

## Review

# Structural Specificity of Mucosal-Cell Transport and Metabolism of Peptide Drugs: Implication for Oral Peptide Drug Delivery

Jane P. F. Bai<sup>1,3</sup> and Gordon L. Amidon<sup>2</sup>

---

The brush border membrane of intestinal mucosal cells contains a peptide carrier system with rather broad substrate specificity and various endo- and exopeptidase activities. Small peptide (di-/tripeptide)-type drugs with or without an N-terminal  $\alpha$ -amino group, including  $\beta$ -lactam antibiotics and angiotensin-converting enzyme (ACE) inhibitors, are transported by the peptide transporter. Polypeptide drugs are hydrolyzed by brush border membrane proteolytic enzymes to di-/tripeptides and amino acids. Therefore, while the intestinal brush border membrane has a carrier system facilitating the absorption of di-/tripeptide drugs, it is a major barrier limiting oral availability of polypeptide drugs. In this paper, the specificity of peptide transport and metabolism in the intestinal brush border membrane is reviewed.

---

**KEY WORDS:** peptide transporter; angiotensin-converting enzyme (ACE) inhibitors;  $\beta$ -lactam antibiotics; peptide-type drugs; oral availability;  $\alpha$ -methyldopa-pro; brush border membrane peptidases; N-terminal  $\alpha$ -amino group; cytosolic peptidases; endopeptidase-24.11; aminopeptidase M.

---

## INTRODUCTION

With absorption area enhanced by villi and microvilli, and with various exo- and endo-peptidases anchored in the brush border membrane, the small intestine is an important digestion and absorption organ in the body. In addition to passive diffusion, there are specific carrier-mediated systems in the intestine to facilitate absorption of nutrient. Since absorption of intact dipeptides into the portal vein was observed in 1968, it has been found that di-/tripeptides are transported by a carrier-mediated transport process (1,2). Oral delivery of drugs, especially peptide-type drugs, benefits from the unique absorption capability of the intestine but also suffers from its intensive digestive ability. Though hydrophilic and ionized at the intestinal pHs, small peptide-type drugs, such as  $\beta$ -lactam antibiotics and angiotensin-converting enzyme (ACE) inhibitors, are absorbed orally through the peptide transporter (3–8). Targeting the peptide transporter, with its rather broad substrate specificity, in order to achieve significant oral efficacy of di-/tripeptide drugs is an appealing strategy but requires delineation of its specificity.

The exopeptidases and endopeptidases in the intestinal brush border membrane are able to cleave polypeptides and protein fragments, resulting from the actions of gastric and pancreatic enzymes, to amino acids and di-/tripeptides (9).

Polypeptide drugs are subject to the attack by the brush border peptidases even if pancreatic proteolysis is avoided. Consequently, oral availability of bioactive polypeptides is, in general, very poor (10). However, cyclosporin is an orally active peptide; further significant membrane transport of enkephalin and renin inhibitors was observed when peptidase inhibitors were used, and the oral efficacy of insulin and vasopressin is improved, though still too low to be useful, using inhibitors and a stable analogue, respectively. These results indicate that oral delivery is feasible if proteolysis is avoided (11–14). Through an understanding of the substrate specificity of mucosal-cell proteolytic enzymes, the proteolytic metabolism of peptide-type drugs or prodrugs can be predicted. Strategies to target or to avoid these enzymatic actions can then be designed accordingly. With increasing knowledge of physiological functions and pharmacophores of peptide drugs, such as renin inhibitors, ACE inhibitors, human growth hormone, luteinizing hormone-releasing hormone (LHRH), insulin, calcitonin, and vasopressin, their therapeutic use is also becoming more important. This paper reviews our current knowledge of the structural requirements for the peptide transporter and the substrate specificity of mucosal-cell peptidases.

## INTESTINAL MUCOSAL-CELL TRANSPORT OF AMINO ACID AND PEPTIDE-TYPE DRUGS

### Carrier-Mediated Transport of Small Peptides

Small peptides (di-/tripeptides) are absorbed by a carrier-mediated system, which has broad substrate specificity, in various species (2). Di-/tripeptides share a common trans-

<sup>1</sup> University of Minnesota, College of Pharmacy, 308 Harvard Street S.E., Minneapolis, Minnesota 55455.

<sup>2</sup> University of Michigan, College of Pharmacy, Ann Arbor, Michigan 48109-1065.

<sup>3</sup> To whom correspondence should be addressed.

port mechanism, while the absorption of Gly-Gly-Gly-Gly and Gly-Sar-Sar-Sar is concluded to be passive (2,15,16). However, characterization of the small peptide transporter in the intestinal mucosal cell is complicated by hydrolysis. Moreover, there are more than 480 possible dipeptides and  $10^4$  possible tripeptides, and the number of existing peptide carriers is unknown. However, currently only one protein isolated from the brush border membrane has been reported as the peptide transporter (17). Di-/tripeptides are cotransported with proton and  $\text{Na}^+$  is only indirectly involved in the process (18). According to Leibach's hypothesis (Fig. 1), the  $\text{Na}^+-\text{H}^+$  exchanger generates and maintains the inward proton gradient, while the  $\text{Na}^+-\text{K}^+$ -ATPase in the basolateral membrane maintains a low intracellular sodium concentration. Thus the  $\text{Na}^+-\text{H}^+$  exchanger coupled with the  $\text{Na}^+-\text{K}^+$ -ATPase drives the transport of di-/tripeptides into the intestinal epithelial cells.

### Substrate Structural Requirements

**N-Terminal  $\alpha$ -Amino Group.** Imidopeptides, Pro-X, where X is an amino acid, are compatible with the peptide carrier (19,20). Methylation, acetylation, or other modifications of the N-terminal  $\alpha$ -amino group reduce or abolish the capability to inhibit peptide transport. The examples include Sar-Gly, *N*-benzyloxycarbonyl-Gly-Leu, and *N*-acetyl-Gly-Gly (19–22). A  $\beta$ -amino acid at either side of the amide bond of dipeptides is compatible with the peptide transporter. For example  $\beta$ -alanyl-histidine (carnosine),  $\beta$ -Ala-Gly-Gly, bestatin, and His- $\beta$ -Ala are recognized by this carrier system (19,22,23). However, small peptides containing a  $\gamma$ -amino acid are incompatible with the peptide transporter (20,22).

The N-terminal  $\alpha$ -amino group is not required for the peptide transporter as shown with a series of dipeptide analogues without an N-terminal  $\alpha$ -amino group (including phenylpropionylproline, phenylacetylproline, *N*-benzoylproline, phenylacetyl- $\alpha$ -methyl-dopa, and hippuric acid) (24). Their structures and intestinal permeability are summarized in Table I. Absorption of the peptide analogue is nonlinear and inhibited by small peptides and/or by cephadrine but not by amino acids (Figs. 2 and 3). The results indicate that peptide analogues are indeed recognized by the peptide transporter. These findings parallel results with  $\beta$ -lactam antibiotics and ACE inhibitors, which also lack an N-terminal  $\alpha$ -amino group.

**Other Functional Groups.** Modification of the C-terminal carboxyl group leads to a reduction or an abolition of

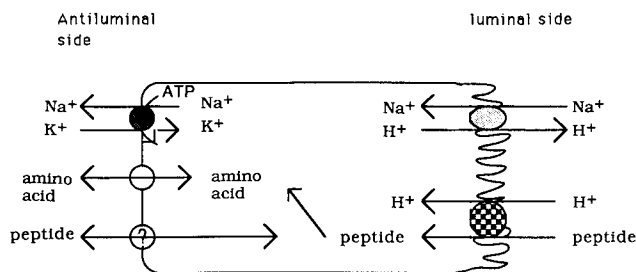


Fig. 1. The transport of di-/tripeptides are energized by a proton ( $\text{H}^+$ ) gradient across the brush border membrane. Referred to the model proposed by Leibach *et al.* (18).

affinity to transporter. Examples include Gly-GlyNH<sub>2</sub>, Gly-Gly-GlyNH<sub>2</sub>, and Asp-PheOCH<sub>3</sub> (19,22). More studies are required to establish the significance of a free C-terminal carboxyl group for the peptide transporter. Gly-Sar, Gly-Sar-Sar, and dipeptides of X-Pro and X-Hyp are absorbed by the peptide transporter (17,22,25), suggesting that di-/tripeptides with an imide bond are compatible.

**Stereospecificity.** Dipeptides with a D-amino acid on either side of the amide bond are compatible with the peptide transporter (15,26,27). Nevertheless, the uptake of small peptides is stereoselective (17,26–28). Dipeptides of L-L form have the highest uptake rate, followed by mixed isomers (L-D and D-L) and then D-D-isomers.

### Carrier-Mediated Transport of Peptide-Type Drugs and Prodrugs

#### $\beta$ -Lactam Antibiotics

With free N-terminal  $\alpha$ -amino and C-terminal carboxyl groups, amino- $\beta$ -lactam antibiotics (including cyclacillin, amoxicillin, ampicillin, cefaclor, cefadroxil, cephalexin, cefatrizine, and cephradine) are all transported by the peptide transporter (7,29). The Michaelis-Menten parameters describing their transport are summarized in Table II. The intestinal absorption of amino- $\beta$ -lactam antibiotics is inhibited by di- and tri-peptides, but not by amino acids, and shows mutual inhibition (Fig. 4), demonstrating that these amino- $\beta$ -lactam antibiotics share the peptide transporter.  $\beta$ -Lactam antibiotics without a free N-terminal  $\alpha$ -amino group (including cefixime, FK089, and ceftibuten) are also transported by the transporter (8,30,31). The transport parameters of cefixime are as follows:  $J_{\text{max}}$ ,  $0.02 \pm 0.01 \text{ mM}$ ;  $K_m$ ,  $0.03 \pm 0.01$ ;  $P_c$ ,  $0.52 \pm 0.05$ ; and  $P_m$ ,  $0.18 \pm 0.04$  (30). Its absorption is energized by a proton gradient, as is that of small peptides (8). The uptake of D-cephalexin is saturable and inhibited by its L-isomer, which is absorbed by the peptide transporter (32).

#### ACE Inhibitors

Captopril, SQ 29,852, enalapril, lisinopril, benazepril, and quinapril are transported by the peptide transporter (3–5,33). Without a free N-terminal  $\alpha$ -amino group, these compounds all show nonlinear absorption, as summarized in Table III. Moreover their uptake is significantly inhibited by small peptides and cephadrine. The diacid ACE inhibitors (including enalaprilat, quinaprilat, and benazeprilat) are poorly absorbed, while their ester prodrugs (including enalapril, quinapril, and benazepril) are absorbed by the transporter. However, lisinopril, with two free carboxyl groups at similar positions as in enalaprilat, is absorbed by the transporter with a low carrier permeability, as is FK089, a  $\beta$ -lactam antibiotic (3,4,31,33). Further investigation of the substrate specificity of the peptide transporter is needed to understand this binding/transport phenomenon.

#### Renin Inhibitors and Thyrotropin-Releasing Hormone (TRH)

Without having free N-terminal  $\alpha$ -amino and C-terminal

Table I. Permeabilities of Dipeptide Analogues Investigated for the Need of a N-Terminal  $\alpha$ -Amino Group

Compound	Chemical structure	$P_w^a$
Phe-Pro		$2.01 \pm 0.54$
Phenylpropionylproline		$1.19 \pm 0.16$
Phenylacetylproline		$0.84 \pm 0.15$
N-Benzoylproline		$0.90 \pm 0.06$
Hippuric acid		$0.18 \pm 0.03$
Phenylacetyl- $\alpha$ -methyldopa		$0.61 \pm 0.07$

<sup>a</sup> All compounds were studied at 0.1 mM, except phenylacetyl- $\alpha$ -methyldopa, which was studied at 0.05 mM. Adapted from Bai *et al.* (24).

carboxyl groups, TRH was still found to be absorbed by the peptide transporter (34,35). Its analogue DN 1417, with a carboxybutyrolactone replacing the pyroglutamyl residue, however, is only passively absorbed. Pro-Leu-Gly-NH<sub>2</sub> was suggested to be incompatible with the peptide transporter (22). Nevertheless, it was recently reported that two renin inhibitors, tripeptide analogues without free amino and carboxy termini, were transported by the peptide transporter (36). More studies are necessary to clarify whether tripeptide analogues with modified termini are compatible with the transporter.

#### Amino Acid-Type Drugs and Their Peptide Prodrugs

Unlike small peptides (di-/tripeptides) sharing a com-

mon transport system, amino acids are transported by four transporters with narrow and distinct substrate specificities (37,38). Amino acid-type drugs, such as  $\alpha$ -methyldopa, L-dopa, and baclofen, all exhibit nonlinear absorption (39–41). The transport of the former two compounds is inhibited by amino acids, suggesting that they are absorbed by the amino acid carriers. Baclofen is rapidly and well absorbed, but  $\alpha$ -methyldopa has variable and low intestinal absorption (41,42). L-Dopa has high intestinal absorption but is metabolized extensively to dopamine (43). Dipeptide prodrugs of L-dopa have a higher oral availability than L-dopa itself (43). Phe- $\alpha$ -methyldopa,  $\alpha$ -methyldopa-Phe, and  $\alpha$ -methyldopa-Pro all demonstrate more than 10 times higher intestinal permeabilities than  $\alpha$ -methyldopa (Table IV) (40). Hence the increase in membrane permeability is achieved by the pep-

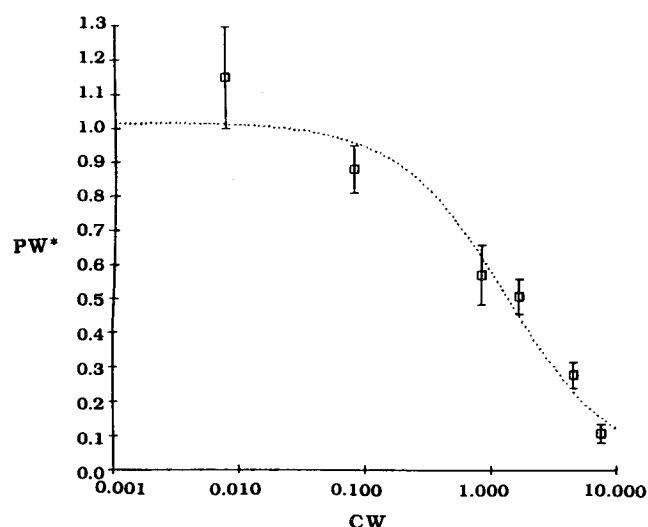


Fig. 2. Transport kinetics of *N*-benzoylproline.  $PW^*$  is the dimensionless membrane permeability, and  $CW$  the membrane surface concentration (mM). The values of  $PW^*$  shown are mean  $\pm$  SE. (Adapted from Ref. 24.)

peptide prodrug strategy targeting the peptide transporter. *L*- $\alpha$ -Methyldopa-Pro is absorbed via the peptide carrier and hydrolyzed by the cytosolic prolidase, a potential peptide-prodrug converting enzyme, with Michaelis-Menten parameters  $K_m$  and  $V_{max}$  of  $0.09 \pm 0.022$  mM and  $3.98 \pm 0.250$   $\mu$ mol/min/mg protein, respectively. Substrates of prolidase are usually poorly hydrolyzed by the brush border membrane peptidases (44). This result demonstrates that oral

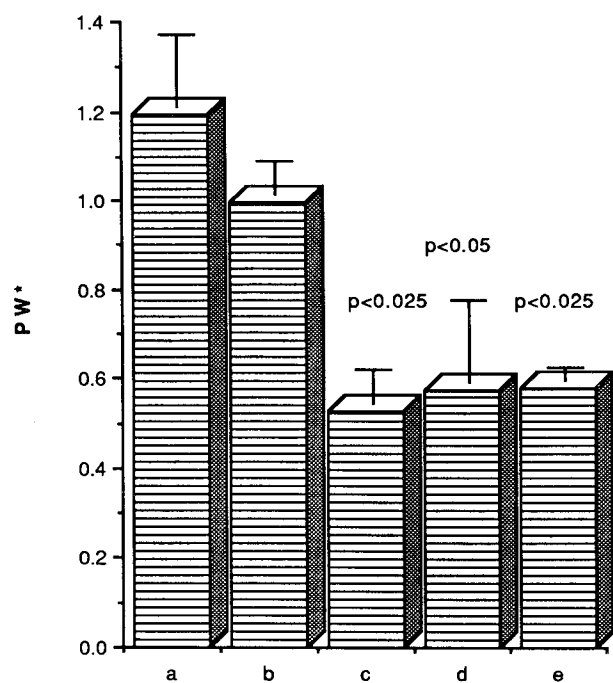


Fig. 3. Inhibition of 0.1 mM phenylpropinylproline permeability: (a) control; (b) 27 mM *L*-methionine; (c) mixed dipeptides (60 mM Gly-Gly, 2 mM Gly-Phe, 2 mM Pro-Phe); (d) 1 mM cephadrine; (e) 27 mM Gly-Pro. (Adapted from Ref. 24.)

Table II. Summary of the Membrane Absorption Parameters<sup>a</sup> of  $\beta$ -Lactam Antibiotics

Compound	$J_{max}^*$ (mM)	$K_m$ (mM)	$P_c^*$	$P_m^*$
Cyclacillin <sup>b</sup>	16.30 (3.40)	14.00 (3.30)	1.14 (0.05)	0
Amoxicillin <sup>b</sup>	0.04 (0.02)	0.06 (0.03)	0.56 (0.06)	0.76 (0.09)
Ampicillin <sup>b</sup>	11.78 (1.88)	15.80 (2.92)	0.75 (0.04)	0
Cefaclor <sup>c</sup>	21.30 (4.00)	16.10 (3.60)	1.30 (0.10)	0
Cefadroxil <sup>c</sup>	8.40 (0.80)	5.90 (0.80)	1.40 (0.10)	0
Cephalexin <sup>c</sup>	9.10 (1.20)	7.20 (1.10)	1.30 (0.10)	0
Cefatrizine <sup>c</sup>	0.70 (0.20)	0.60 (0.20)	1.30 (0.10)	0.20 (0.03)
Cephadrine <sup>c</sup>	1.60 (0.80)	1.50 (0.80)	1.10 (0.10)	0.30 (0.10)

<sup>a</sup> Reported values are mean  $\pm$  SD.

<sup>b</sup> From Oh *et al.* (29).

<sup>c</sup> From Sinko *et al.* (6).

availability of poorly absorbed drugs can be improved by utilizing the peptide carrier to increase membrane permeability and then the cytosolic enzymes to release parent drugs, as illustrated in Fig. 5 (45).

## INTESTINAL MUCOSAL-CELL METABOLISM OF PEPTIDE-TYPE DRUGS

### Distribution and Development of Mucosal Peptidases

For tetrapeptides and higher peptides, more than 90% of the proteolytic activity is in the brush border membrane, whereas for tripeptides it is 10 to 60%, and for dipeptides,

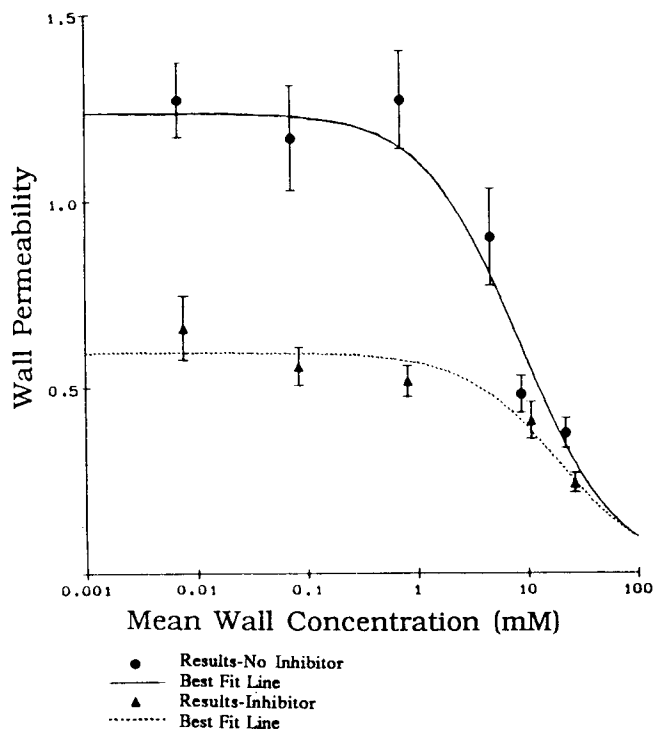


Fig. 4. Plot of the wall permeability of cephalexin perfused alone and in the presence of a competitive inhibitor, cefadroxil (7 mM). The results are reported as the mean wall permeability  $\pm$  SE. (Adapted from Ref 7.)

Table III. Intestinal Transport Parameters of ACE Inhibitors

Compound	$J_{max}$ (mM)	$K_m$ (mM)	$P_c$	$P_m$
Captopril <sup>a</sup>	12.3 (2.8)	5.91 (1.65)	2.08 (0.19)	0.75
SQ29,852 <sup>b</sup>	0.16 (0.04)	0.08 (0.01)	2.00 (0.20)	0.25 (0.07)
Lisinopril <sup>b</sup>	0.18 (0.004)	0.056 (0.003)	0.33 (0.03)	0.06 (0.05)
Enalapril <sup>c</sup>	0.13	0.07	1.9	0.35
Benazepril <sup>d</sup>	0.072	0.075	0.962	0.749
Quinapril <sup>d</sup>	1.703	2.341	0.728	0.621

<sup>a</sup> From Hu *et al.* (5).

<sup>b</sup> From Friedman *et al.* (4).

<sup>c</sup> From Friedman *et al.* (3).

<sup>d</sup> From Yee *et al.* (33).

only 10% of the proteolytic activity in the brush border membrane (44,46,47). The activities of brush border membrane peptidases change as the cells migrate from the crypt to the tip of a villi (48,49). Furthermore, the activities of peptidases are not uniform along the intestine; the distribution profiles vary from peptidase to peptidase. There is also species difference in the longitudinal distribution of peptidase activities (48,50–53).

### Brush Border Membrane Peptidases

#### EXOPEPTIDASES

**Aminopeptidases.** *Aminooligopeptidase* (EC 3.4.11.2). This is also known as aminopeptidase N, aminopeptidase M, oligoaminopeptidase, and L-leucyl- $\beta$ -naphthylamide hydrolase. This enzyme, a zinc protein, is the most abundant peptidase in the intestinal and renal microvilli (54–56). Its standard substrates include Ala-X and Leu-X, where X can be 2-naphthylamide, 4-nitroanilide, or 7-amido-4-methylcoumarin. Aminooligopeptidase releases the N-terminal amino acid from oligopeptides and can hydrolyze oligopeptides for size from dipeptides to octapeptides (57), as shown in Table V. An L-configuration and a free N-terminal  $\alpha$ -amino group are required for its activity (44,58). Upon perfusion through the rat jejunum, Leu-enkephalin was hydrolyzed to Tyr and Gly-Gly-Phe-Leu, suggesting that aminopeptidase is involved (59). In contrast, the enzyme exhibits no activity against insulin B chain, casein, and bradykinin (57).

**Aspartate Aminopeptidase** (EC 3.4.11.7). This is also known as acid aminopeptidase and aminopeptidase A. Its standard substrates are  $\alpha$ -Glu-2-naphthylamide and  $\alpha$ -Asp-

Table IV. Wall Permeabilities ( $P_w \pm SE$ ) of L- $\alpha$ -Methyldopa and Its Dipeptidyl Derivatives<sup>a</sup>

Compound	Concentration (mM)		
	1.0	0.1	0.01
L- $\alpha$ -Methyldopa (I)	0.41 (0.11)	0.4 (0.22)	0.43 (0.14)
Gly-I		4.34 (0.27)	
Pro-I		1.68 (0.23)	
I-Pro		5.41 (0.55)	
Phe-I		5.29 (1.57)	
I-Phe	4.30 (0.30)	10.22 (0.45)	10.19 (1.8)

<sup>a</sup> Adapted from Hu *et al.* (40).

### Peptide prodrug strategy

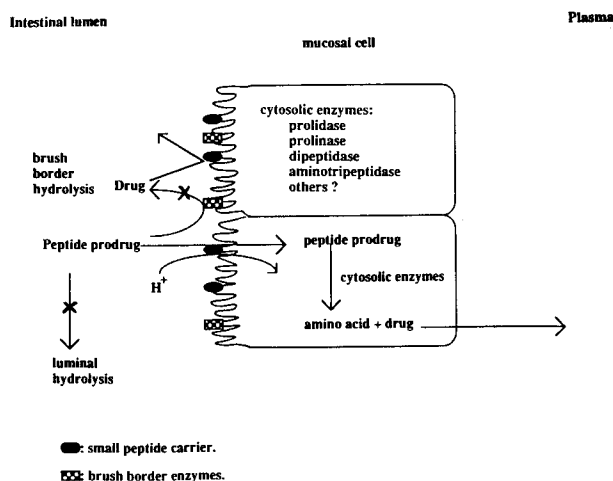


Fig. 5. Schematic presentation of a peptide prodrug strategy for improving oral absorption. (Adapted from Ref. 45.)

2-naphthylamide. This enzyme releases the N-terminal aspartic acid and glutamic acid from tripeptides and has limited activity against dipeptides (60) (Table V).

**Dipeptidyl Aminopeptidase IV** (3.4.14.5). This is also known as dipeptidyl peptide hydrolase and post-proline dipeptidyl aminopeptidase. Its standard substrates are Gly-Pro-X, where X can be 2-naphthylamide, 4-nitroanilide, or 7-amido-4-methylcoumarin. This enzyme is a serine peptidase and is inhibited by diisopropylphosphorofluoridate (DIP-F) (55,61). Its typical peptide substrates are shown in Table V. A dipeptide from the N terminus of the peptide is released by the enzyme if proline or alanine is at the penultimate position from the N terminus (61,62). It has an absolute requirement for a free N-terminal  $\alpha$ -amino group.

**$\gamma$ -Glutamyltransferase** (EC 2.3.2.2). This is also known as  $\gamma$ -glutamyltranspeptidase. The standard substrates include  $\gamma$ -Glu-X, where X can be 2-naphthylamide, 4-nitroanilide, or 7-amido-4-methylcoumarin (55). The serine reagent phenylmethanesulfonyl fluoride irreversibly inhibits this enzyme if maleate is present, hence a seryl or threonyl residue may be involved in its activity. This enzyme catalyzes the transfer of a  $\gamma$ -glutamyl group from glutathione or  $\gamma$ -glutamyl-containing molecules to the N terminus of an acceptor, an amino acid, or a peptide, to form a  $\gamma$ -glutamyl amino acid or peptide derivative (49) (Table V). However, its physiological function remains unclear.

**Aminopeptidase P** (EC 3.4.11.9). This is also known as prolyl aminopeptidase, X-prolyl aminopeptidase and aminoacyl-peptide hydrolase. Its standard substrates include Gly-Pro-Pro and Gly-Pro-Hyp, and  $Mn^{2+}$  is required for its activity (63). Aminopeptidase P from the human serum and rat lung are inhibited by metal chelating agents. The enzyme has a high activity in the human testis, lung, and kidney and a low activity in the human serum (64); and it remains attached to the intestinal brush border membrane after the papain treatment (63,65). Aminopeptidase P exclusively cleaves the N-terminal imido bonds of oligopeptides with proline but not hydroxyproline as the N-terminal second res-

Table V. The Typical Substrates of the Intestinal Mucosal-Cell Peptidases

Peptidase (location)	Typical substrate	Mode of action <sup>a</sup>	Reference
Aminooligopeptidase (brush border)	Oligopeptides with an N-terminal L-amino acid	$X_1 - X_2 - X_3 - -$ ↑	Adibi and Kim (9)
Aspartate aminopeptidase (brush border)	Tripeptides with an N-terminal L-Asp or L-Glu	Asp - $X_2 - X_3$ ↑	Tobey <i>et al.</i> (60)
Dipeptidylaminopeptidase IV (brush border)	Peptides with proline or alanine as the penultimate residue from the free N terminus	$X_1 - \text{Pro} - X_3 - -$ ↑	Walter <i>et al.</i> (61)
$\gamma$ -Glutamyltransferase (brush border)	Glutathione + acceptor, acceptor: amino acids or small peptides	Glutathione + acceptor → $\gamma$ -glutamyl-acceptor + Cys - Gly	Garvey <i>et al.</i> (49)
Carboxypeptidase P (brush border)	Peptides with proline as the penultimate residue from the free C terminus	$X_1 - - - - \text{Pro} - X_n$ ↑	Walter <i>et al.</i> (61)
ACE (brush border)	Peptides with a carboxyl-terminal proline	$- - - X_{n-1} - X_n - \text{Pro}$ ↑	Yoshioka <i>et al.</i> (50,67)
Aminopeptidase P (brush border)	Peptides with proline as the penultimate residue from the free N terminus	$X_1 - \text{Pro} - X_3 - -$ ↑	Walter <i>et al.</i> (61)
Endopeptidase-24.11 (brush border)	Peptides containing a peptide bond on the N-terminal side of hydrophobic amino acids	Tyr - Gly - Gly - Phe - Met ↑	Bunnett <i>et al.</i> (71)
Dipeptidase (cytosol)	Neutral dipeptides	Gly - Leu ↑	Adibi and Kim (9)
Aminotripeptidase (cytosol)	Tripeptides with proline as the N-terminal residue	Pro - $X_2 - X_3$ ↑	Doumeng and Maroux (73)
Prolidase (cytosol)	Imidodipeptides with Pro or Hyp as the C-terminal residue	$X_1 - \text{Pro}$ ↑	Myara <i>et al.</i> (79)
Prolinase (cytosol)	Imidodipeptides with Pro or Hyp as the N-terminal residue	Pro - $X_2$ ↑	Myara <i>et al.</i> (79)
Carnosinase (cytosol)	Carnosine	$\beta - \text{Ala} - \text{His}$ ↑	Lenny <i>et al.</i> (80,81)

<sup>a</sup>  $X_i$  is the amino acid at the  $i$  position.

idue (61) (Table V). Dipeptides such as Gly-Pro and Val-Pro are hydrolyzed as well, but at a much slower rate (61,66). With a substituted N-terminal imino group, dinitrophenyl-poly-L-proline is resistant to this enzyme as opposed to the rapid hydrolysis of poly-L-proline.

**Carboxypeptidases.** *Carboxypeptidase P* (EC 3.4.17). The standard substrate is *N*-carbobenzoxy-L-prolyl-alanine (NCBZ-Prolyl-L-Ala). Its activity is stimulated by  $\text{Mn}^{2+}$ , and the enzyme can be completely solubilized by the papain

treatment (53,61). The enzyme preferentially releases the C-terminal amino acids from polypeptides containing a proline residue penultimate to the C terminus (Table V); and it can hydrolyze dipeptides Pro-Ala and Pro-Phe as well. However, hydroxyproline could not replace proline in the penultimate position. Further, *Z*-Pro-Pro is not hydrolyzed by this enzyme (67).

*Angiotensin-Converting Enzyme* (EC 3.4.15.1). This is also known as dipeptidyl carboxypeptidase, kininase II, and

peptidyl dipeptidase. The standard substrates include N-terminal blocked peptides such as Z-Gly-Gly-Pro (benzyloxycarbonyl-glycyl-glycyl-proline), Z-Gly-His-Leu, and *N*-[3-[2-furyl]acryloyl]-L-phenylalanyl-glycyl-glycine (50,68). Angiotensin-converting enzyme releases dipeptides from the carboxyl terminus of polypeptides; activity is highest against those substrates containing proline at the carboxyl terminus (50,67,69) (Table V).

#### ENDOPEPTIDASES

**Endopeptidase-24.11 (EC 3.4.24.11).** This is also known as enkephalinase and enkephalin-dipeptidyl-carboxypeptidase. The standard substrates include insulin B chain and enkephalin. The enzyme is a  $Zn^{2+}$ -metalloenzyme, and its activity is found in the renal and intestinal microvilli and several other organs (55). The enzymes from various tissues are sensitive to phosphoramidon and 1,10-phenanthroline (55,70,71). The enzyme has a broad substrate specificity and degrades peptides by hydrolyzing peptide bonds on the N-terminal side of hydrophobic amino acids, such as leucine, tyrosine, phenylalanine, valine, and tryptophan (Table V) (71). Enkephalinase is involved in the inactivation of bioactive peptides. Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu) was hydrolyzed to Tyr-Gly-Gly and Phe-Leu when perfused through jejunum, suggesting that the action of endopeptidase may be involved (59).

**Angiotensin-Converting Enzyme (EC 3.4.15.1).** With the capability to hydrolyze a C-terminal blocked peptide such as substance P and LHRH, ACE seems to possess an endopeptidase activity as well (69,72). The specificity of its endopeptidase action is not clear.

#### DIPEPTIDASES

**Dipeptidases.** Two dipeptidases were suggested to be present in the brush border membrane: one is Gly-Leu peptidase; the other, zinc stable Asp-Lys peptidase (60). Further studies are required to confirm the existence of these membrane dipeptidases.

#### *Cytoplasmic Peptidases*

#### DIPEPTIDASE (EC 3.4.13.2)

Cytosolic dipeptidases from several species all have similar and broad substrate specificity (44). Its typical substrates are shown in Table V. Nearly all neutral peptides except Gly-Gly are hydrolyzed rather readily, especially Gly-Leu, thus this enzyme is also known as Gly-Leu-dipeptidase. Dipeptides consisting of acidic amino acids, such as Asp, or basic amino acids, such as His, are not readily hydrolyzed as neutral peptides. Iminodipeptides (Pro-X) are readily hydrolyzed but not X-Pro peptides.

#### AMINOTRIPEPTIDASE

Aminotripeptidase can only release the N-terminal res-

idue from a tripeptide substrate. Dipeptides and peptides larger than tetrapeptides are not substrates. Its activity is primarily toward tripeptides with an N-terminal proline, as shown in Table V. Nevertheless, it can also hydrolyze tripeptides with other N-terminal amino acids. An L-configuration for the first two amino acids seems to be required for the enzyme activity (73).

#### PROLIDASE (EC 3.4.13.9)

This is also known as imidodipeptidase, peptidase D, proline dipeptidase, and aminoacyl-L-proline hydrolase. Prolidases from various animals and tissues have similar enzyme properties, molecular weights, and substrate specificities (74-77). The enzyme can hydrolyze only the *trans* isomers of dipeptides with an imino acid, such as proline or hydroxyproline, at the C terminus (Table V) (78). An N-terminal  $\alpha$ -amino group on the substrate is required for prolidase activity (45). The activity of porcine kidney prolidase, however, is significantly inhibited by phenylpropionyl-proline and *N*-benzoylproline.

#### PROLINASE

As shown in Table V, prolinase cleaves iminodipeptides with an N-terminal proline or hydroxyproline and has no activity against tripeptides, prolinamide, and iminodipeptides with the imino group acylated (61,79).

#### CARNOSINASE (EC 3.4.13.3)

This is also known as aminoacyl-L-histidine hydrolase. Its typical substrate is L-carnosine ( $\beta$ -Ala-His) (Table V). Serum carnosinase hydrolyzes homocarnosine and anserine, while cellular carnosinase cannot (80,81). Anserine,  $\beta$ -Ala-Ala, and  $\beta$ -Ala-Gly are incapable of inhibiting human tissue carnosinase (82).

#### *Peptidases in Other Subcellular Fractions*

In the rabbit, activities of aminopeptidases N and A are observed in the basolateral membrane and Golgi apparatus but not in the rough and smooth endoplasmic reticulum (83). In the rat, the hydrolysis patterns of substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH<sub>2</sub>) by the intestinal brush border and basolateral membrane are similar (84). Endopeptidase-24.11 is the major responsible enzyme. However, the basolateral membrane has much lower enzyme activities than the brush border membrane.

#### CONCLUSION

In the intestine, polypeptides or protein fragments are hydrolyzed to amino acids and di-/tripeptides by the brush border membrane peptidase, and di-/tripeptides are absorbed by the peptide transporter. Energized by a proton (H<sup>+</sup>) gradient, the intestinal peptide transporter has a rather

broad substrate specificity. Moreover, an N-terminal  $\alpha$ -amino group is not required for this transporter. Peptide-type drugs,  $\beta$ -lactam antibiotics, and ACE inhibitors, are transported by the transporter. Containing various exo- and endopeptidases, the brush border membrane of intestinal mucosal cells is the major location limiting oral efficacy of polypeptide drugs. Having a broad substrate specificity, aminopeptidase M and endopeptidase-24.11 are the key membrane peptidases that hydrolyze polypeptide drugs.

## REFERENCES

1. S. A. Adibi and E. Phillips. Evidence for greater absorption of amino acid from peptide than from free form by human intestine. *Clin. Res.* 16:446-448 (1968).
2. D. M. Matthews and J. W. Payne. Transmembrane transport of small peptides. In *Current Topics in Membrane and Transport*, Academic Press, New York, 1980, Vol. 14, pp. 331-425.
3. D. I. Friedman and G. L. Amidon. Intestinal absorption mechanism of two prodrug ACE inhibitors in rats: Enalapril maleate and fosinopril sodium. *Pharm. Res.* 6:1043-1047 (1989).
4. D. I. Friedman and G. L. Amidon. The intestinal absorption mechanism of dipeptide ACE inhibitors of the lysyl-proline type: Lisinopril and SQ 29,852. *J. Pharm. Sci.* 78:995 (1989).
5. M. Hu and G. L. Amidon. Passive and carrier-mediated intestinal absorption components of captopril. *J. Pharm. Sci.* 77:1007-1011 (1988).
6. P. J. Sinko and G. L. Amidon. Characterization of the oral absorption of  $\beta$ -lactam antibiotics. I. Cephalosporins. Determination of intrinsic membrane absorption parameters in the rat intestine *in situ*. *Pharm. Res.* 5:645-650 (1988).
7. P. J. Sinko and G. L. Amidon. Characterization of the oral absorption of  $\beta$ -lactam antibiotics. II. Competitive absorption and peptide carrier specificity. *J. Pharm. Sci.* 78:723-726 (1989).
8. A. Tsuji, T. Terasaki, I. Tamai, and H. Hirooka.  $H^+$ -gradient-dependent and carrier-mediated transport of cefixime, a new cephalosporin antibiotic, across brush-border membrane vesicles from rat small intestine. *J. Pharmacol. Exp. Ther.* 241:594-601 (1987).
9. S. A. Adibi and Y. S. Kim. Peptide absorption and hydrolysis. In L. R. Johnson (ed.), *Physiology of the Gastrointestinal Tract*, 1st ed., Raven Press, New York, 1981, p. 1073.
10. V. H. L. Lee, S. Dodda-Kashi, G. M. Grass, and W. Rubas. Oral route of peptide and protein drug delivery. In V. H. L. Lee (ed.), *Peptide and Protein Drug Delivery*, Marcel Dekker, New York, 1991, pp. 691-738.
11. M. Saffran, C. Bedra, G. S. Kumar, and D. C. Neckers. Vaspressin: A model for the study of effects of additives on the oral and rectal administration of peptide drugs. *J. Pharm. Sci.* 77:33-38 (1988).
12. K. Takaori, J. Burton, and M. Donawitz. The transport of an intact oligopeptide across adult mammalian jejunum. *Biochem. Biophys. Res. Comm.* 137:682-687 (1986).
13. A. J. Wood, G. Maurer, W. Niederberger, and T. Beveridge. Cyclosporine: Pharmacokinetics, metabolism, and drug interactions. *Transplant. Proc.* 15:2409 (1983).
14. M. Kidron, H. Bar-On, E. M. Berry, and E. Ziv. The absorption of insulin from various regions of the rat intestine. *Life Sci.* 31:2937-2841 (1982).
15. C. A. R. Boyd and M. R. Ward. A micro-electrode study of oligopeptide absorption by the small intestinal epithelium of *Necturus maculosus*. *J. Physiol.* 324:411-428 (1982).
16. J. M. Addison, D. Burston, J. W. Payne, S. Wilkison, and D. M. Matthews. Evidence for active transport of tripeptides by hamster jejunum *in vitro*. *Clin. Sci. Mol. Med.* 49:305-312 (1975).
17. W. Kramer, U. Gutjahr, F. Girbig, and I. Leipe. Intestinal absorption of dipeptides and  $\beta$ -lactam antibiotics. II. purification of the binding protein for dipeptides and  $\beta$ -lactam antibiotics from rabbit small intestinal brush border membrane. *Biochim. Biophys. Acta* 1030:50-55 (1990).
18. V. Ganapathy and F. H. Leibach. Is intestinal peptide transport energized by a proton gradient? *Am. J. Physiol.* 249:G153-G160 (1985).
19. J. M. Addison, D. M. Matthews, and D. Burston. Evidence for active transport of the dipeptide carnosine ( $\beta$ -alanyl-L-histidine) by hamster jejunum *in vitro*. *Clin. Sci. Mol. Med.* 46:707-714 (1974).
20. M. Das and A. N. Radhakrishnan. Studies on a wide-spectrum intestinal dipeptide uptake system in the monkey and in the human. *Biochem. J.* 146:133-139 (1975).
21. A. Rubino, M. Field, and H. Schwachman. Intestinal transport of amino acid residues of dipeptides. I. Influx of the glycine residue of glycyl-L-proline across mucosal border. *J. Biol. Chem.* 246:3542-3548 (1971).
22. J. M. Addison, D. Burston, J. A. Dalrymple, D. M. Matthews, J. W. Payne, M. H. Sleisenger, and S. Wilkinson. A common mechanism for transport of di- and tripeptides by hamster jejunum *in vitro*. *Clin. Sci. Mol. Med.* 49:313-322 (1975a).
23. Y. Tomita, T. Katsura, T. Okano, K. I. Inui, and R. Hori. Transport mechanisms of bestatin in rabbit intestinal brush-border membrane: Role of  $H^+$ /dipeptide cotransport system. *J. Pharmacol. Exp. Ther.* 252:859-862 (1990).
24. P. F. Bai, P. Subramanian, H. I. Mosberg, and G. L. Amidon. Structural requirements for the intestinal mucosal cell peptide transporter: The need for N-terminal  $\alpha$ -amino group. *Pharm. Res.* 8:593-599 (1991).
25. V. M. Rajendran, S. A. Ansari, J. M. Harig, M. B. Adams, A. H. Khan, and K. Ramaswamy. Transport of glycyl-L-proline by human intestinal brush border membrane vesicles. *Gastroenterology* 89:1298-1304 (1985).
26. A. M. Asatoor, A. Chadha, M. D. Milne, and D. I. Prosser. Intestinal absorption of stereoisomers of dipeptides in the rat. *Clin. Sci. Mol. Med.* 45:199-212 (1973).
27. C. I. Cheeseman and D. H. Smyth. Specific transfer process for intestinal absorption of peptides. *Pro. Physiol. Soc.* Nov:45p-46p (1972).
28. D. Burston, J. M. Addison, and D. M. Matthews. Uptake of dipeptides containing basic and acidic amino acids by rat small intestine *in vitro*. *Clin. Sci.* 43:823-837 (1972).
29. D. M. Oh, P. J. Sinko, and G. L. Amidon. Characterization of the oral absorption of some penicillins: Determination of intrinsic membrane absorption parameters in the intestine *in situ*. *Pharm. Res.* 6:S-91 (1989).
30. D. M. Oh, P. J. Sinko, and G. L. Amidon. Peptide transport of  $\beta$ -lactam antibiotics: structural requirements for an  $\alpha$ -amino group. *Pharm. Res.* 7:S-119 (1990).
31. A. Tsuji, H. Hirooka, I. Tamai, and T. Terasaki. Evidence for a carrier-mediated transport system in the small intestine available for FK089, a new cephalosporin antibiotic without an amino group. *J. Antibio.* 39:1592-1597 (1986).
32. I. Tamai, H. Y. Ling, S. M. Timbul, J. Nishikido, and A. Tsuji. Stereospecific absorption and degradation of cephalixin. *J. Pharm. Pharmacol.* 40:320-324 (1988).
33. S. Yee and G. L. Amidon. Intestinal absorption mechanism of three angiotensin-converting enzyme inhibitors: Quinapril, benazepril and CGS16617. *Pharm. Sci.* 7:S-155 (1990).
34. S. Yokohama, T. Yoshioka, K. Yamashita, and N. J. Kitamori. Intestinal absorption mechanisms of thyrotropin-releasing hormone. *Pharm. Dyn.* 7:445-451 (1984).
35. M. J. Humphrey and P. S. Ringrose. Peptides and related drugs: A review of their absorption, metabolism, and excretion. *Drug Metab. Rev.* 17:283-310 (1986).
36. W. Kramer, F. Girbig, U. Gutjahr, H. W. Kleemann, I. Leipe, H. Urbach, and A. Wagner. Interaction of renin inhibitors with the intestinal uptake system for oligopeptides and  $\beta$ -lactam antibiotics. *Biochim. Biophys. Acta* 1027:25-30 (1990).
37. B. G. Munck. Intestinal absorption of amino acids. In L. R. Johnson (ed.), *Physiology of the Gastrointestinal Tract*, Raven Press, New York, 1981, p. 1097.
38. G. Esposito. In T. Z. Csaky (ed.), *Pharmacology of Intestinal*



- Permeation I*, Springer-Verlag, Berlin, Heidelberg, 1984, p. 567.
39. D. N. Wade, P. T. Mearrick, and J. I. Morris. Active transport of L-dopa in the intestine. *Nature* 242:463-465 (1972).
  40. M. Hu, P. Subramanian, H. I. Mosberg, and G. L. Amidon. Use of the peptide carrier system to improve intestinal absorption of L- $\alpha$ -methyl-dopa: Carrier kinetics, intestinal permeabilities, and *in vitro* hydrolysis of dipeptidyl derivatives of L- $\alpha$ -methyl-dopa. *Pharm. Res.* 6:66-70 (1989).
  41. M. Merino, I. L. Peris-Ribera, F. Torres-Molina, A. Sánchez-Picó, M. C. García-Carbonell, V. G. Casabó, A. Martín-Villodre, and J. M. Plá-Delfina. Evidence for a specialized transport mechanism for the intestinal absorption of baclofen. *Biopharm. Drug Dispos.* 10:279-297 (1989).
  42. A. E. Merfeld, A. R. Mlodozieniec, M. A. Cortese, J. B. Rhodes, J. B. Dressman, and G. L. Amidon. The effect of pH and concentration on  $\alpha$ -methyl-dopa absorption in man. *J. Pharm. Pharmacol.* 38:815-822 (1986).
  43. N. Border, K. B. Sloan, and T. Higuchi. Improved delivery through biological membrane. 4. Prodrugs of L-dopa. *J. Med. Chem.* 20:1435-1445 (1977).
  44. D. Burston, J. M. Addison, and D. M. Matthews. Uptake of dipeptides containing basic and acidic amino acids by rat small intestine *in vitro*. *Clin. Sci.* 43:823-837 (1972).
  45. P. F. Bai, M. Hu, P. Subramanian, H. I. Mosberg, and G. L. Amidon. Utilization of peptide carrier system to improve the intestinal absorption: Targeting prolidase as a prodrug converting enzyme. *J. Pharm. Sci.* 80:1-4 (1991b).
  46. Y. S. Kim. Intestinal mucosal hydrolysis of proteins and peptides. In K. Elliot and M. O'Connor (eds.), *Peptide Transport and Hydrolysis*, Ciba Foundation Symposium, Associated Scientific, Amsterdam, 1977, p. 151.
  47. E. E. Sterchi and J. F. Woodley. Peptide hydrolases of the human small intestinal mucosa: Distribution of activities between brush border membranes and cytosol. *Clin. Chim. Acta* 102:49-56 (1980).
  48. S. Miura, I. S. Song, A. Morita, R. H. Erickson, and Y. S. Kim. Distribution and biosynthesis of aminopeptidase N and dipeptidyl aminopeptidase IV in rat small intestine. *Biochim. Biophys. Acta* 761:66-75 (1983).
  49. T. Q. Garvey, P. E. Hyman, and K. J. Isselbacher.  $\gamma$ -Glutamyl transpeptidase of rat intestine: Localization and possible role in amino acid transport. *Gastroenterology* 71:778-785 (1976).
  50. M. Yoshioka, R. H. Erickson, J. F. Woodley, R. Gullis, D. Guan, and Y. S. Kim. Role of rat intestinal brush-border membrane angiotensin-converting enzyme in dietary protein digestion. *Am. J. Physiol.* 253:G781-G786 (1987).
  51. H. Skovbjerg. Immunoelectrophoretic studies on human small intestinal brush border proteins—the longitudinal distribution of peptidases and disaccharidases. *Clin. Chim. Acta* 112:205-212 (1981).
  52. N. Triadou, J. Bataille, and J. Schmitz. Longitudinal study of the human intestinal brush border membrane proteins: Distribution of the main disaccharidases and peptidases. *Gastroenterology* 85:1326-1332 (1983).
  53. S. Auricchio, L. Greco, B. D. G. Vizia, and V. Buonocore. Dipeptidylaminopeptidase and carboxypeptidase activities of the brush border of rabbit small intestine. *Gastroenterology* 75:1073-1079 (1978).
  54. R. K. Kania, N. A. Santiago, and G. M. Gray. Intestinal surface amino-oligopeptidases. II. Substrate kinetics and topography of the active site. *J. Biol. Chem.* 252:4929-4934 (1977).
  55. A. J. Kenny and S. Maroux. Topology of microvillar membrane hydrolases of kidney and intestine. *Physiol. Rev.* 62:91-128 (1982).
  56. Y. S. Kim and E. J. Brophy. Rat intestinal brush border membrane peptidases. I. Solubilization, purification, and physicochemical properties of two different forms of the enzyme. *J. Biol. Chem.* 251:3199-3205 (1976).
  57. Y. S. Kim, E. J. Brophy, and J. A. Nicholson. Rat intestinal brush border membrane peptidases. II. Enzymatic properties, immunochemistry and interactions with lectins of two different forms of the enzyme. *J. Biol. Chem.* 251:3206-3212 (1976).
  58. S. T. Suresh. Microvillus membrane peptidases that catalyze hydrolysis of cysteinylglycine and its derivatives. In *Methods in Enzymology*, Academic Press, New York, 1985, Vol. 113, p. 471.
  59. D. I. Friedman and G. L. Amidon. Oral absorption of peptides: Influence of pH and inhibitors on the intestinal hydrolysis of leu-enkephalin and analogues. *Pharm. Res.* 8:93-96 (1991).
  60. N. Tobey, W. Heizer, R. Yeh, T. I. Huang, and C. Hoffner. Human intestinal brush border peptidases. *Gastroenterology* 88:913-926 (1985).
  61. R. Walter, W. H. Simmons, and T. Yoshimoto. Proline specific endo- and exopeptidases. *Mol. Cell. Biochem.* 30:111-127 (1980).
  62. B. Svensson, M. Danielsen, M. Staun, L. Jeppesen, O. Noren, and H. Sjostrom. An amphiphilic form of dipeptidyl peptidase IV from pig small-intestinal brush-border membrane. *Eur. J. Biochem.* 90:489-498 (1978).
  63. J. Lasch, R. Koelsch, A. M. Ladhoff, and B. Hartrodt. Is the proline-specific aminopeptidase P of the intestinal brush border an integral membrane enzyme? *Biomed. Biochim. Acta* 45:833-843 (1986).
  64. E. J. Holtzman, G. Pillay, T. Rosenthal, and A. Yaron. Aminopeptidase P activity in rat organs and human serum. *Anal. Biochem.* 162:476-484 (1987).
  65. J. Lasch, R. Koelsch, T. Steinmetzer, U. Neumann, and H. U. Demuth. Enzymic properties of intestinal aminopeptidase P: A new continuous assay. *FEBS* 227:171-174 (1988).
  66. W. Sidorowicz, J. Szechinski, P. C. Canizaro, and F. J. Behal. Cleavage of the Arg<sup>1</sup>-Pro<sup>2</sup> bond of bradykinin by a human lung peptidase: Isolation, characterization, and inhibition by several  $\beta$ -lactam antibiotics (41828). *Proc. Soc. Exp. Biol. Med.* 175:503-509 (1984).
  67. M. Yoshioka, R. H. Erickson, and Y. S. Kim. Digestion and assimilation of proline-containing peptides by rat intestinal brush border membrane carboxypeptidases. *J. Clin. Invest.* 81:1090-1095 (1988).
  68. B. R. Stevens, A. Fernandez, C. Kneer, J. J. Cerda, M. I. Phillips, and E. R. Woodward. Human intestinal brush border angiotensin-converting enzyme activity and its inhibition by antihypertensive ramipril. *Gastroenterology* 94:942-947 (1988).
  69. H. Yokosawa, S. Endo, Y. Ohgaki, J. I. Maeyama, and S. I. Ishii. Hydrolysis of substance p and its analogs by angiotensin-converting enzyme from rat lung: Characterization of endopeptidase activity of the enzyme. *J. Biochem.* 98:1293-1299 (1985).
  70. E. M. Danielsen, J. P. Vyas, and A. J. Kenny. A neutral endopeptidase in the microvillar membrane of pig intestine. *Biochem. J.* 191:645-648 (1980).
  71. N. W. Bunnett, A. J. Turner, J. Hryszko, R. Kobayashi, and J. H. Walsh. Isolation of endopeptidase-24.11 (EC 3.4.24.11, "enkephalinase") from the pig stomach. *Gastroenterology* 95:952-957 (1988).
  72. R. A. Skidgel and E. G. Erdős. Novel activity of human angiotensin I converting enzyme: Release of the NH<sub>2</sub>- and COOH-terminal tripeptides from the luteinizing hormone-releasing hormone. *Proc. Natl. Acad. Sci.* 82:1025-1029 (1985).
  73. C. Doumeng and S. Maroux. Aminopeptidase, a cytosol enzyme from rabbit intestinal mucosa. *Biochem. J.* 177:801-808 (1979).
  74. F. Endo, I. Matsuda, A. Ogata, and S. Tanaka. Human erythrocyte prolidase and prolidase deficiency. *Pediatr. Res.* 16:227-231 (1982).
  75. G. Mano, P. Nassi, G. Cappugi, G. Camici, and G. Ramponi. Swine kidney prolidase: Assay, isolation procedure, and molecular properties. *Physiol. Chem. Phys.* 4:75-87 (1972).
  76. H. Sjöström and O. Norén. Structural properties of pig intestinal proline dipeptidase. *Biochim. Biophys. Acta* 359:177-185 (1974).
  77. T. Yoshimoto, F. Matsubara, E. Kawano, and D. Tsuru. Prolidase from bovine intestine: Purification and characterization. *J. Biochem.* 94:1889-1896 (1983).
  78. L. N. Lin and J. F. Brandts. Evidence suggesting that some proteolytic enzymes may cleave only the trans form of the peptide bond. *Biochemistry* 18:43-47 (1979).

79. I. Myara, C. Charpentier, and A. Lemonnier. Minireview: Prolidase and prolidase deficiency. *Life Sci.* 34:1985-1998 (1984).
80. J. F. Lenney, P. W. H. Chan, R. P. George, C. M. Kucera, G. S. Rinzler, and A. M. Weiss. (1982). Human-serum carnosinase, and activation by cadmium. *Clin. Chim. Acta* 123:221-231 (1982).
81. J. F. Lenney, R. P. George, S. C. Peppers, and C. M. Kucera. Characterization of human-tissue carnosine. *Biochem. J.* 228:653-660 (1985).
82. S. C. Peppers and J. Lenney. Bestatin inhibition of human tissue carnosinase, a non-specific cytosolic dipeptidase. *Biol. Chem. Hoppe-Seyler* 368:1281-1286 (1988).
83. B. Colas and S. Maroux. Simultaneous isolation of brush border and basolateral membrane from rabbit enterocytes: Presence of brush border hydrolases in the basolateral membrane of rabbit enterocytes. *Biochim. Biophys. Acta* 600:406-420 (1980).
84. R. Nau, G. Schäfer, and J. M. Colon. Proteolytic inactivation of substance P in the epithelial layer of the intestine. *Biochem. Pharmacol.* 34:4019-4023 (1985).