gradient is generated and maintained by the combined action of a Na^+/K^+ ATPase at the basolateral membrane and the Na^+/H^+ exchanger at the apical membrane. Reviews on the transport mechanism are presented in the following articles: Daniel (1996), Leibach and Ganapathy (1996), Meredith and Boyd (2000), Herrera-Ruiz and Knipp (2003), Daniel and Kottra (2004), and Terada and Inui (2004).

PEPT1 and PEPT2 show similarities concerning their substrate spectrum, but differ in structure, capacity, and affinity. PEPT2 is a high-affinity, low-capacity transporter, whereas PEPT1 works with low-affinity, high-capacity (Silbernagl et al., 1987; Ramamoorthy et al., 1995). It is generally assumed that PEPT2 displays a 10–15 times higher affinity to its substrates than PEPT1. hPEPT2 consists of 729 amino acid residues and hPEPT1 of 708. The primary structures of the proteins exhibit 50% identity and 70% similarity to each other. Both peptide transporters are

polytopic integral membrane proteins with 12 membrane-spanning domains with their N- and C-terminus facing the cytosol.

The substrate specificity of PEPT1 and PEPT2 has been the subject of intense studies, but focused so far mainly on PEPT1 (Meredith and Boyd, 1995, 2000; Adibi, 1997; Daniel and Herget, 1997; Inui and Terada, 1999; Nielsen et al., 2002; Brandsch et al., 2003, 2004; Daniel and Rubio-Aliaga, 2003; Daniel and Kottra, 2004; Terada and Inui, 2004).

In this review we compile and discuss available affinity and transport data measured at PEPT2 and the corresponding values for PEPT1. The data show that the peptide transporters PEPT1 and PEPT2 do not transport every possible di- and tripeptide, as often postulated. Moreover, the general assumption that substrate affinities of PEPT2 are 10 to 15 fold higher compared to PEPT1 is re-evaluated.

Determination of structure-activity relationships

A large number of studies using various tissue preparations and species have been performed to analyze peptide uptake and transport via PEPT2. In early studies, cortex slices of rats were used to study uptake of peptides into the kidney (Nutzenadel and Scriver, 1976). Later, Leibach's group employed the brush-border-membrane vesicle (BBMV) technique and demonstrated that in the apical membrane of tubular cells of cortex and medulla a specific electrogenic peptide transporter is present (Ganapathy and Leibach, 1982, 1983, 1986). Microperfusion experiments at rat renal tubule to study the H^+ gradient-driven dipeptide reabsorption were performed by Silbernagl and co-workers (Silbernagl et al., 1987). BBMV, which are prepared from rat or rabbit renal cortex, usually contain membranes from multiple types of cells from the nephron segment. Both peptide transporters, PEPT1 and PEPT2, are present in the kidney (Daniel et al., 1992; Daniel and Adibi, 1993). PEPT1 can be found in the distal part of the nephron, whereas PEPT2 is expressed in the proximal part of the tubule (Smith et al., 1998). PEPT2 is also expressed in several cell lines: SKPT-0193 Cl.2 cells, which constitutively express PEPT2, were obtained from SV40 transformation of rat proximal tubular cells (Brandsch et al., 1995). After cloning, the transport protein has been expressed heterologously for example in the mammalian cell lines HeLa and LLC-PK_1, in the yeast *Pichia pastoris*, and in *Xenopus laevis* oocytes (Ganapathy et al., 1995; Liu et al., 1995; Boll et al., 1996; Terada et al., 1997a; Döring et al., 1998a). With all these methods, affinity constants of PEPT2 substrates have been determined.

How do we define "high-affinity" PEPT2 substrates as opposed to "low-affinity" substrates? In this review, the classification of substrates/inhibitors suggested by Luckner and Brandsch (2005) is used. We consider (i) affinity constants lower than 0.1 mM as high affinity, (ii) constants between 0.1 and 1 mM as medium affinity and (iii) affinity constants above 1 mM as low affinity. Those compounds with inhibition constants above 5 mM should not be considered as PEPT2 substrates, although higher K_i values

Table 1. Classification of apparent affinity data of PEPT2 substrates and inhibitors determined at SKPT cells

Category, K_i range	Substrate/Inhibitor	K_i (SKPT)	Reference
High affinity <0.1 mM	Lys[Z(NO_2)]-Lys[Z(NO_2)]	10 ± 1 nM	Theis et al. (2002b)
	Trp-Gly-Tyr	1.7 ± 0.3 μM	this study[a]
	Cefadroxil	3.0 ± 1.0 μM	Luckner and Brandsch (2005)
	Ala-Asp	14 ± 1 μM	Brandsch et al. (1995)
	Fosinopril	29 ± 1 μM	Shu et al. (2001)
	Valganciclovir	46 ± 5 μM*	Sugawara et al. (2000)
Medium affinity 0.1–1 mM	Ala-12-Adc	0.13 ± 0.02 mM	this study
	Ala-D-Ala	0.27 ± 0.04 mM	this study
	δ-Aminolevulinic acid	0.23 ± 0.09 mM	Bravo et al. (2005)
	Ceftibuten	0.28 ± 0.01 mM	Luckner and Brandsch (2005)
	D-Leu-Gly-Gly	0.59 ± 0.02 mM	this study
Low affinity 1–5 mM	β-Ala-Ala	2.1 ± 0.2 mM	this study
	Pro-Glu	2.6 ± 0.3 mM	this study
	Cefamandole	2.8 ± 1.1 mM	Luckner and Brandsch (2005)
	Ala-D-Ala-Ala	4.2 ± 0.2 mM	this study

[a] Uptake of 10 μM [^{14}C]Gly-Sar was measured for 10 min in SKPT monolayer cultures at pH 6.0 in the absence or presence of increasing concentrations of substrates (0–31.6 mM or concentration of maximal solubility, respectively). Uptake of [^{14}C]Gly-Sar measured in the absence of the inhibitors was taken as 100%. Data are shown as means ± SE, n = 4. * IC_{50} value

Table 2. Affinity data and transport of dipeptides by PEPT1 and PEPT2

Dipeptide	K_i (mM)		K_i (mM)		K_i (mM)		K_i (mM)		Transport		Ratio [#]
	Caco-2 (PEPT1)	SKPT (PEPT2)	BBMV (PEPT1)	BBMV (PEPT2)	LLC-PK_1 (PEPT1)	LLC-PK_1 (PEPT2)	*P. pastoris* (PEPT1)	*P. pastoris* (PEPT2)	*X. laevis* (PEPT1)	*X. laevis* (PEPT2)	PEPT1/ PEPT2
Ala-Ala	0.10 [a]	0.006 [b]		0.062 [c]			0.16 [d]	0.10	+	+	17
Ala-Asp	0.26 [e,f]	0.014 [b]					0.21 [f]	0.20	+ [f]	+	19
Ala-D-Ala	4.2 [a,d]	0.27 [g]					6.1 [d]	2.3	+	+	16
Ala-D-Lys	>30 (~49) [a,d]						<30 (~43) [d]	16	−	−	
Ala-D-Pro	15 [a,d,f]	15 [g]					<30 [d]	44	−	+	1
Ala-Dab	1.4	0.13									11
Ala-Glu	0.32 [e]	0.025					0.20	0.20	+	+	13
Ala-Gly	0.14 [e]	0.007 [g]		0.027 [c]			0.22	0.070 [h]	+	+ [h]	20
Ala-His	0.33						0.16	0.070	+	+	
Ala-Leu				0.009 [c]							
Ala-Lys	0.21 [a,f]	0.012 [b]/0.023 [g]					0.22 [f]	0.060	+ [f]	+	9
Ala-Nle	0.09 [e]	0.003 [g]					0.17	0.090	+	+	30
Ala-Orn	0.97 [a,f]						0.36 [f]	0.16	+ [f]	+	
Ala-Pip	0.035 *,[i]						0.11	0.15	+	+	
Ala-Pro	0.10 *,[i]	0.012 *,[g]					0.10*	0.040*	+	+	8
Ala-Sar	0.14 *,[i]						0.20	0.040	+	+	
Ala-Ser	0.14 [f]	0.006 [g]					0.32 [f]	0.09	+ [f]	+	23
Ala-Tyr	0.15 [e,f]			0.029 [j,§]			0.22 [f]	0.07	+ [f]	+	
Arg-Ala	0.28 [i]						0.24	0.18	+	+	
Arg-Gly				0.037 [c]							
Arg-Pro	0.27 *,[i]	0.012 *,[g]					0.14*	0.11*	+	+	23
Asn-Gly				0.078 [c]							
Asp-Ala	0.32 [f]						0.35 [f]	0.37	+ [f]	+	
Asp-Asp	0.41 [e]				0.99 [k]	0.15 [k]	0.62	0.51	+	+	
Asp-Gly	0.56	0.019 [g]		0.23 [c]	0.83 [k]	0.046 [k]	0.26	0.040	+	+	30
Asp-Lys	0.86 [l]	0.020 [b]									43
Asp-Phe	0.18	0.011									16
Cys-Gly	0.20 [e]	0.029 [g]									7
D-Ala-Ala	2.1 [a,d]	0.13 [g]					0.78 [d]	0.24	+	+	16
D-Ala-Gly				1.9 [c]							
D-Ala-D-Ala	>30 (~100) [a,d,e]	>10 (~42) [g]		>15 [c]			>30 [d]	23	−	+	2
D-Ala-D-Lys	>30 (~72) [a,d,e]						>30 [d]	4.5	−	−	
D-Ala-D-Pro	>30 (~65) [a,d,e]						>30 [d]	>5	−		
D-Ala-Leu				0.041 [c]							
D-Ala-Lys	7.0 [a,d]						0.88 [d]	0.34	+	+	
D-Ala-Pro	5.0 [a,d]						1.5 [d]	0.69	+	−	
D-Leu-Tyr				0.032 [c]							
D-Phe-Ala	7.0 [e]	0.097 [g]					1.1	0.26	+	+	72
Dab-Ala	0.25 [f]	0.025							+ [f]		10
Glu-Ala	0.25 [i]	0.030					0.62	0.52	+	+	8

Glu-Gly			0.21[c]							
Glu-Lys	0.51[l]					0.31	0.07	+	+	
Gly-Ala	0.38[i]	0.035[g]	0.032[c]			0.24	0.11[h]	+	+[h]	11
Gly-Asn				0.27[k]	0.022[k]					
Gly-Asp	0.35[e]	0.08	0.15[c]	0.30[k]	0.060[k]/0.008[o]	0.61	0.34[$,m]	+[n]	+	4
Gly-Arg			0.14[c]							
Gly-D-Ala			12[c]							
Gly-D-Phe			0.68[c]							
Gly-D-Val			4.6[c]							
Gly-Gln	0.26				0.030[o]	0.17	0.070	+[n]	+	
Gly-Glu			0.092[c]							
Gly-Gly	1.0[e]	0.054[g]	0.091[c]	0.86[k]	0.042[k]	0.20	0.16[h]	+	+[h]	19
Gly-His	1.0[e]			1.4[k]	0.039[k]	0.20	0.040	+	+	
Gly-Leu	0.16[e]			0.11[k]	0.006[k]			+	+	
Gly-Lys	2.5		0.15[c]	2.2[k]	0.21[k]/0.15[o]	0.24	0.090[$,m]	+[n]	+	
Gly-Phe			0.019[c]	0.17[k]	0.008[k]					
Gly-Pro	0.16*[,i]	0.027*[,g]	0.38[c]			0.19*	0.18*	+	+	6
Gly-Sar	0.51*[,f,i]	0.037*[,b]	0.37[c]			0.21*	0.20*	+	+	14
Gly-Ser			0.086[c]							
Gly-Trp				1.4[k]	0.007[k]					
Gly-Tyr			0.077[j,$]	0.21[k]	0.014[k]					
Gly-Val			0.032[c]							
His-Ala	0.48[e]					0.21	0.060	+	+	
Ile-Tyr	0.12	0.008[g]								15
Leu-Ala	0.12[i]					0.20	0.090	+	+	
Leu-Arg	0.41[g]	0.009[g]								46
Leu-Leu			0.012[c]							
Leu-Pro	0.11*[,i]	0.010*[,g]				0.20*	0.080*	+	+	11
Leu-Tyr			0.008[c]							
Lys-Ala	0.34[f,i]	0.041[g]				0.21[f]	0.060	+[f]	+	8
Lys-Asp	0.33[l]					0.40	0.12	+	+	
Lys-Glu	1.3[l]					0.51	0.30	+	+	
Lys-Gly	0.62		0.067[c]	0.91[k]	0.021[k]	0.18	0.010	+	+	
Lys-Lys	6.7[a]	0.012[a,p]		8.0[k]	0.096[k]	0.47	0.004[p]	+[a,n]	+[a]	558
Lys-Pro	0.26*[,b,i]	0.034*[,g]				0.18*	0.080*	+	+	8
Met-Ala				0.15[k]	0.017[k]					
Met-Met	0.080[g]	0.003[g]								27
Nle-Ala	0.14[i]					0.17	0.12	+	+	
Nle-Pro	0.10*[,l]					0.19*	0.040*	+	+	
Orn-Ala	0.48[f]					0.32[f]	0.15	+[f]	+	
Phe-Ala	0.11[i]/0.18[q]	0.016[g]/0.013[q]				0.19	0.030	+	+	7
Phe-Asp	0.17	0.011								16
Phe-Glu	0.18	0.022								8
Phe-Gly	0.20[q]	0.014[q]		0.22[k]	0.007[k]					14
Phe-Phe	0.11[e]	0.037				0.080	0.060	+	+	3
Phe-Pro	0.13*[,i]					0.24*	0.10*	+	+	

(*continued*)

Table 2 (*continued*)

Dipeptide	K_i (mM)		K_i (mM)		K_i (mM)		K_i (mM)		Transport		Ratio [#]
	Caco-2 (PEPT1)	SKPT (PEPT2)	BBMV (PEPT1)	BBMV (PEPT2)	LLC-PK_1 (PEPT1)	LLC-PK_1 (PEPT2)	*P. pastoris* (PEPT1)	*P. pastoris* (PEPT2)	*X. laevis* (PEPT1)	*X. laevis* (PEPT2)	PEPT1/ PEPT2
Pro-Ala	9.5[i]	2.6[g]					4.3	0.46	+	+	4
Pro-Arg	2.5[i]	1.6[g]					2.0	0.63	−	+	2
Pro-Asp	9.8[i]	2.1[g]					6.2	4.1	−	−	5
Pro-Glu	20[i]	2.6[g]					8.4	4.2	−	−	8
Pro-Gly	22[i]			6.0[c]	13[k]	2.0[k]	6.4	3.5	−	+	
Pro-Hpr	8.5[i]						2.6	1.7	+	+	
Pro-Leu	0.47[i]						0.26	0.08	+	+	
Pro-Lys	3.2[i]						3.6	0.25	−	+	
Pro-Phe	1.9[i]						0.51	0.11	−	−	
Pro-Pro	0.61*,[i]						0.17*	0.09*	+	+	
Pro-Ser	14[i]						3.2	0.74	−	+	
Pro-Tyr	0.73[i]						0.25	0.23	−	+	
Sar-Gly					13[k]	2.2[k]					
Sar-Pro	1.6*,[i]						0.29*	0.16*	+	+	
Sar-Sar	31[e]/>30[i]			16[c]			>10 (~14)	3.1	−	+	
Ser-Ala	0.14[f,i]	0.007[g]					0.32[f]	0.10	+[f]	+	20
Ser-Pro	0.13*,[i]						0.11*	0.050*	+	+	
Trp-Ala	0.16[i]	0.004[g]					0.16	0.03	+	+	40
Trp-Pro	0.13*,[i]						0.070*	0.040*	+		
Trp-Trp	0.090[g]	0.0008[g]							+	+	113
Tyr-Ala	0.090[f,i]						0.22[f]	0.16	+[f]	+	
Tyr-Arg		0.018[b]/0.029[r]									
Tyr-Phe	0.14	0.009[g]									16
Tyr-Pro	0.16*,[i]						0.15*	0.13*	+	+	
Val-Ala	0.090[e]	0.004[g]									23
Val-Phe	0.050[e]	0.004[g]									13
Val-Pro	0.080	0.039[g]									2
Val-Tyr	0.10[e]	0.003[g]									33

Values without an assigning letter are unpublished data, for experimental conditions see Knütter et al. (2004) and Theis et al. (2002b). [#] Ratios are calculated from inhibition constants measured at Caco-2 and SKPT cells and calculated before rounding off the values; [a] Brandsch et al. (2003); [b] Brandsch et al. (1995); [c] Daniel et al. (1992); [d] Hartrodt et al. (2001); [e] Gebauer et al. (2003); [f] Knütter et al. (2004); [g] this study; [h] Theis et al. (2002a); [i] Brandsch et al. (1999); [j] Daniel et al. (1994); [k] Terada et al. (2000); [l] Biegel et al. (2005); [m] Döring et al. (1998a); [n] Amasheh et al. (1997); [o] Wenzel et al. (1998); [p] Theis et al. (2002b); [q] Vabeno et al. (2004); [r] Bravo et al. (2005); * by the *trans*-content corrected K_i value; [$] EC_{50} value

can be measured (Luckner and Brandsch, 2005). Examples for this classification are shown in Table 1.

Values compiled in Tables 2–8 were measured in very different PEPT2 expressing systems. Interestingly, for certain substrate subgroups, data vary from assay to assay. This is due to subtle differences between expression systems (Bill, 2001) and different experimental conditions employed in the different studies such as extracellular pH value and membrane potential.

Dipeptides

The interaction of dipeptides with PEPT2 has been subject of many publications. The first structure-activity studies were performed with BBMV (Ganapathy et al., 1981; Tiruppathi et al., 1987; Daniel et al., 1992). Later, the groups around Leibach, Brandsch and Luthmann used the SKPT expression system, whereas Daniel's group expressed PEPT2 routinely in the yeast *Pichia pastoris* (Döring et al., 1998a). The LLC-PK_1 expression system was used e.g. by Inui's group (Terada et al., 1997b).

The inhibitory constants of dipeptides studied at SKPT cells vary from 0.8 μM for Trp-Trp to >10 mM (~42 mM) for D-Ala-D-Ala (Table 2). Most of the natural dipeptides are high affinity substrates of PEPT2. The highest affinities have been found for dipeptides containing hydrophobic side chains (Daniel et al., 1992). Among the numerous dipeptides listed in Table 2, the hydrophobic dipeptides Trp-Trp, Val-Tyr, Met-Met, Trp-Ala, Val-Ala, and Ile-Tyr show the highest affinities to PEPT2 with K_i values in the range from 0.8 μM to 8 μM at SKPT cells.

At BBMV it has been shown that the transporter differentiates between charged and uncharged residues. This has been tested using Gly-Xaa and Xaa-Gly dipeptides, where Xaa is Glu, Asp, Arg or Lys (Table 2, Daniel et al., 1992). The presence of acidic amino acids in the amino terminus resulted in greater reduction in affinity than the presence of the same amino acids in the C-terminus. For basic residues, however, the reverse effect has been shown. The presence of basic amino groups in the C-terminus caused a greater reduction in affinity than in the N-terminal position. Using SKPT cells major differences in affinities between substrates with charged amino acid residues in the N-terminal compared to the C-terminal position did not emerge when the measurements where performed at an outside pH of 6.0. At this model, the affinity constants for Ala-Lys ($K_i = 12$ μM), Lys-Ala ($K_i = 41$ μM), Ala-Asp ($K_i = 14$ μM), Ala-Glu ($K_i = 25$ μM), Glu-Ala ($K_i = 30$ μM) were in the same range, but they were all higher than the K_i value of Ala-Ala ($K_i = 6$ μM) (Table 2). Other studies measured similar values (Amasheh et al., 1997; Terada et al., 2000).

Dipeptides with glycine and proline in N-terminal position show lower affinities than the other dipeptides. This becomes evident when comparing the inhibition constants of Ala-Ala ($K_i = 6$ μM), Gly-Ala ($K_i = 35$ μM) and Gly-Gly ($K_i = 54$ μM). In addition, the K_i value of Ala-Asp (14 μM) is increased to 80 μM by substitution of alanine with N-terminal glycine. Nonetheless, Gly-Xaa dipeptides are still high affinity substrates of PEPT2, whereas Pro-Xaa dipeptides, which contain no free N-terminal amino group, have low affinity to PEPT2, e.g. Pro-Ala ($K_i = 2.6$ mM), Pro-Asp ($K_i = 2.1$ mM), Pro-Glu ($K_i = 2.6$ mM).

The H^+/peptide symporter PEPT2 is stereoselective (Daniel et al., 1992; Theis et al., 2002a). PEPT2 favors dipeptides with an amino acid in LL-configuration over those in DL-configuration. In the case of Phe-Ala ($K_i = 16$ μM), the affinity constant is 6 times higher for D-Phe-Ala ($K_i = 97$ μM). The stereoselectivity of the carrier protein is even more pronounced for dipeptides in LD-configuration. Ala-D-Ala displays a 45 times lower affinity to PEPT2 than Ala-Ala. Ala-D-Pro ($K_i = 15$ mM) has even a 1,250 times lower affinity than Ala-Pro ($K_i = 10$ μM). DD-Dipeptides are not recognized by PEPT2, e.g. D-Ala-D-Ala, (K_i >10 mM ~42 mM). Daniel and co-workers concluded furthermore that PEPT2 prefers substrates with a *trans* peptide bond (Daniel et al., 1992).

Transport studies at *Xenopus laevis* oocytes expressing PEPT2 revealed that most dipeptides with the exception of some Pro-Xaa dipeptides and several dipeptides containing D-amino acids are transported (Table 2).

Tripeptides

There are only very few reports on the affinity of tripeptides to PEPT2. The first evidence for intact tripeptide transport via PEPT2 has been provided by Tiruppathi and co-workers (Tiruppathi et al., 1990). They determined a K_m value of Phe-Pro-Ala of 48 μM. Other groups showed, that further tripeptides interact with PEPT2 (Daniel et al., 1992; Brandsch et al., 1995; Terada et al., 2000). K_i values of the tripeptides studied at SKPT cells range from 0.3 μM (Trp-Trp-Trp) to 11 mM (Pro-Gly-Gly) as shown in Table 3. Interestingly, the largest tripeptide Trp-Trp-Trp is the natural substrate with the highest affinity to PEPT2 known so far ($K_i = 0.3$ μM). Transport studies, however, revealed that Trp-Trp-Trp did not elicit any currents in oocytes expressing PEPT2 (data not shown). Hence, it is not transported via PEPT2 but represents a natural high affinity inhibitor of PEPT2.

Table 3. Affinity data and transport of tripeptides by PEPT1 and PEPT2

Tripeptides	K_i (mM)		K_i (mM)		K_i (mM)		Ratio [#]
	Caco-2 (PEPT1)	SKPT (PEPT2)	BBMV (PEPT1)	BBMV (PEPT2)	LLC-PK_1 (PEPT1)	LLC-PK_1 (PEPT2)	PEPT1/ PEPT2
Ala-Ala-Ala	0.18 [a]	0.018 [b]		0.066 [c]			11
Ala-D-Ala-Ala	8.4	4.2 [d]					2
Ala-Ala-Asp	0.82 [d]	0.16 [d]					5
Ala-Ala-Glu	0.81 [d]	0.069 [d]					12
Ala-Asp-Ala	0.72 [d]	0.079 [d]					9
Ala-Glu-Ala	0.48 [d]	0.046 [d]					10
Ala-Pro-Gly		0.017 [b]					
Ala-Val-Leu	0.14 [d]	0.012 [d]					12
D-Ala-Ala-Ala	7.9 [a]	1.0 [d]					8
D-Ala-D-Ala-D-Ala	>31	>31					
D-Leu-Gly-Gly	25 [a]	0.59 [d]					42
D-Met-Met-Met	0.52 [a]	0.006 [d]					88
D-Tyr-Val-Gly	14 [a]	0.72 [d]					19
Glu-Phe-Tyr	0.20 [a]	0.052 [d]					4
Gly-Gly-Gly				0.22 [c]	1.3 [e]	0.16 [e]	
Gly-Gly-Sar				0.21 [c]			
Gly-Gly-Phe					0.61 [e]	0.029 [e]	
Gly-His-Lys	4.1 [a]	3.1 [d]					1
Gly-Leu-Tyr					0.19 [e]	0.008 [e]	
Gly-Sar-Sar				2.4 [c]			
Ile-Pro-Pro	0.28 [a]	0.027 [d]					10
Ile-Val-Tyr	0.20 [a]	0.014 [d]					14
Leu-Arg-Pro	0.30 [a]	0.001 [d]					300
Leu-Gly-Gly	0.39 [a]	0.018 [d]					22
Leu-Leu-Leu				0.008 [c]			
Leu-Thr-Leu	0.11 [a]	0.01 [d]					11
Met-Met-Met	0.10 [a]	0.002 [d]					50
Phe-Pro-Ala				0.048 *,[f]			
Pro-Gly-Gly	16 [a]	11 [d]					1
Pro-Phe-Lys	2.0 [a]	0.90 [d]					2
Ser-Pro-Ile	0.17 [a]	0.019 [d]					8
Thr-Lys-Tyr	1.1 [a]	0.039 [d]					28
Trp-Gly-Tyr	0.24 [a]	0.0017 [d]					141
Trp-Trp-Trp [$]	0.17 [a]	0.0003 [d]					567
Tyr-Pro-Ile	0.25 [a]	0.0054 [d]					46
Tyr-D-Ala-Gly	>10 (~14) [a]	>3 (~6.5) [d]					2
Val-Ala-Leu	0.14 [a]	0.009 [d]					16
Val-Pro-Pro	0.06	0.023 [d]					3
Tyr-Pro-Phe-NH_2		>3 (~4.6)					
Pro-Leu-Gly-NH_2	>31	>31 (~38)					

Values without an assigning letter are unpublished data, for experimental conditions see Knütter et al. (2004) and Theis et al. (2002b). [#] Ratios are calculated from inhibition constants measured at Caco-2 and SKPT cells; * K_m-value; [$] is not transported by PEPT1 and PEPT2; [a] Biegel et al. (2005); [b] Brandsch et al. (1995); [c] Daniel et al. (1992); [d] this study; [e] Terada et al. (2000); [f] Tiruppathi et al. (1990)

As already observed for dipeptides, those tripeptides consisting of hydrophobic amino acid residues show the highest affinity to PEPT2. Terada and coworkers reported that the tripeptide Gly-Gly-Gly displays a 5 times lower affinity than Gly-Gly-Phe and 20 times lower affinity than Gly-Leu-Tyr (Terada et al., 2000). The high affinity H^+/peptide symporter differentiates between tripeptides that consist of charged and uncharged amino acids. Tripeptides containing a charged amino acid in third position show lower affinities to PEPT2 than those with uncharged side chains. Glu and Asp in C-terminal position lead to 4 to 9 fold lower affinities than Ala, e.g. Ala-Ala-Ala ($K_i = 18\,\mu M$), Ala-Ala-Asp ($K_i = 160\,\mu M$) and Ala-Ala-Glu ($K_i = 69\,\mu M$). PEPT2 seems to dislike positive charges at the third amino acid side chain of tripeptides. Affinities of Gly-His-Lys ($K_i = 3.1\,mM$), and Pro-Phe-Lys ($K_i = 0.9\,mM$) are low. For our knowledge, affinity constants of only two tripeptides with N-terminal

proline have been measured; Pro-Gly-Gly ($K_i = 11$ mM) and Pro-Phe-Lys ($K_i = 0.9$ mM) (Table 3).

Comparable to dipeptides, tripeptides with a D-configured N-terminal amino acid show lower affinities to PEPT2 than tripeptides in LLL-configuration. However, the affinity constants range from 6 μM for D-Met-Met-Met to 1 mM for D-Ala-Ala-Ala. The inhibition constant of D-Met-Met-Met is unexpectedly low (L-Met-Met-Met: 2 μM, D-Met-Met-Met: 6 μM). Tripeptides with LDL-configuration are low affinity substrates of PEPT2 (Ala-D-Ala-Ala, Tyr-D-Ala-Gly). DDD-configured tripeptides are not recognized (D-Ala-D-Ala-D-Ala; K_i >31.6 mM).

The carboxylic groups of tripeptides seem to be an important recognition feature for PEPT2, since the affinities of the tripeptide-derivatives Pro-Leu-Gly-NH_2 and Tyr-Pro-Phe-NH_2 are low (Table 3).

It should be noted that in transport studies of tripeptides, accompanying measurements of their stability during uptake experiments are obligatory because most often dipeptides as possible hydrolyzation products are PEPT2 substrates themselves.

Minimal substrate recognition structure of PEPT2

Backbone modifications

Modifications at the peptide backbone gave insight into the minimal structural requirements of the peptide transporter PEPT2. Theis and co-workers reported that the main features of substrate recognition and transport by PEPT2 are: i) a free N-terminus, ii) a correctly positioned backbone carbonyl group, and iii) a carboxylic group that is in a suitable distance from the intramolecular carbonyl function and the amino terminal head group (Theis et al., 2002a). A free N-terminal α-amino group is essential for recognition by PEPT2. The substitution of this amino group by hydroxy or mercapto groups leads to loss of affinity (Table 2 and 4; Ala-Ala ($K_i = 6$ μM); lactyl-Ala ($K_i = 18$ mM), and Ala-Gly ($K_i = 7$ μM); thiolactyl-Gly ($K_i = 12$ mM)). Compounds lacking this amino group are not recognized by PEPT2, e.g. acetyl-Gly ($K_i = 19$ mM). Extending the backbone at the N-terminus with methylene groups, e.g. Gly-Ala (0.035 mM) vs. β-Ala-Ala (2.1 mM), 4-Abu-Ala (18 mM); 5-Apt-Ala (5.6 mM), 6-Ahx-Ala (1.2 mM), 7-Ahp-Ala (11 mM), and 8-Aoc-Ala (14 mM), leads to a reduction or loss of affinity. An extension at the C-terminal side, however, has less dramatic effects on affinity (Ala-Gly 7 μM, Ala-β-Ala 0.98 mM, Ala-4-Abu 0.43 mM, Ala-5-Apt 0.28 mM, Ala-6-Ahx 0.57 mM, Ala-12-Adc 0.13 mM).

The replacement of the carbonyl group of the peptide bond by a CH_2 group shows that the carbonyl oxygen is an important feature for recognition by PEPT2 (SKPT assay: N-β-aminoethyl-Gly >30 ~91 mM, 5-Apt 23 mM, 8-Aoc 16 mM). Theis and co-workers measured affinity constants at *Pichia pastoris* expressing PEPT2 and came to similar results, although the K_i values between SKPT and yeast cells vary. Actual transport across the membrane was measured in *Xenopus laevis* oocytes expressing PEPT2 (Table 4, Theis et al., 2002a). Vabeno and co-workers analyzed Phe-Gly dipeptidomimetics and measured high affinity to PEPT2 for 5-(*S*)-amino-4-oxo-benzenehexanoic acid ($K_i = 19$ μM), a ketomethylene isoster of Phe-Gly (Vabeno et al., 2004). The *trans*-hydroxyethylidene and hydroxyethylene isosters, however, showed low affinities to the carrier (Table 4). The isosteric replacement of the amide NH by CH_2 (δ-aminolevulinic acid) leads to medium affinity to PEPT2 (Table 4, Theis et al., 2002a).

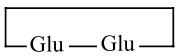
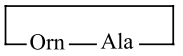
It has been shown that the carboxylic group can be substituted by a phosphonic group as observed for alafosfalin (Table 8, $K_i = 80$ μM, Neumann et al., 2004). Dipeptide derivatives containing a C-terminal amide group show low affinity to PEPT2, e.g. Gly-Gly-NH_2 $K_{i\ BBMV} = 4.7$ mM (Daniel et al., 1992).

Cyclo(Asp-Gly) and cyclo(Asp-Asp) showed weak inhibitory effect on reference substrate uptake into PEPT2 transfected LLC-PK_1 cells (Terada et al., 2000). Cyclo (Glu-Glu), and cyclo(Orn-Ala) did not interact with PEPT2 expressed in *Pichia pastoris* cells (Table 4).

Amino acid amides

Another group of compounds which proved to be useful for the determination of the minimal recognition structure of peptide transporters are amino acid amides. The high affinity of nitroanilide analogs of dipeptides has been described using BBMV and LLC-PK_1 cells (Table 5, Daniel and Adibi 1994; Terada et al., 2000). Theis and co-workers tested several amino acid amides with regard to their interaction with PEPT2 in *Pichia pastoris* (Table 5, Theis et al., 2002a). The compounds showed affinity to PEPT2 in the range from 8 μM to 2.9 mM. The basic structure in this study, Ala-anilide, is a PEPT2 substrate with a moderate affinity constant of 130 μM. By introducing a methoxycarbonyl, a chloro, or a nitro group in *para*-position of the phenyl ring, affinity was increased (e.g. Ala-4-nitroanilide $K_i = 8$ μM; in LLC-PK_1 cells 29 μM, Terada et al., 2000). Interestingly, among these anilides, only Ala-anilide and Ala-4-chloroanilide were transported

Table 4. Affinity data and transport of compounds with modified backbone structures by PEPT1 and PEPT2

Derivative	Structure	K_i (mM)		K_i (mM)		K_i (mM)		Transport		Ratio [#]
		Caco-2 (PEPT1)	SKPT (PEPT2)	LLC-PK_1 (PEPT1)	LLC-PK_1 (PEPT2)	*P. Pastoris* (PEPT1)	*P. Pastoris* (PEPT2)	*X. laevis* (PEPT1)	*X. laevis* (PEPT2)	PEPT1/ PEPT2
5-Apt	$H_2N–CH_2–CH_2–CH_2–CH_2–COOH$	25[a]	23			1.1[$, b]/2.3	7.3[c]	+[b]	+[c]	1
8-Aoc	$H_2N–(CH_2)_7–COOH$	7.9	16	4.4[d]	7.9[d]	0.30[$, b]		+[b]		0.5
δ-ALA	$H_2N–CH_2–CO–CH_2–CH_2–COOH$	0.79[a]	0.23[e]/0.39	2.2[d]	0.23[d]	0.50	0.22[c]	+	+[c]	2
N-β-Aminoethyl-Gly	$H_2N–CH_2–CH_2–NH–CH_2–COOH$	>30[a]	>30 (∼91)			>10	>10[c]	−	−[c]	
Ac-Gly	$H–CH_2–CO–NH–CH_2–COOH$	>30[a]	19							
Ala-ethylamide	$H_2N–CH(CH_3)–CO–NH–CH_2–CH_3$	>30 (∼37)[a]	>30 (∼37)							1
N-Ethylacetamid	$H–CH_2–CO–NH–CH_2–CH_3$	>30[a]	>30							
Lactyl-Ala	$HO–CH_2(CH_3)–CO–NH–CH_2(CH_3)–COOH$	>30	18							
Thiolactyl-Gly	$HS–CH_2(CH_3)–CO–NH–CH_2–COOH$	>30	12							
5(*S*) Aobh	$H_2N–CH(CH_2–Ph)–CO–(CH_2)_2–COOH$	0.40[f]	0.019[f]							21
5(*S*), 4(*R*) Ahbh	$H_2N–CH(CH_2–Ph)–CH(OH)–(CH)_2–COOH$		6.9[f]							
5(*R*), 4(*S*) Ahbh	$H_2N–CH(CH_2–Ph)–CH(OH)–(CH)_2–COOH$		7.5[f]							
5(*S*), 4(*R*) Ahph	$H_2N–CH(CH_2–Ph)–CH(OH)–(CH_2)_2–COOH$	40[f]	1.3[f]							29
5(*S*), 4(*S*) Ahph	$H_2N–CH(CH_2–Ph)–CH(OH)–(CH_2)_2–COOH$	26[f]	3.5[f]							7
Carbamoyl-β-Ala	$H_2N–(CH_2)_0–CO–NH–CH_2–CH_2–COOH$	>30	>30			>10	>10[c]	−	−[c]	
β-Ala-Ala	$H_2N–(CH_2)_2–CO–NH–CH_2(CH_3)–COOH$	4.8[g]	2.1[h]			1.1	0.93[c]	+	+[c]	2
4-Abu-Ala	$H_2N–(CH_2)_3–CO–NH–CH_2(CH_3)–COOH$	24	18			18	5.6[c]		−[c]	1
5-Apt-Ala	$H_2N–(CH_2)_4–CO–NH–CH_2(CH_3)–COOH$	7.7	5.6			7.6	3.6[c]	+	−[c]	1
6-Ahx-Ala	$H_2N–(CH_2)_5–CO–NH–CH_2(CH_3)–COOH$	7.9	1.2			4.3	6.5[c]	−	+[c]	7
7-Ahp-Ala	$H_2N–(CH_2)_6–CO–NH–CH_2(CH_3)–COOH$	>30 (∼50)	11			10	25[c]	−	+[c]	5
8-Aoc-Ala	$H_2N–(CH_2)_7–CO–NH–CH_2(CH_3)–COOH$	26	14			>50	47			2
Gly-β-Ala	$H_2N–CH_2–CO–NH–(CH_2)_2–COOH$	12				6.3	2.1	+	−	
Ala-β-Ala	$H_2N–CH_2(CH_3)–CO–NH–(CH_2)_2–COOH$	2.7[g]	0.98[h]			0.99	0.39[c]	+	+[c]	3
Ala-4-Abu	$H_2N–CH_2(CH_3)–CO–NH–(CH_2)_3–COOH$	0.39	0.43			0.50	0.12[c]	+	+[c]	1
Ala-5-Apt	$H_2N–CH_2(CH_3)–CO–NH–(CH_2)_4–COOH$	0.47	0.28			0.24	0.06[c]	+	+[c]	2
Gly-6-Ahx	$H_2N–CH_2–CO–NH–(CH_2)_5–COOH$	4.0	10							0.4
Ala-6-Ahx	$H_2N–CH_2(CH_3)–CO–NH–(CH_2)_5–COOH$	0.27	0.57			0.44	0.76[c]	−	+[c]	0.5
Ala-8-Aoc	$H_2N–CH_2(CH_3)–CO–NH–(CH_2)_7–COOH$	0.64				0.61	0.37[c]	+	+[c]	
Ala-12-Adc	$H_2N–CH_2(CH_3)–CO–NH–(CH_2)_{11}–COOH$	0.54	0.13			1.2	0.38			4
β-Ala-β-Ala	$H_2N–(CH_2)_2–CO–NH–(CH_2)_2–COOH$	>30 (∼77)[g]	>10			>10	>10	−	−	
6-Ahx-6-Ahx	$H_2N–(CH_2)_5–CO–NH–(CH_2)_5–COOH$	>10	>10 (∼13)			>10	4.4	−	+	
Ala-Aib	$H_2N–CH_2(CH_3)–CO–NH–CH(CH_3)_2–COOH$	1.1				0.32	0.18	+	+	
Ala-α-Aba	$H_2N–CH_2(CH_3)–CO–NH–CH_2(CH_2–CH_3)–COOH$	0.07[g]				0.11	0.05	+	+	
β-Asp-Ala	$H_2N–CH_2(COOH)–CH_2–CO–NH–CH_2(CH_3)–COOH$	>30				>20	24	−	−	
β-Asp-Gly	$H_2N–CH_2(COOH)–CH_2–CO–NH–CH_2–COOH$	>30 (∼98)		>50[d]	28[d]					
cyclo(Glu-Glu)		>10					>20			
cyclo(Orn-Ala)		2.3				9.0	>10 (∼20)	−	−	

Values without an assigning letter are unpublished data, for experimental conditions see Knütter et al. (2004) and Theis et al. (2002b). [#] Ratios are calculated from inhibition constants measured at Caco-2 and SKPT cells; [a] Brandsch et al. (2004); [b] Döring et al. (1998c); [c] Theis et al. (2002a); [d] Terada et al. (2000); [e] Bravo et al. (2005); [f] Vabeno et al. (2004); [g] Gebauer et al. (2003); [h] this study; [$] EC_{50} value

Table 5. Affinity data and transport of amino acid derivatives by PEPT1 and PEPT2. Affinities are presented as K_i values, unless otherwise indicated

Amino acid derivative	K_i (mM)		K_i (mM)		K_i (mM)		Transport		Ratio[#]
	Caco-2 (PEPT1)	SKPT (PEPT2)	BBMV (PEPT1)	BBMV (PEPT2)	*P. pastoris* (PEPT1)	*P. pastoris* (PEPT2)	*X. laevis* (PEPT1)	*X. laevis* (PEPT2)	PEPT1/ PEPT2
Ala-anilide	2.9[a]				0.08	0.13[b]	+[a]	+[b]	
Ala-benzylamide	14[a]				0.34	1.96	+	−	
Ala-cyclohexylamide	1.5				0.14	0.32	+	+	
Ala-2-aminobenzoic acid	2.8[c]				0.44	0.54	+	−	
Ala-3-aminobenzoic acid	0.31[c]				0.57	0.056[b]	+	+[b]	
Ala-4-aminobenzoic acid	9.1[c]				5.3	2.9[b]		−[b]	
Ala-2-aminobenzoic acid methyl ester	0.89[c]				0.15	0.19	+	+	
Ala-3-aminobenzoic acid methyl ester	6.9[c]				0.62	0.19[b]	+	−[b]	
Ala-4-aminobenzoic acid methyl ester	1.1[c]				0.17	0.03[b]	−	−[b]	
Ala-2-chloroanilide	7.8[c]				1.2	0.88	−	−	
Ala-3-chloroanilide	0.46[c]				0.070	0.53	+	+	
Ala-4-chloroanilide	0.33[a]				0.060	0.02[b]	+	+[b]	
Ala-2,3,4,5,6-penta-fluoroanilide	3.1				1.6	0.27	+	−	
Ala-2-methylanilide	14				0.32	0.16	+	+	
Ala-3-methylanilide	0.41[c]				0.04	0.019	+	+	
Ala-4-methylanilide	0.34[a]				0.024	0.015	+[a]	+	
Ala-2-nitroanilide	5.0				2.2	1.9	−	−	
Ala-3-nitroanilide	0.50[c]				0.092	0.062	+	+	
Ala-4-nitroanilide	0.08[a]				0.021	0.008[b]	+[a]	−[b]	
Ala-3,5-dinitroanilide	0.39				0.64	0.24	+	+	
Ala-2-phenylanilide	17[c]				7	0.11	−	−	
Ala-3-phenylanilide	0.29				0.10	0.19	+	+	
Ala-4-phenylanilide	0.030[a]				0.028	0.14[b]	+	−[b]	
Gly-anilide	>30 (∼103)				0.45	0.21	−	+	
Gly-4-nitroanilide					0.050	0.022	+	−	
Arg-4-nitroanilide				0.056[d,$]					
Glu-4-nitroanilide				0.767[d,$]					
Leu-4-nitroanilide				0.046[d,$]					
Glu-thiazolidide					1.2[e,*]	4.4[e,*]	+[e]	−[e]	
Ile-thiazolidide	6.3[f]				0.51[e,*]	0.53[e,*]	+[e]	−[e]	
Leu-thiazolidide					0.34[e,*]	0.18[e,*]	+[e]	−[e]	
Val-thiazolidide					0.10[e,*]	0.06[e,*]	+[e]	−[e]	
Lys[Z(NO_2)]-thiazolidide	0.44	0.015							29
Lys[Z(NO_2)]-pyrrolidide	16	0.23							70
(Z)Lys					1.5	0.12	−	−	
Val-OMe	8.0	2.0							4
Lys[Z(NO_2)]-OMe	17					0.17	−	−	

Values without an assigning letter are unpublished data, for experimental conditions see Knütter et al. (2004) and Theis et al. (2002b). [#] Ratios are calculated from inhibition constants measured at Caco-2 and SKPT cells. [*] IC_{50} values; [$] EC_{50} value; [a] Börner et al. (1998); [b] Theis et al. (2002a); [c] Gebauer et al. (2003); [d] Daniel and Adibi (1994); [e] Foltz et al. (2004); [f] Brandsch et al. (1999)

across the membrane as determined at *Xenopus laevis* oocytes. In contrast to PEPT1, Ala-4-nitroanilide (and also Gly-4-nitroanilide) was shown to be not transported by PEPT2 but to represent a high affinity inhibitor (Börner et al., 1998; Theis et al., 2002a). Ala-4-aminobenzoic acid, which contains a carboxylic group in *para*-position, shows low affinity ($K_i = 2.9$ mM), while the isomeric Ala-3-aminobenzoic acid has a 52 fold higher affinity ($K_i = 56\,\mu M$). Furthermore, transport of the dipeptide mimetic Ala-3-aminobenzoic acid was measurable in oocytes. The isomer with the carboxylic group in *ortho*-position (Ala-2-aminobenzoic acid) represents a medium affinity inhibitor ($K_i = 540\,\mu M$).

In Table 5 IC_{50} values of several amino acid thiazolidides, which were measured at *Pichia pastoris* are presented (Foltz et al., 2004). The data of these dipeptidylpeptidase

Table 6. Affinity data and transport of dipeptide derivatives by PEPT1 and PEPT2

Dipeptide derivative	K_i (mM)		K_i (mM)		Transport		Ratio[#]
	Caco-2 (PEPT1)	SKPT (PEPT2)	*P. pastoris* (PEPT1)	*P. pastoris* (PEPT2)	*X. laevis* (PEPT1)	*X. laevis* (PEPT2)	PEPT1/ PEPT2
Ac-Ala-Ala	>30 (~90)[a]		>50	44			
Ac-Lys-Ala	6.4		7	10			
Z-Ala-Ala	5.6		8.0	8.7	−	+	
Z-Ala-Lys	5.3		3.1	0.46		−	
Z-Lys-Ala	2.3		2.8	1.5	−	−	
Boc-Ala-Ala	>30 (~62)[a]		>50	43			
Boc-Ala-Lys	>10 (~28)		9.7	4.0			
Boc-Lys-Ala	>30 (~73)		>50	>30 (~60)			
Ala-Asp(OBzl)	0.11[b,c,d]		0.17[c]/0.18[d]	0.045	+[c]	+	
D-Ala-D-Asp(OBzl]	11[b]/7.8[d]		1.3[d]	0.69	−	+	
Asp(OBzl)-Ala	0.10[c]		0.07[c]	0.044	+[c]	+	
D-Asp(OBzl)-Ala	2.9		0.42	0.13	+	+	
Asp(Ala-D-Ala-)-Ala	2.7[c]		4.2[c]	0.09	+[c]	−	
Asp(Ala-D-Ala-OBzl)-Ala	0.65[c]		0.18[c]	0.002	−[c]	−	
Asp-(Ala-OH)-OBzl	8.3[a]		3.7	0.24	+	+	
Ala-Ser(Bzl)	0.11[c]		0.15[c]	0.038	+[c]	+	
Ser(Bzl)-Ala	0.10[c]		0.15[c]	0.040	+[c]	+	
Ala-Tyr(Bzl)	0.13[c]		0.08[c]	0.12		−	
Tyr(Bzl)-Ala	0.01[c]		0.10[c]	0.004	+[c]	−	
D-Tyr(Bzl)-Ala	1.4[a]		1.6	0.21	−	−	
Ala-Lys(Ac)	0.21[b,d]		0.21[d]	0.035	+	+	
Ala-Lys(Z)	0.18[b,c,d]	0.002[e]	0.19[c,d]	0.009[e]	+[c]	+[e]	106
Ala-D-Lys(Z)	2.5[b,d]		1.7[d]	2.1	+	+	
D-Ala-Lys(Z)	2.3[b,d]		0.5[d]	0.16	+	+	
D-Ala-D-Lys(Z)	5.5[b,d]		3.3[d]	3.4	−	−	
D-Ala-D-Lys(Ac)	>30[b,d]		24[d]	15	+	−	
Ala-Orn(Z)	0.52[b,c]		0.10[c]	0.019	+[c]	+	
Ala-D-Orn(Z)	6.6[b]		0.78	0.70	−	+	
D-Ala-Orn(Z)	1.9[b]		0.27	0.087	+	+	
D-Ala-D-Orn(Z)	5.5[b]		1.6	2.4	−	+	
Orn(Z)-Ala	0.17[c]	0.002[e]	0.10[c]	0.026[e]	+[c]	+[e]	89
Orn[Z(NO_2)]-Ala	0.02[c]	0.002[e]	0.05[c]	0.002[e]	−[c]	−[e]	13
Dab(Z)-Ala	0.08[c]	0.004[e]	0.18[c]	0.05[e]	+[c]	+[e]	20
Dab[Z(NO_2)]-Ala	0.05[c]	0.004[e]	0.07[c]	0.027[e]	−[c]	−[e]	13
Lys(Ac)-Ala	0.26[c]	0.016[e]	0.26[c]	0.064[e]	+[c]	+[e]	16
Lys(Boc)-Ala	0.56[c]	0.003[e]	0.25[c]	0.026[e]	−[c]	−[e]	175
Lys(Bz)-Ala	0.26[c]	0.006	0.02[c]	0.0008	−[c]	−	43
Lys(BzBz)-Ala	0.033	0.002	0.098	0.0003	−	−	22
Lys(Pac)-Ala	0.41[c]	0.003	0.034[c]	0.003	−[c]	−	120
Lys(Ppr)-Ala	0.65[c]	0.004	0.13[c]	0.006	−[c]	−	162
Lys(Pbu)-Ala	0.20[c]	0.0032	0.07[c]	0.0013	−[c]	−	63
Lys(Phx)-Ala	0.28[c]	0.002	0.049[c]	0.0002	−[c]	−	140
Lys(Z)-Ala	0.11[b,c,d]	0.001[e]	0.090[c,d]	0.023[e]	−[c]	−[e]	110
Lys(biotinyl)-Ala	1.1		0.54	0.007	+	−	
Lys[Bz(NO_2)]Ala	0.21[c]	0.01	0.010[c]	0.004	−[c]	−	22
Lys[Pac(NO_2)]-Ala	0.21[c]	0.003	0.015[c]	0.0008	−[c]	−	75
Lys[Pbu(NO_2)]-Ala	0.10[c]	0.001	0.008[c]	0.0002	−[c]	−	100
Lys[Z(NO_2)]-Ala	0.042[b,c]	0.0007[b,e]	0.034[c]	0.0007[e]	−[b,c]	−[b,e]	60
Lys(Z)-Lys	0.94[b]	0.002[b,e]	0.11	0.0008[e]	−[b]	−[b,e]	470
Lys(Z)-Lys(Z)	0.063[b]	2.3 nM[b]	0.004	0.0002	−[b]	−[b]	27400
Lys(Z)-Lys[Z(NO_2)]	n.s.[b]	10 nM[b,e]	0.039	40 nM[e]	−[b]	−[b,e]	
Lys[Z(NO_2)]-Lys	0.280[b,c]	0.002[b,e]	0.05[c]	0.001[e]	−[b,c]	−[b,e]	140
Lys[Z(NO_2)]-Lys[Z(NO_2)]	0.013[b]	10 nM[b,e]		10 nM[e]	−[b]	−[b,e]	2280
Lys(Z)-Asp	0.61		0.28	0.024	−	+	
Lys(Z)-Glu	0.40		0.066	0.015	−	+	
Lys(Z)-Glu(OBzl)			0.033	0.002	−	+	

(*continued*)

Table 6 (*continued*)

Dipeptide derivative	K_i (mM)		K_i (mM)		Transport		Ratio [#]
	Caco-2 (PEPT1)	SKPT (PEPT2)	*P. pastoris* (PEPT1)	*P. pastoris* (PEPT2)	*X. laevis* (PEPT1)	*X. laevis* (PEPT2)	PEPT1/PEPT2
Lys(Z)-Pro	0.033 [b]	0.0007 [b]	0.079	0.002	− [b]	− [b]	47
Lys(Z)-Sar	0.26		0.20	0.028	−	−	
Lys[Z(NO_2)]-Pro	0.007 *[,b,c]	0.0005 *[,b,e]	0.003 *[,c]	0.002 [e]	− [b,c]	− [b,e]	14
Lys[Z(NO_2)]-Sar	0.083 [b,c]	0.0009 [b,e]	0.04 [c]	0.004 [e]	− [b,c]	− [b,e]	92
Lys[Z(NO_2)]-Val	0.002 [b,c]	0.0001 [b,e]	0.002 [c]	0.002 [e]	− [b,c]	− [b,e]	20
Ala-Ala-Lys[Z(NO_2)]	0.13	0.062			+	+	2
Ala-Lys[Z(NO_2)]	0.09 [c]	0.004 [e]	0.17 [c]	0.01 [e]	+ [c]	+ [e]	23
Val-Lys[Z(NO_2)]	0.07 [c]	0.0005 [e]	0.03 [c]	0.013 [e]	+ [c]	+ [e]	140

Values without an assigning letter are unpublished data, for experimental conditions see Knütter et al. (2004) and Theis et al. (2002b). [#] Ratios are calculated from inhibition constants measured at Caco-2 and SKPT cells. [a] Gebauer et al. (2003); [b] Brandsch et al. (2003); [c] Knütter et al. (2004); [d] Hartrodt et al. (2001); [e] Theis et al. (2002b); * by the *trans*-content corrected K_i value

IV inhibitors demonstrate that the higher the hydrophobicity of the N-terminal amino acid, the higher is the affinity to PEPT2.

Dipeptide derivatives with blocked functional groups

Extensive studies with modified side chains of dipeptides have been performed to examine systematically the effect of side chain modifications at dipeptides on their transport via PEPT2 (Theis et al., 2002b). Dipeptide derivatives differing in the structure of the side chain protecting groups such as Lys(Z)-Ala, Lys(Boc)-Ala and Lys(Ac)-Ala were studied. These compounds possess high affinity to PEPT2. Whereas Lys(Z)-Ala and Lys(Boc)-Ala evoked no inward currents in oocytes, Lys(Ac)-Ala is a substrate of PEPT2. Theis and co-workers reported that lysine containing dipeptide derivatives can be converted from transported substrates of PEPT2 into effective, very high affinity inhibitors (Theis et al., 2002b). Dipeptide derivatives containing Lys[Z(NO_2)] in N-terminal position combined with C-terminal Val, Pro, Ala, or Sar show K_i values at PEPT2 in the range of 0.1 to 0.9 μM (SKPT, Table 6), but transport could not be detected. Introducing Lys[Z(NO_2)] at the C-terminus as in Ala-Lys[Z(NO_2)] or Val-Lys[Z(NO_2)] resulted in peptides with high affinity to PEPT2 and proven membrane translocation (Theis et al., 2002b; Brandsch et al., 2003). Thus, for the inhibition of PEPT2 mediated transport the protected lysine residue has to be in the N-terminal position, which became also evident by obtaining transport currents for Ala-Lys(Z). Furthermore, Orn(Z)-Ala and Dab(Z)-Ala, which are only one and two CH_2 units shorter, respectively, are high affinity substrates of PEPT2. Introducing a NO_2-group into the side chain blocking group, leads to high affinity inhibitors of PEPT2 (Table 6, Lys[Z(NO_2)]-Ala, Orn[Z(NO_2)]-Ala, and Dab[Z(NO_2)]-Ala).

Again, the more hydrophobic a compound, the higher is the affinity to PEPT2. The K_i values of unprotected dipeptides, e.g. Ala-Asp ($K_{i\ P.\ pastoris} = 0.20$ mM; Table 2) compared to those of the corresponding, more hydrophobic protected derivatives such as Ala-Asp(OBzl) ($K_{i\ P.\ pastoris} = 0.045$ mM; Table 6) confirm this phenomenon. Theis and co-workers systematically increased the hydrophobicity of the dipeptide Lys-Lys by introducing Z or Z(NO_2) to the N- or/and C-terminal side chains. Lys[Z(NO_2)]-Lys[Z(NO_2)] shows with $K_i = 10$ nM the highest binding affinity to PEPT2 known at *P. pastoris* so far. The inhibitory mechanism of Lys[Z(NO_2)]-Lys[Z(NO_2)] was reported to be competitive. It was shown that the compound is a nontransported inhibitor of PEPT2 (Theis et al., 2002b).

β-Lactam antibiotics

β-Lactam antibiotics administered orally or intravenously are almost completely excreted into the renal primary filtrate. Since PEPT2 in the kidney plays a key role in the reabsorption of these drugs to the blood, interaction of β-lactam antibiotics with PEPT2 has been studied intensively (Daniel and Adibi, 1993; Ganapathy et al., 1995, 1997; Terada et al., 1997a; Takahashi et al., 1998; Luckner and Brandsch, 2005). The first reports about uptake of β-lactam antibiotics into renal BBMV were presented in the 1980s (Inui et al., 1984; Kramer et al., 1988). The structure of β-lactam antibiotics resembles the backbone of tripeptides with a C-terminal peptide bond incorporated into a four-membered β-lactam ring.

Penicillins contain a penam ring system, whereas cephalosporins consist of a cephem ring. Structural variations of different drugs are mostly done at their side chains. Daniel and Adibi (1993) determined affinity data of 17 β-lactam antibiotics for PEPT2 using renal BBMV. The K_i values ranged from 60–250 μM for aminocephalosporins and 0.78–3.0 mM for the aminopenicillins (Table 7; Daniel and Rubio-Aliaga, 2003). The authors showed that those

Table 7. Affinity data and transport of β-lactam antibiotics by PEPT1 and PEPT2. Affinities are presented as K_i values, unless otherwise indicated

β-Lactam antibiotics	K_i (mM)		K_i (mM)		K_i (mM)		Transport		Ratio [#]
	Caco-2 (PEPT1)	SKPT (PEPT2)	BBMV (PEPT1)	BBMV (PEPT2)	LLC-PK_1 (PEPT1)	LLC-PK_1 (PEPT2)	*X. laevis* (PEPT1)	*X. laevis* (PEPT2)	PEPT1/ PEPT2
Acidocillin	15[a]	0.72[b]							21
Amoxicillin	>10 (~25)[c]	0.43[c]		0.78[d]	13[e]	0.18[e]			60
Ampicillin	15[f]	1.3[c,g,*]		3.0[d]	48[e]	0.67[e]			11
Benzylpenicillin	>30 (~40)[f]	>10[g,*]/11[c]		>10[d]					4
Carbenicillin				>10[d]					
Cefaclor	>10 (~11)[f]	0.03[c]		0.06[d]					380
Cefadroxil	7.2[f]/1.5[h]/5.4[g,*]	0.003[c,g]		0.06[d]	2.2[e]	0.003[e]/0.015[i]	+[h]		2400
Cefamandole	8.1[f]	2.8[c]							3
Cefapirin	>10 (~20)[f]	>10 (~11)[c]		>10[d]					2
Cefazoline	>30 (~31)[a]	>30 (~31)[b]							1
Cefepime	>30 (~70)[f]	11[c]							6
Cefixime	12[f]/1.3[h]/~10[j,*]	2.6[c]/3.2[j,*]			6.9[e]	12[e]	+[h]		5
Cefdinir	>10[j,*]	~10[j,*]			12[e]	20[e]			
Cefmetazole	28[f]	4.3[c]							7
Cefodizime	22[f]	9[c]							2
Cefotaxime	>30 (~50)[f]	20[c]							3
Cefoxitin	10[a]	8.6[b]							1
Cefpirome	>30 (~45)[f]	10[c]							5
Cefpodoxime	>30 (~110)[b]	>30 (~31)[b]							4
Cefroxadine				0.21[d]					
Cefsulodin	>30 (~150)[f]	>30 (~55)[c]							3
Ceftazidime	>10 (~40)[f]	>10 (~18)[c]							2
Ceftibuten	0.34[f]/0.87[j]	0.28[c]/0.38[j]			0.60[e]	1.3[e]	+[j]		1
Ceftriaxone	>30 (~40)[f]	>20 (~28)[c]							1
Cefuroxime	26[f]	13[c]							2
Cefuroxime-Axetil	>5 (~12)[f]	>3 (~9)[c]							1
Cephalexin	14[f]	0.08[c]/0.05[k]/ 0.04[l]/0.07[g]		0.06[d]	4.5[e]	0.05[e]			190
Cephaloglycin				1.6[d]					
Cephaloridine	>30 (~100)[f]	8.5[c]		>10[d]					12
Cephalothin	>10 (~14)[f]	8.3[c]/7.5[g,*]		>10[d]					2
Cephamycin C				>10[d]					
Cephradine	9.8[f]	0.07[c]		0.12[d]	8.5[e]	0.05[e]			150
Cloxacillin	3.0[c]	0.95[c]							3
Cyclacillin	0.6[g,*]/0.5[f]	0.04[g,j]/0.04[c]			0.17[e]	0.03[e]			11
Dicloxacillin	7.2[c]	0.42[c]							17
Epicillin				2.1[d]					
Flucloxacillin	7.0[b]	1.6[b]							4
Loracarbef				0.08[d]					
Metampicillin	13[c]	0.73[c]		2.6[d]					17
Moxalactam	12[c]	0.09[c]							140
Oxacillin	12[c]	3.3[c]							4
7-Aminocephalosporanic acid	>10 (~15)[c]	4.9[c]							3
6-Aminopenicillanic acid	>30 (~50)[c]	19[c]							2

Values without an assigning letter are unpublished data, for experimental conditions see Knütter et al. (2004) and Theis et al. (2002b). [#] Ratios are calculated from inhibition constants measured at Caco-2 and SKPT cells. * IC_{50} values; [a] Biegel et al. (2005); [b] this study; [c] Luckner and Brandsch (2005); [d] Daniel and Adibi (1993); [e] Terada et al. (1997b); [f] Bretschneider et al. (1999); [g] Ganapathy et al. (1995); [h] Wenzel et al. (1996); [i] Wenzel et al. (1998); [j] Ganapathy et al. (1997); [k] Bravo et al. (2005); [l] Vabeno et al. (2004)

Table 8. Affinity data of other drugs to PEPT1 and PEPT2. Affinities are presented as K_i values, unless otherwise indicated

Other drugs	K_i (mM)		K_i (mM)		K_i (mM)		K_i (mM)		K_i (mM)		K_i (mM)		Ratio [#]
	Caco-2 (PEPT1)	SKPT (PEPT2)	BBMV (PEPT1)	BBMV (PEPT2)	LLC-PK_1 (PEPT1)	LLC-PK_1 (PEPT2)	*P. pastoris* (PEPT1)	*P. pastoris* (PEPT2)	*X. laevis* (PEPT1)	*X. laevis* (PEPT2)	HeLa (PEPT2)	HeLa (PEPT2)	PEPT1/ PEPT2
δ-Aminolevulinic acid [$]	0.79[a]	0.23[b]			2.2[c]	0.23[c]	0.5	0.22[d]					3
Alafosfalin [$]	0.2[e]	0.08[e]											3
Arphamenine A				0.012[f]									
Arphamenine B				0.030[f,*]									
Bestatin				0.099[f]	0.5[g]/1.5[c]	0.02[c,g]							
Captopril				19[h,*]									
Carnosine					8.0[c]	0.07[c]							
Enalapril				7.0[h,*]					11[i]	4.3[i]			
Enalaprilat				52[h,*]									
Fosinopril	0.04[j]	0.03[j]		0.06[h,*]									1
Lisinopril				22[h,*]									
Quinapril				0.89[h,*]					0.81[i]	0.42[i]			
Quinaprilat				4.7[h,*]									
Ramipril				0.89[h,*]									
Valacyclovir	0.49[k]	0.17[k]			2.7[g]/2.5[c]	0.22[c,g]					0.74[k]	0.39[k]	3
Valganciclovir	1.7[l]	0.04[l]									2.7[l]	0.11[l]	43
Zofenopril				0.08[h,*]									

Values without an assigning letter are unpublished data, for experimental conditions see Knütter et al. (2004) and Theis et al. (2002b). [#] Ratios are calculated from inhibition constants measured at Caco-2 and SKPT cells. * IC_{50} value; [$] is transported by PEPT1 and PEPT2; [a] Brandsch et al. (2004); [b] Bravo et al. (2005); [c] Terada et al. (2000); [d] Theis et al. (2002a); [e] Neumann et al. (2004); [f] Daniel and Adibi (1994); [g] Inui et al. (2000); [h] Lin et al. (1999); [i] Zhu et al. (2000); [j] Shu et al. (2001); [k] Ganapathy et al. (1998); [l] Sugawara et al. (2000)

drugs lacking the α-amino group are low affinity substrates of PEPT2. Ganapathy and co-workers measured affinity data of 3 selected penicillins and 3 cephalosporins at SKPT cells (Ganapathy et al., 1995). They reported that cefadroxil, cyclacillin and cephalexin are high affinity substrates of PEPT2 with IC_{50} values of 3.0, 42, and 73 μM, respectively. Ampicillin, cephalothin, and benzylpenicillin show low affinity to PEPT2 with IC_{50} values of 1.3, 7.5, and >10 mM, respectively (Ganapathy et al., 1995). Two years later the same authors showed that also the anionic cephalosporins ceftibuten, cefixime, and cefdinir are transported by PEPT2 using SKPT cells and BBMV as well as transfected HeLa cells and *Xenopus laevis* oocytes (Ganapathy et al., 1997). Terada and coworkers (1997b) studied the binding affinities of 9 β-lactam antibiotics to PEPT2 at transfected LLC-PK_1 cells (Table 7). The K_i values of ampicillin, cephalothin and cefadroxil are comparable to those determined at SKPT cells (Ganapathy et al., 1995; Terada et al., 1997b). Takahashi and co-workers concluded that along the nephron, β-lactam antibiotics at therapeutic concentrations interact predominantly with PEPT2 rather than PEPT1 (Takahashi et al., 1998). Uptake inhibition of [^{14}C]Gly-Sar into renal SKPT cells by 31 β-lactam antibiotics was measured by Luckner and Brandsch (2005). They confirmed that PEPT2 prefers those β-lactam antibiotics containing an α-amino group (e.g. cefadroxil). Moreover, an hydroxyl group at the N-terminal phenyl ring seems to be important for high affinity, e.g. cefadroxil, amoxycillin, and moxalactam. The N-terminal part of the β-lactam determines the degree of affinity decisively (Luckner and Brandsch, 2005).

Other drugs

Besides β-lactam antibiotics, PEPT2 transports a variety of other drugs (Table 8). PEPT2 recognizes for example some ACE inhibitors, antiviral and anticancer drugs. Inui's group was first to report uptake of bestatin, an antineoplastic drug, by PEPT2 (Saito et al., 1996). Döring and co-workers reported about δ-aminolevulinic acid which matches the minimal substrate structural requirements characterized for PEPT2 (Döring et al., 1998b). PEPT2 recognizes ester prodrugs such as enalapril (low affinity), fosinopril (high affinity), as well as amino acid prodrugs like valacyclovir, valganciclovir (Boll et al., 1996; Akarawut et al., 1998; Ganapathy et al., 1998; Chen et al., 1999; Lin et al., 1999; Sugawara et al., 2000; Shu et al., 2001). Enalaprilat, quinaprilat, glibenclamide, and nateglinide are PEPT2 inhibitors (Akarawut et al., 1998; Lin et al., 1999; Sawada et al., 1999). Drugs like captopril, lisinopril, quinapril, and ramipril display only low or no affinity to PEPT2 (Lin et al., 1999).

Molecular modeling investigations

In the absence of information about the three-dimensional structure of a biological macromolecule, ligand-based computational investigations are the method of choice to understand ligand-receptor interactions. Especially three-dimensional quantitative structure-activity relationship (3D QSAR) models may help to explain differences and similarities in the binding affinities of structurally diverse compounds. While this approach has been recently used for PEPT1 (Gebauer et al., 2003; Biegel et al., 2005), such investigations have not yet been performed for PEPT2. Considering the importance of this carrier for the transport of drugs via the respiratory system and the drug reabsorption in the kidney, we have performed a systematic 3D QSAR study of the binding of di- and tripeptides as well as of β-lactam antibiotics to identify those regions and properties in the substrates which are essential for their affinity (Biegel et al., 2006). The same alignment as described for PEPT1 was used. The heterogeneous data set contained high, medium and low affinity substrates comprising altogether 83 compounds.

Using the Comparative Molecular Similarity Indices Analysis (CoMSIA) (Klebe, 1998) of the SYBYL molecular modeling program (Version SYBYL 7.0; Tripos Associates, Inc.) a mathematical model with high statistical significance was created which by graphical representation helps to understand how steric, electrostatic, hydrophobic as well as hydrogen bond donor and acceptor properties contribute to the affinity of a given substrate to PEPT2. Thus, favorable steric fields delineate the dimension of the binding pocket which is most probably large in the region of the side chain of the second amino acid in di- and tripeptides but small for the third side chain of tripeptides and the C3 substituents of cephalosporins. The latter might explain why cephalosporins bearing larger substituents at C3 such as cefazoline, cefamandole or cefoxitin have low affinities to PEPT2. The analysis of the electrostatic properties revealed that a positively charged N-terminus is necessary for a high affinity while basic chains in the third amino acid of tripeptides reduce the affinity as in Gly-His-Lys or Pro-Phe-Lys. Notably, electrostatic and hydrogen bond donor fields suggest that D-configuration is tolerated in the N-terminal region while the transporter seems to be selective for L-configured amino acids in the second position.

General structural requirements for substrate/inhibitor recognition by PEPT2

Summarizing the known biological data for substrate recognition by PEPT2, we conclude that the following structural features are required for high affinity interaction:

- a free N-terminal α-amino group in L-configuration;
- a peptide bond which can be replaced by a ketomethylene group but not by a $–CH_2–NH–$ group and which should be separated by one or two methine groups or methylene carbon atoms from the N-terminal nitrogen;
- in case of dipeptides, *trans* conformation of the peptide bond;
- a C-terminal acid group, e.g. a carboxylic group; the distance between the carbonyl group of the first peptide bond and the carboxylic group is less relevant;
- high hydrophobicity;
- for tripeptides: an uncharged amino acid residue in position 3.

Comparison of PEPT1 and PEPT2 substrate specificity

A number of studies concerning the substrate recognition of PEPT1 and PEPT2 as well as reports directly comparing the two transporter isoforms have been published (Daniel et al., 1992; Daniel and Adibi, 1993; Ganapathy et al., 1995, 1997; Amasheh et al., 1997; Terada et al., 1997b, 2000; Döring et al., 1998c; Takahashi et al., 1998; Sugawara et al., 2000; Theis et al., 2002a, b; Shu et al., 2001; Knütter et al., 2004; Vabeno et al., 2004; Luckner and Brandsch, 2005). It has been established that PEPT2 has similar but not identical requirements for substrate recognition and transport compared to PEPT1.

Comparing affinity data between PEPT1 and PEPT2, $K_{i\ PEPT1}/K_{i\ PEPT2}$ ratios can be used to highlight differences. It is generally believed and often written in reviews, that PEPT2 transports its substrates with a 10 to 15 fold higher affinity than PEPT1. However, as shown in Tables 2–8, the $K_{i\ PEPT1}/K_{i\ PEPT2}$ ratios derived from K_i values measured at Caco-2 and SKPT cells, vary from 0.4 for Gly-6-Ahx to 27400 for Lys(Z)-Lys(Z). Using these ratios, the major differences in substrate recognition between the two proteins are easily assessable. When comparing affinity constants derived from Caco-2 and SKPT cells, one has to keep in mind that these cell lines originate from different species, human and rat, respectively. However, differences between hPEPT1 and rPEPT2 could so far all be confirmed using hPEPT1 and hPEPT2 expression systems. Hence, the Caco-2/SKPT comparison is a well accepted procedure (Ganapathy et al., 1995, 1997; Brandsch et al., 1997; Shu et al., 2001; Luckner and Brandsch, 2005).

The main differences in substrate recognition between PEPT1 and PEPT2 are the following:

- In general PEPT2 accepts the same substrates as PEPT1 but in case of natural dipeptides with higher affinity and lower maximal uptake rates.
- The more hydrophobic a substrate the higher is the binding affinity to PEPT2. This phenomenon was not observed for PEPT1. This difference is illustrated by exceptional large $K_{i\ PEPT1}/K_{i\ PEPT2}$ ratios found for PEPT2 high affinity ligands such as Trp-Trp-Trp, Trp-Trp, Trp-Gly-Tyr, and Lys(Z)-Lys(Z).
- PEPT2 has disproportionally higher affinities for those β-lactam antibiotics that contain an α-amino group than PEPT1.
- PEPT2 displays a wide range of affinities and different transport characteristics for amino acid aryl amides.
- Tripeptides containing a charged amino acid in positon 3 are medium to low affinity substrates for PEPT2, whereas they show high binding affinities to PEPT1. This is obvious from small $K_{i\ PEPT1}/K_{i\ PEPT2}$ ratios in the range of 1 to 5 found for Ala-Ala-Asp, Gly-His-Lys, and Pro-Phe-Lys.

In general it can be said that PEPT2 is more selective than PEPT1 and that PEPT2 has more specific, narrower requirements for substrate recognition.

Acknowledgements

This work was supported by Land Sachsen-Anhalt grant 3505A/0403L, grant PAXB3599HP/0105T and a Ph.D fellowship to A.B. We thank Reiner Reichlmaier for excellent technical assistance. This work will be part of the doctorial thesis of Annegret Biegel.

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