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STUDIES ON THE PROPERTIES OF PEPTIDE HYDROLASES IN THE BRUSH-BORDER AND SOLUBLE FRACTIONS OF SMALL INTESTINAL MUCOSA OF RAT AND MAN

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SUMMARY

The properties of intestinal peptide hydrolases from two main subcellular loci, the brush-border and the cytosol fractions from man and rat, were studied.

Peptide hydrolases were partially purified from the brush-border and cytosol fractions of small intestinal mucosa and the properties of these enzymes were examined using the following peptide substrates: L-alanylglycine, L-alanyldiglycine, L-alanyltriglycine, L-alanyltetraglycine, glycyl-L-alanine, diglycyl-L-alanine, di-, tri- and tetraglycine and a heterohexapeptide. Analysis of the reaction products after incubation of these peptides with intestinal peptide hydrolases indicated that in man and rat, both the brush-border and the cytosol enzymes are amino-oligopeptidases. When these peptides were used as substrates, the cytosol enzymes of both rat and human small intestinal mucosa were capable of hydrolyzing di- and tripeptides but could not hydrolyze peptides of greater chain length. In contrast, the brush-border enzymes prepared from both rat and man hydrolyzed all the peptide substrates including a heterohexapeptide.

Data obtained from studies of heat stability and competition with various peptide substrates suggest that two enzymes probably are involved in the hydrolysis of L-alanylglycine and L-alanyldiglycine by the cytosol fraction. A similar study obtained with a brush-border preparation suggests that a single binding site may be involved in the hydrolysis of peptides of varying chain length.

The data presented herein indicate that the peptide hydrolases of the brush-border and the cytosol fraction are distinct and further suggest that these enzymes from two subcellular loci play important roles in different stages of digestion and absorption of dietary proteins.

INTRODUCTION

It has been shown that the peptide hydrolases (EC 3.4.–.–) of small intestinal mucosal cells are primarily found in two main subcellular locations, the brush-border

and the cytosol [1, 2]. Though considerable work has been carried out on these hydrolases in both subcellular loci [3–5], information concerning kinetic properties and functional aspects of these enzymes has been lacking.

That the cytosol peptide hydrolases may be distinct from those in the brush-border is suggested by recent studies indicating differential heat stability [6, 7], effect of *p*-hydroxymercuribenzoate [6] and electrophoretic mobilities [2] of these enzymes from two different subcellular locations. Recent studies on the effect of starvation [8], of small bowel resection [9] or of feeding diets of varying protein contents [10] on the brush-border and cytosol peptide hydrolases of rat small intestinal mucosa suggest that the biological roles of peptide hydrolases in these two subcellular loci may also be distinct.

Thus it is evident that a further elucidation of the properties of peptide hydrolases in two subcellular loci is needed for a proper understanding of the functional roles that these enzymes may play. The purpose of the present study was to examine in detail, three aspects of the action of these enzymes: First, which end of the peptide molecule is preferentially cleaved, i.e. do the enzymes release amino acids from the amino terminal or from the carboxylterminal of the peptides? Second, what effect has the chain length of a peptide substrate on its susceptibility to hydrolysis? Third, are there differences in the kinetics and other biochemical parameters of peptide hydrolysis between cytosol and brush-border enzymes?

METHODS

Preparation of brush-border and cytosol fractions

Male albino Sprague–Dawley (Simonson Labs, Inc., Gilroy, Calif.) rats (250–300 g) were fasted overnight and were killed by decapitation. The whole small intestine was quickly removed and mucosa scraped with a spatula after rinsing in ice-cold isotonic saline at 4 °C. Rat brush-border preparations were prepared by the method of Eichholz and Crane [11]. For the preparation of the cytosol fraction, mucosal scrapings were homogenized in 14% glycerol (4 ml/g) in a Potter–Elvehjem tissue homogenizer by eight strokes of teflon pestle with a 0.004–0.006 cm clearance driven by Con-Torque (Eberbach Corp., Ann Arbor, Mich.) stirrer at medium speed. The homogenate was passed through two layers of cheesecloth and then centrifuged at 20 000 × *g* for 20 min. The resultant supernatant fluid was centrifuged at 105 000 × *g* for 1 h and the postmicrosomal supernatant fraction was designated as the cytosol fraction [2]. Full-thickness sections of human small intestine obtained at surgery were sealed in foil and frozen on dry ice immediately after removal. 2 weeks later, the whole specimen was thawed, washed in cold isotonic saline and the brush-borders prepared according to the method of Welsh et al. [12]. For preparing the human cytosol fraction, the mucosa was scraped, homogenized in 14% glycerol and centrifuged in a manner similar to that described above for preparing the rat cytosol fraction.

Enzyme preparation

For the solubilization of brush-border enzymes, the membranes were suspended in 14% glycerol. The brush-border preparation containing 3.5 mg protein/ml was added to an equal volume of 0.1 M sodium phosphate buffer, pH 7.0, containing

papain (1 mg/ml) and L-cysteine hydrochloride hydrate (0.5 mg/ml). The reaction mixture was incubated at 37 °C for 1 h and centrifuged at 45 000 × *g* for 30 min. The supernatant fraction was reduced to one-third of its volume by dialysis against polyethylene glycol (20%, w/v) in 14% glycerol. The solubilized brush-border enzymes and the cytosol fraction were then applied to a 1.2 cm × 65 cm column of Biogel A 0.5 M and eluted with 0.02 M borate buffer, pH 7.4, in 14% glycerol at a flow rate of 7.5 ml/h. The elution profile was similar to that described previously [2]. The eluted fractions containing peptide hydrolase activity were pooled and used as a source of enzyme.

Enzyme assay

To obtain an elution profile of peptide hydrolase activity from the gel-filtration step, a modification [2] of the method of Josefsson and Lindberg [3] was used. For the assay of peptide hydrolase activities in the remainder of the study, a method was developed utilizing a Beckman amino acid analyzer (Model 120C). The standard assay mixture contained 375 nmoles of peptide substrate and the enzymes (0.8 μg protein from the cytosol fraction or 2.4 μg from the brush-border preparation) in 0.05 M KCl–borate buffer, pH 7.6, in a final volume of 100 μl. The reaction mixture was incubated at 37 °C for 10 min in a shaking water bath and the reaction stopped by quickly adding 200 μl of 7.5% sulfosalicylic acid. The acidified reaction mixture was diluted as desired by adding sodium citrate (0.2 M, pH 2.2), and stored at −20 °C until analysis on the amino acid analyzer. Under these assay conditions, the linearity of the enzyme activity was achieved with respect to time and the amount of enzyme. Control incubations consisting of either enzyme alone or substrate alone were carried out and were also analyzed on the amino acid analyzer. Enzyme samples from cytosol or brush-border fractions did not yield measurable amounts of amino acids or peptides. Protein was determined by the method of Lowry et al. [13].

Chromatography

Separation and quantitation of amino acids and peptides of varying chain lengths were performed by the use of a Beckman amino acid analyzer. The sample (0.25 ml) was applied to a UR-30 column (56 cm × 0.9 cm) maintained at 55.5 °C and eluted with sodium citrate buffer (0.2 M, pH 3.25) for 250 min at a flow rate of 68 ml/h.

Chemicals

The following amino acids and peptides were used in this study: L-alanylglycine, L-alanyldiglycine, L-alanyltriglycine, L-alanyltetraglycine, glycyl-L-alanine, diglycyl-L-alanine and tetraglycine were purchased from Cyclo Chemical Corp., Los Angeles, Calif., and L-alanine, glycine, diglycine and triglycine from Calbiochem, Los Angeles, Calif. A heterohexapeptide, L-leucyl-L-tryptophanyl-L-methionyl-L-arginyl-L-phenylalanyl-L-alanine was obtained from Schwartz/Mann, Orangeburg, New York. All the peptides and amino acids used in this study yielded single peaks on the amino acid analyzer indicating chromatographic purity.

RESULTS

Chromatographic analysis of substrates and reaction products

Optimal separation and quantitation of substrates and possible reaction products to be expected in this study were achieved as shown in Fig. 1. The only exception was that L-alanyldiglycine and L-alanylglycine were eluted at the same time.

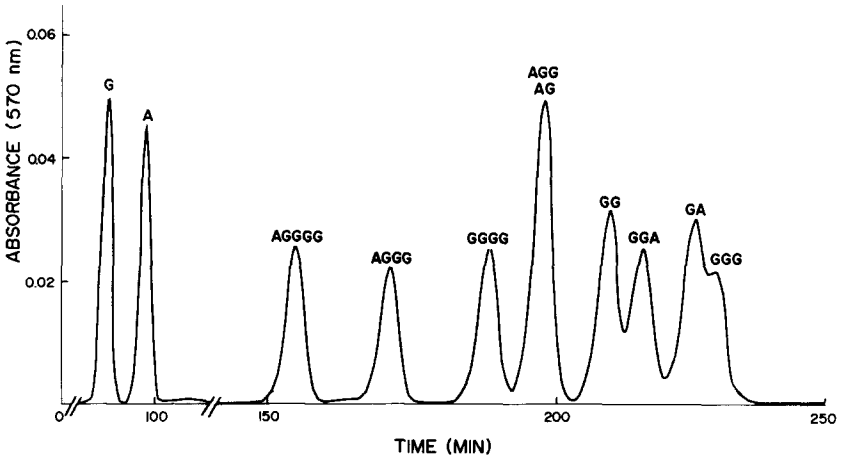

Fig. 1. Elution profile of substrates examined and possible products of hydrolysis on UR-30 resin column of Beckman 120C amino acid analyzer. A, alanine; G, glycine.

Hydrolysis of peptide substrates by enzymes from cytosol and brush-border fractions

The products of hydrolysis by peptide hydrolases from cytosol and brush-border fractions of rat small intestinal mucosa are shown, respectively, in Tables I and II. As can be seen in Table I, cytosol enzymes from rat intestine were capable of

TABLE I

EFFECT OF CYTOSOL ENZYMES OF RAT SMALL INTESTINAL MUCOSA ON PEPTIDE SUBSTRATES

Substrates	Products*					
	Ala	Gly	Gly–Ala	Gly–Gly	Gly–Gly–Gly	Gly–Gly–Gly–Gly
Gly–Gly	**	6.4				
Gly–Gly–Gly		17.9		18.3		
Gly–Gly–Gly–Gly		—***		—	—	
Ala–Gly	33.1	32.9				
Ala–Gly–Gly	58.4	0.7		57.2		
Ala–Gly–Gly–Gly	—	—		—	—	
Ala–Gly–Gly–Gly–Gly	—	—		—	—	—
Gly–Ala	10.7	11.0				
Gly–Gly–Ala	1.1	28.7	28.2	—		

* Numbers represent nmoles of products formed after 10 min of incubation.

** Spaces are left blank where values would represent the substrate under investigation or where the product could not result from hydrolysis of substrate.

*** — represents non-measurable amount, i.e. less than 0.5 nmole of product formed after 10 min of incubation.

TABLE II

EFFECT OF BRUSH-BORDER ENZYMES OF RAT SMALL INTESTINAL MUCOSA ON PEPTIDE SUBSTRATES

Substrates	Products*					
	Ala	Gly	Gly–Ala	Gly–Gly	Gly–Gly–Gly	Gly–Gly–Gly–Gly
Gly–Gly	**	2.2				
Gly–Gly–Gly		3.6		3.9		
Gly–Gly–Gly–Gly		3.0		—***	2.7	
Ala–Gly	8.1	8.1				
Ala–Gly–Gly	12.2	—		11.4		
Ala–Gly–Gly–Gly	12.1	—		—	11.6	
Ala–Gly–Gly–Gly–Gly	16.9	—		—	—	16.4
Gly–Ala	4.7	4.4				
Gly–Gly–Ala	—	7.4	7.2	—		

* Numbers represent nmoles of products formed after 10 min of incubation.

** Spaces are left blank where values would represent the substrate under investigation or where the product could not result from hydrolysis of substrate.

*** — represents non-measurable amount, i.e. less than 0.5 nmole of product formed after 10 min of incubation.

hydrolyzing only di- and tripeptides. Tetraglycine was not hydrolyzed. This was true even when incubations were carried out for up to 10 h. Since with a glycine homopeptide substrate it was not possible to determine whether amino or carboxyterminal amino acids were released, glycylpeptides having alanine either at the N-terminal or at the C-terminal end were studied. Again cytosol enzymes hydrolyzed di- and tripeptides but not longer peptides. When L-alanyldiglycine was the substrate, L-alanine and diglycine were produced with only a trace amount of glycine. The hydrolysis products of the brush-border enzymes from rat small intestinal mucosa are shown in Table II. In contrast to the cytosol enzymes, brush-border enzymes were effective in hydrolyzing peptides containing more than three amino acids. Tetraglycine which was not cleaved by the cytosol fraction was hydrolyzed by the brush-border enzymes at a rate comparable to that of triglycine. When the terminal specificity of the brush-border fraction was examined, N-terminal amino acids were released as was the case for the cytosol fraction. For instance when L-alanyldiglycine was the substrate, only L-alanine and diglycine were produced. Except when L-alanylglycine was the substrate, no glycine was detected from any other substrate in this group. When diglycyl-L-alanine was the substrate only glycine and glycyl-L-alanine were produced; no L-alanine was detected. Similar results were obtained with cytosol fractions and brush border fractions of human small intestinal mucosa as shown in Table III.

Hydrolysis of a heterohexapeptide

Peptide hydrolases from cytosol fractions of both rat and human small intestinal mucosa failed to release amino acids from a hexapeptide, L-leucyl-L-tryptophanyl-L-methionyl-L-arginyl-L-phenylalanyl-L-alanine even after 10 h of incubation. By contrast, the brush-border enzymes from rat intestine hydrolyzed the hexapeptide as shown in Fig. 2. There was a gradual increase in all amino acids released from the

TABLE III

EFFECT OF PEPTIDE HYDROLASES OF HUMAN SMALL INTESTINAL MUCOSA ON PEPTIDE SUBSTRATES

Substrates	Cytosol enzymes		Brush-border enzymes	
	Alanine*	Glycine*	Alanine	Glycine
Gly–Gly	**	4.3***		8.1
Gly–Gly–Gly		13.4		6.8
Gly–Gly–Gly–Gly		—****		9.5
Ala–Gly	2.7	2.2	3.7	3.5
Ala–Gly–Gly	76.0	—	51.8	—
Ala–Gly–Gly–Gly	—		51.9	—
Ala–Gly–Gly–Gly–Gly	—	—	41.5	—
Gly–Ala	3.4	3.6	5.1	5.9
Gly–Gly–Ala	—	32.2	—	37.2

* Alanine and glycine are amino acids produced as a result of the enzymatic hydrolysis.
** Spaces are left blank where the product could not result from hydrolysis of substrate.
*** Numbers represent nmoles of products formed after 10 min of incubation.
**** — represents non-measurable amount, i.e. less than 0.5 nmole of product formed after 10 min of incubation.

substrate with time. Proportionately more of the amino acids close to the N-terminus were released with progressively lesser amounts of those derived from the C-terminal end. With both rat and human brush-border enzymes, nearly 50% of L-leucine, L-tryptophan, L-methionine and L-arginine were released from the hexapeptide after 1 h of incubation while virtually no L-phenylalanine or L-alanine was detected at that time. By 6 h of incubation, however, 30% to 50% of L-phenylalanine and L-alanine had been released. The brush-border enzymes from human intestine showed a similar result.

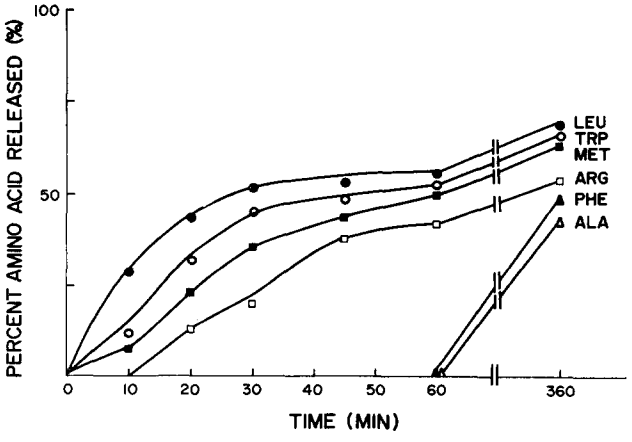

Fig. 2. Effect of rat brush-border peptide hydrolases on a hexapeptide (L-leucyl-L-tryptophanyl-L-methionyl-L-arginyl-L-phenylalanyl-L-alanine). The time represents the number of minutes the reaction mixture was incubated at 37 °C. LEU, L-leucine; TRP, L-tryptophan; MET, L-methionine; ARG, L-arginine; PHE, L-phenylalanine; and ALA, L-alanine.

Effect of pH

The effects of pH on enzyme activity in brush-border and cytosol fractions of rat small intestinal mucosa are shown in Figs 3A and 3B. Cytosol enzymes showed optimum activity at pH 8.5 when L-alanyldiglycine or L-alanylglycine was used as substrate. Optimal pH for brush-border peptide hydrolase activity against peptide substrates of varying chain length was also between 8 and 8.5.

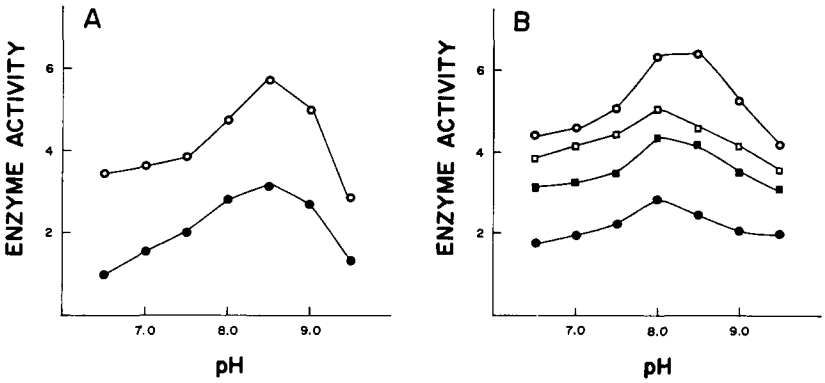

Fig. 3. Influence of pH on peptide hydrolase activities of rat small intestinal mucosa. (A) Peptide hydrolase activities of the cytosol fraction were examined at different pH. (B) Peptide hydrolase activities of rat brush-border fraction were examined at different pH. Peptide substrates used were L-alanylglycine (●); L-alanyldiglycine (○); L-alanyltriglycine (■); and L-alanyltetraglycine (□).

The effect of bivalent ions and EDTA

Tables IV and V illustrate the effect of bivalent metal ions and EDTA on the peptide hydrolase activities of brush-border and cytosol fractions of rat small intestinal mucosa. Additions of EDTA, Mg^{2+} or Mn^{2+} at 50 or 500 μM did not affect either the cytosol or brush-border peptide hydrolase activities of rat small intestinal mucosa using L-alanylglycine, L-alanyldiglycine, L-alanyltriglycine or L-alanyltetraglycine as substrate. Though not shown in Table V, these ions had no effect on the cytosol enzyme activity even at 1 mM. The brush-border enzyme activity with these substrates was not affected by the addition of Co^{2+}, while the cytosol enzyme activity with either L-alanylglycine or L-alanyldiglycine was significantly inhibited by Co^{2+} at a concentration of 500 μM but not at 50 μM. Zn^{2+}, however, inhibited both the cytosol and brush-border enzyme activities; the enzyme activity in the cytosol fraction was nearly completely abolished by Zn^{2+} even at 50 μM. Though not shown in Table V, the cytosol enzyme activity was inhibited by Zn^{2+} at a 1 μM concentration; L-alanylglycine and L-alanyldiglycine, 71% and 19% inhibition, respectively. When di- and triglycine were used as substrates somewhat different results were obtained. Zn^{2+} inhibited both the brush-border and cytosol enzyme activity against these substrates. EDTA caused varying degrees of inhibition of the enzyme activity in both subcellular fractions when diglycine was used as the substrate. When triglycine was used as the substrate, however, only the brush-border enzyme activity was inhibited by EDTA. The addition of Co^{2+} did not affect the brush-border enzyme activity against di- and triglycine but the cytosol enzyme activity with these substrates was

TABLE IV

EFFECT OF BIVALENT METAL IONS AND EDTA ON PEPTIDE HYDROLASE ACTIVITIES OF BRUSH-BORDER FRACTION OF RAT SMALL INTESTINAL MUCOSA

Metal ion added	Concentration (μM)	Substrates (%)*					
		Ala–Gly	Ala–Gly–Gly	Ala–Gly–Gly–Gly	Ala–Gly–Gly–Gly–Gly	Gly–Gly	Gly–Gly–Gly
Co^{2+}	50	101	104	95	101	101	99
	500	99	99	99	100	103	97
Mg^{2+}	50	99	99	100	103	100	94
	500	98	102	97	98	98	101
Mn^{2+}	50	98	98	98	104	95	95
	500	100	98	97	100	99	100
Zn^{2+}	50	89	74	78	75	83	44
	500	73	32	17	28	67	7
EDTA	50	98	100	96	101	78	98
	500	98	98	100	100	63	31

* Enzyme activity is expressed as percentage of control incubation in the absence of metals or EDTA.

TABLE V

EFFECT OF BIVALENT METAL IONS AND EDTA ON PEPTIDE HYDROLASE ACTIVITIES OF CYTOSOL FRACTION OF RAT SMALL INTESTINAL MUCOSA

Metal ion added	Concentration (μM)	Substrates (%)*			
		Ala–Gly	Ala–Gly–Gly	Gly–Gly	Gly–Gly–Gly
Co^{2+}	50	90	76	136	109
	500	49	35	213	75
Mg^{2+}	50	98	96	102	99
	500	100	94	96	96
Mn^{2+}	50	99	99	96	100
	500	101	93	92	105
Zn^{2+}	50	0	11	86	24
	500	0	0	58	0
EDTA	50	103	98	63	96
	500	98	99	51	101

* Enzyme activity is expressed as percentage of control incubation in the absence of metals or EDTA.

markedly increased by the addition of this ion. Other ions had little effect on the activity of these enzymes irrespective of their subcellular loci.

Heat stability

Cytosol enzymes showed differential thermal stabilities depending on the substrate used. The rat cytosol enzymes were relatively stable at 40 and 50 °C when L-alanyldiglycine was the substrate, while virtually no enzyme activity remained when L-alanylglycine was the substrate (Fig. 4). Human cytosol enzymes showed similar but less marked difference in thermal stability with L-alanylglycine and L-alanyl-

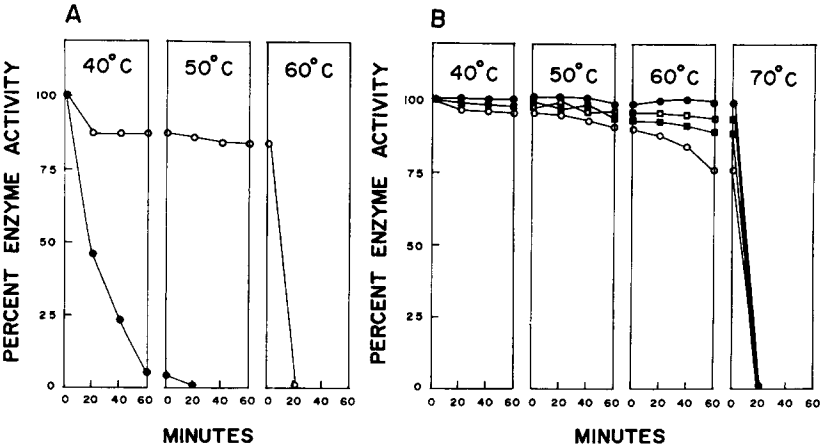

Fig. 4. Stability of peptide hydrolase activities of rat small intestinal mucosa for various peptide substrates at 40, 50, 60 and 70 °C. (A) Cytosol enzymes and (B) brush-border enzyme. Peptide substrates of varying chain length were used: L-alanylglycine (●); L-alanyldiglycine (○); L-alanyltriglycine (■); and L-alanyltetraglycine (□).

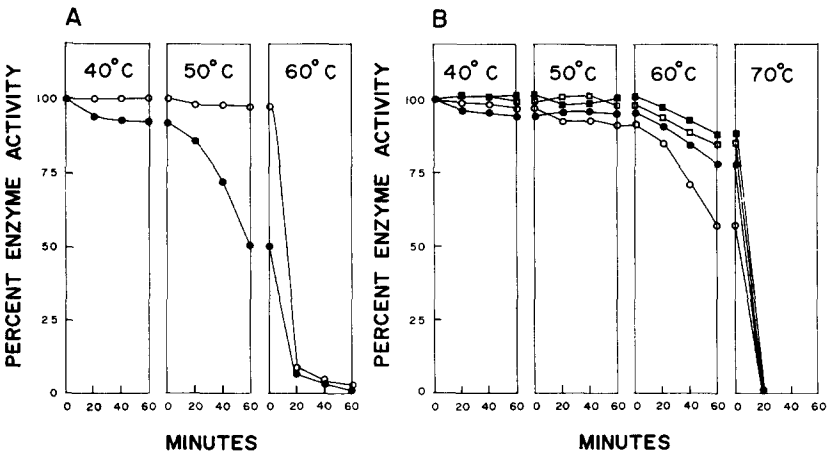

Fig. 5. Stability of peptide hydrolase activities of human small intestinal mucosa for various peptide substrates at 40, 50, 60 and 70 °C. (A) Cytosol enzymes and (B) brush-border enzyme. The same peptide substrates used in Fig. 4 were used.

diglycine as substrates (Fig. 5). In marked contrast to the cytosol enzymes, the brush-border enzymes of both rat and human intestine showed considerable thermal stability even at 60 °C (Figs 4 and 5).

Kinetics of peptide hydrolases

The kinetic parameters of peptide hydrolases from the two subcellular fractions of rat small intestinal mucosa are shown in Table VI. With cytosol enzymes the apparent K_m value was smaller and the apparent V value was greater with L-alanyldiglycine than the values obtained with L-alanylglycine. The values for apparent K_m and V seen with the rat brush-border enzymes differed from those of the cytosol and varied with peptide chain length.

TABLE VI

KINETIC PARAMETERS OF PEPTIDE HYDROLASES

Substrate	Peptide hydrolases			
	Cytosol		Brush border	
	K_m (mM)	V (nmoles hydrolyzed/min)	K_m (mM)	V (nmoles hydrolyzed/min)
Ala–Gly	2.35	5.25	0.65	1.65
Ala–Gly–Gly	0.95	6.95	1.29	3.92
Ala–Gly–Gly–Gly	—	—	1.01	2.15
Ala–Gly–Gly–Gly–Gly	—	—	0.84	3.25

Competition among peptide hydrolases

To determine whether the same or different enzymes hydrolyze the peptide substrates of different chain length, competition studies were carried out with the enzymes from the cytosol and the brush-border preparations of rat small intestinal

TABLE VII

COMPETITION AMONG CYTOSOL PEPTIDE HYDROLASES OF RAT SMALL INTESTINAL MUCOSA

Substrates		Enzyme activity (nmoles/min)	
Ala–Gly	Ala–Gly–Gly	Found**	Expected for two enzymes***
+*	–	2.78	
–	+	3.72	
+	+	6.40	6.50

* +, added; – omitted.
** Numbers in this column represent actual assay values.
*** Number in this column represents a value obtained by adding the enzyme assay value when a single peptide is used as a substrate.

mucosa using peptides of different chain length. The results are shown in Tables VII and VIII. It appears from Table VII that in the cytosol fraction an enzyme capable of hydrolyzing L-alanylglycine is distinct from that capable of hydrolyzing L-alanyldiglycine since the actual assay values of the enzyme preparation when both substrates were present was equal to the sum of those obtained with each individual substrate. Table VIII shows that a different result was obtained with the brush-border enzyme; the actual assay values of the enzymes when two substrates were present were less than the sum of those obtained with the individual substrate.

TABLE VIII

COMPETITION AMONG BRUSH-BORDER PEPTIDE HYDROLASES OF RAT SMALL INTESTINAL MUCOSA

Substrates				Enzyme activity (nmoles/min)	
Ala–Gly	Ala–Gly–Gly	Ala–Gly–Gly–Gly	Ala–Gly–Gly–Gly–Gly	Found**	Expected for two enzymes***
+*	–	–	–	0.84	
–	+	–	–	3.44	
–	–	+	–	3.00	
–	–	–	+	2.42	
+	+	–	–	3.10	4.28
+	–	+	–	2.90	3.84
+	–	–	+	2.56	4.26
–	+	+	–	3.08	6.44
–	+	–	+	3.72	6.86
–	–	+	+	3.60	6.42

* +, added; – omitted.
** Numbers represent actual assay values.
*** Numbers represent values obtained by adding the enzyme assay values when an individual peptide is used as a substrate.

DISCUSSION

We have found in the present studies that, using defined peptides, cytosol enzymes of rat and human intestinal mucosa were capable of hydrolyzing dipeptides and tripeptides but did not hydrolyze peptides of greater chain length. In contrast, brush-border enzymes of rat and man hydrolyzed all of the peptide substrates studied including a heterohexapeptide.

The data obtained in the present study indicate further that rat and human cytosol and brush-border peptide hydrolases release amino acid from the amino-terminus of peptides. This conclusion is based on experiments in which the major hydrolysis products obtained by the action of enzymes from both subcellular fractions of rat and man on L-alanyldiglycine were shown to be L-alanine and diglycine. That this amino-terminal hydrolysis is not due primarily to an affinity of the enzyme for the alanyl peptide bond was supported by experiments showing that glycine and glycyl-L-alanine were the major products of hydrolysis of diglycyl-L-alanine. The brush-border enzymes also released the amino acid from the amino-terminus of the tetra-, penta- and hexapeptides studied. Our results provide direct evidence with a number of peptides of different chain length that the brush-border enzymes are aminopeptidases as have been suggested [14–16]; and, furthermore, demonstrate that the cytosol peptide hydrolases are also aminooligopeptidases.

There have been several studies on the metal-ion requirements of the intestinal peptide hydrolases [1, 3–5, 17–19]. However, the results and the interpretations of these results have often been conflicting and difficult to interpret because of the varying concentrations of metal ions used, of species differences, and of the failure to examine both the brush-border and cytosol enzymes. The results obtained in the present study indicate that over the wide range of concentrations (ranging from 10 μM to 1 mM), the additions of EDTA, Mg^{2+} or Mn^{2+} did not affect the enzyme activity from the brush-border or cytosol fractions of rat small intestinal mucosa, when L-alanylglycine, L-alanyltriglycine and L-alanyltetraglycine were used as substrates. Metal-ion requirements for the enzymes appear to vary with different types of peptide substrates since Co^{2+} increased the cytosol enzyme activity considerably when diglycine was the substrate, while inhibiting the hydrolysis of L-alanylglycine and L-alanyldiglycine. Of interest was the consistent inhibition of all enzyme activities by the addition of Zn^{2+}. Furthermore, some metal ions had differential effects on enzymes from cytosol and brush-border fractions, i.e. the cytosol enzyme activity against L-alanylglycine and L-analyldiglycine was inhibited and that against diglycine was enhanced by the addition of Co^{2+} while the brush-border enzyme activity on these substrates remained unaffected by this ion. EDTA on the other hand inhibited the hydrolysis of triglycine by the brush-border enzyme at 500 μM while it had no effect on the cytosol enzymes against the same substrate.

Differential heat stabilities, metal-ion requirements, distinct kinetic properties and chain-length specificities of enzymes from two subcellular locations observed in the present study together with the distinct electrophoretic mobilities of these enzymes reported previously [2] strongly suggest that the enzymes in these two subcellular locations are two distinct groups of enzymes which can act on the same substrate. Furthermore, the present data from the heat stabilities of partially purified enzymes with peptide substrates of different chain length and from the competition studies

with these peptides suggest that two distinct cytosol enzymes may be involved in the hydrolysis of L-alanylglycine and L-alanyldiglycine in man and rat. A less likely interpretation is that the cytosol enzymes may have multiple binding sites, one for dipeptides and another for tripeptides. Similar studies on brush-border enzymes appear to provide data consistent with the idea that each of the enzymes may be capable of hydrolyzing peptides of varying chain length. Further conclusions with regard to number of enzymes and binding sites must await the final purification of these enzymes.

Caution must be exercised in extending these data on in vitro properties of enzymes obtained using a limited number of peptide substrates to conclusions about their biological function. However, it has been reported that the major products of hydrolysis of various proteins by pancreatic enzymes are peptides of two to six amino acid units and mixtures of free amino acids [20]. The present studies on the brush-border enzymes suggest that these primary products of hydrolysis may be degraded further at the brush-border before entering the cell. Since the cytosol fraction was capable of hydrolyzing only di- and tripeptides among the substrates studied, and only the constituent amino acids appear in the portal circulation following intraluminal administration of protein and most peptides [21, 22], it seems unlikely that larger peptide molecules enter the cell to be degraded intracellularly. Moreover, since it has been reported [23–26] that small peptides can be transported into the cell, it is likely that this transport process is mainly confined to di- and tripeptides and that the digestion of longer peptides is confined to the luminal and brush-border enzymes.

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ENZYMES

1 Peters, T. J. (1970) Biochem. J. 120, 195–203
2 Kim, Y. S., Birtwhistle, W. and Kim, Y. W. (1972) J. Clin. Invest. 51, 1419–1430
3 Josefsson, L. and Lindberg, T. (1965) Biochim. Biophys. Acta 105, 149–161
4 Heizer W. D. and Laster, L. (1969) J. Clin. Invest. 48, 210–228
5 Rhoads, J. B., Eichholz, A. and Crane, R. K. (1967) Biochim. Biophys. Acta 135, 959–965
6 Heizer, W. D., Kerley, R. L. and Isselbacher, K. J. (1972) Biochim. Biophys. Acta 264, 450–461
7 Kim, Y. S., Kim, Y. W. and Sleisenger, M. H. (1973) J. Clin. Invest. 52, 47
8 Kim, Y. S., McCarthy, D. M., Lane, W. and Fong, W. (1973) Biochim. Biophys. Acta 321, 262–273
9 McCarthy, D. M. and Kim, Y. S. (1973) J. Clin. Invest. 52, 942–951
10 Nicholson, J. A., McCarthy, D. M. and Kim, Y. S. (1973) Gastroenterology 64, 778
11 Eichholz, A. and Crane, R. K. (1965) J. Cell Biol. 26, 687–691
12 Welsh, J. D., Preiser, H., Woodley, J. F. and Crane, R. K. (1972) Gastroenterology 62, 572–582
13 Lowry, O. H. Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
14 Maroux, S., Louvard, D. and Baratti, J. (1973) Biochim. Biophys. Acta 321, 282–295
15 Kania, R. J., Santiago, N. A. and Gray, G. M. (1972) Gastroenterology 62, 768
16 Peters, T. J. (1973) Clin. Sci. Mol. Med. 45, 803–816

17 Josefsson, L., Norén, O. and Sjöström, H. (1968) Acta Physiol. Scand. 72, 108–114
18 Fujita, M., Parsons, D. S. and Wonjnarowska, F. (1972) J. Physiol. 227, 377–394
19 Heizer, W. D. and Laster, L. (1969) Biochim. Biophys. Acta 185, 409–423
20 Crampton, R. F., Gangolli, S. D., Mathews, D. M. and Simson, P. (1971) J. Physiol. (Lond.) 213 43
21 Dent, C. E. and Schilling, J. A. (1949) Biochem. J. 44, 318–323
22 Denton, A. E., Gershoff, S. N. and Elvehjem, C. A. (1953) J. Biol. Chem. 204, 731–736
23 Matthews, D. M., Craft, I. L., Geddes, D. M., Wise, I. J. and Hyde, C. W. (1968) Clin. Sci. 35, 415-424
24 Craft, I. L., Geddes, D., Hyde, C. W., Wise, I. J. and Matthews, D. M. (1968) Gut 9, 425–437
25 Adibi, S. A. (1971) J. Clin. Invest. 50, 2266–2275
26 Hellier, M. D., Holdsworth, C. D., Perrett, D. and Thirumalai, C. (1972) Clin. Sci. 43, 659–668