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Comparison of distribution of brush-border exo- and endopeptidases in rat and rabbit intestine

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Abstract—The distribution of brush-border endopeptidase-2, aminopeptidase W, carboxypeptidase P, and aminopeptidase P along the rat and rabbit intestine was examined. In both species, aminopeptidases P and W increased distally and reached the highest in the ileum; their activities in the ileo-caecal junction were the lowest. Endopeptidase-2 had a uniform intestinal distribution in both species with the highest activity in the ileum and little activity in the ileo-caecal junction or caecum. With a distribution similar to that of endopeptidase-2, carboxypeptidase P also had high activity in the ileum in rats and rabbits.

From the point of view of drug delivery, brush-border membrane peptidases metabolically limit intestinal absorption of peptide and peptidiomimetic drugs; release drugs from prodrugs by cleaving the amide bond between the drug and progroup; and digest controlled-release systems, of which polymeric materials are polypeptides, to release drugs. Therefore, a knowledge of longitudinal distribution of brush-border peptidases is important for the rational delivery of drugs and peptide drugs.

There are several endo- and exopeptidases in the intestine; the distribution of some of them in rabbits, rats and man has been reported (Auricchio et al 1978; Skovbjerg 1981; Sterchi 1981; Miura et al 1983; Bai 1993a). However, the distribution of aminopeptidase P, aminopeptidase W, endopeptidase-2, and carboxypeptidase P in rat and rabbit intestine has not been compared. In this study, the distribution of these brush-border enzymes along the rat and rabbit intestine is compared and their activities in the caecum or ileo-caecal junction are also examined.

Materials and methods

Materials. Benzyloxycarbonyl-Pro-Ala, benzyloxycarbonyl-Pro, Ala, Glu-Trp, insulin B-chain, 1,10-phenanthroline, phosphoramidon, $MnCl_2$, Tris, Tyr-D-Ala-Gly, and pentobarbitone were obtained from Sigma Chemical Co. (St Louis, MO). Arg-Pro-Pro was from Bachem Bioscience Inc. (Philadelphia, PA). Cilastatin was a gift from Dr Helmut Krop (Merck Sharp and Dohme Research Laboratories, Rahway, NJ). Bovine γ-globulin and dye reagent for the protein assay were obtained from Bio-Rad Laboratories (Richmond, CA). All other chemical reagents

and buffer components were of analytical grade. Acetonitrile was of HPLC grade. All chemicals were used as obtained.

Preparation of brush-border membranes. Preparation of brush-border membranes from both young male New Zealand white rabbits, 2·5–3 kg, and male Sprague-Dawley rats, 300 g, was as described by Bai (1993b). Five rabbits and thirteen rats were used to prepare membranes. Briefly, brush-border membranes were prepared using the method of Kessler et al (1978). The intestinal mucosa of each segment was scraped off, suspended in a hypotonic solution (50 mM mannitol in the 2 mM pH 7·5 HEPES/Tris buffer), and homogenized using a blender and a glass/Teflon homogenizer. $CaCl_2$ (1 M) was added to the homogenates to achieve a final concentration of 10 mM, and the mixture was centrifuged at 3000 *g* for 15 min. The supernatant was collected and centrifuged at 27 000 *g* for 30 min to obtain pellets. Pellets were resuspended in 150 mM NaCl, 10 mM Tris/HCl (pH 6·8) buffer and homogenized with a glass/Teflon homogenizer. Centrifugation at 3000 and 27 000 *g* was repeated to obtain pure brush-border membranes. All procedures were at 4°C. Protein concentrations were determined using the Bradford method and γ-globulin as the protein standard (Bradford 1976). Activity of alkaline phosphatase was determined to assess the purity of brush-border membranes (Kessler et al 1978). On average, brush-border alkaline phosphatase activity was 15 times enriched in rabbit preparations and 13 times enriched in rat preparations.

Enzyme assay. The incubation mixture (300 μL) consisted of 10 mM pH 6·8 Tris/HCl buffer, NaCl (150 mM), a substrate, and brush-border membrane protein (0·03–0·006 mg) or homogenate protein (0·1–2 mg). Benzyloxycarbonyl-Pro-Ala was used as the substrate for carboxypeptidase P, Arg-Pro-Pro for aminopeptidase P, Glu-Trp for aminopeptidase W, and insulin B-chain for endopeptidase-2 (Auricchio et al 1978; Lasch et al 1988; Barnes et al 1989; Jackson et al 1989). To assay aminopeptidase W activity, 0·05 mM cilastatin and 5 mM 1,10-phenanthroline were included in the reaction mixture to eliminate activities of aminopeptidase N, aminopeptidase A, and microsomal dipeptidase (Jackson et al 1989). Phosphoramidon (0·02 mM) was used to eliminate endopeptidase-24.11 activity so that endopeptidase-2 activity could be determined specifically (Kenny & Ingram 1987; Barnes et al 1989). Kenny & Ingram (1987) suggested that 1 μM phosphoramidon could completely eliminate endopeptidase-24.11 activity against insulin B-chain. In this study, it was observed that an increase of phosphoramidon concentration from 0·002 to 0·06 mM did not significantly reduce proteolytic activity degrading insulin B-chain; and there was no significant difference in insulin B-chain degradation in the presence of 0·002–0·06 mM phosphoramidon. $MnCl_2$ (3 mM) was included in the assay of aminopeptidase P activity (Lasch et al 1988).

The final substrate concentration in the incubation mixture was 0·5 mM for Glu-Trp, 0·2 mM Arg-Pro-Pro, 0·02 mM insulin B-chain, and 0·027 mM benzyloxycarbonyl-Pro-Ala. The concentration of individual substrate was below the reported concentrations or below or close to its K_m value (Auricchio et al 1978; Miura et al 1983; Kenny & Ingram 1987; Jackson et al 1989). The concentration of each substrate was the same for both homogenate and brush-border membrane studies for all the segments, to ensure that results could be compared. Activities of all enzymes were studied at 37°C. Aliquots were taken periodically and 10% trichloroacetic acid (TCA) or 0·1 M HCl was added to stop the reaction. In the control groups, brush-border membrane or homogenate proteins in the buffer were denatured using TCA, or deactivated using 0·1 M HCl before each substrate was added; no hydrolysis was observed for any substrate within the experimental time frame. For each experimental group, the initial hydrolysis rate was obtained from the first 10–20% of proteolysis. Enzyme activity was expressed as the amount of substrate hydrolysed (g protein)$^{-1}$ min^{-1}.

Assay methods. HPLC systems consisted of an SIL autoinjector, an LC-600 pump, an SPD-6A UV spectrophotometric detector, and a CR 601 recorder (Shimadzu Corporation, Kyoto, Japan), reversed phase C_8 (Altex Ultrasphere-ODS, 5μ, 4·6 mm × 25 cm) (Beckman Instrument, Berkley, CA), and C_{18} columns (Vydac 5μ, 4·6 mm x 25 cm) (Vydac, Hesperia, CA) or a strong cation-exchange column (Partisil 10 SCX, 25 cm, Whatman, Maidstone, UK). Glu-Trp and Arg-Pro-Pro were analysed using a cation-exchange column with isocratic mobile phases of pH 2·5 0·005 M $(NH_4)H_2PO_4$: acetonitrile, 90 : 10, and pH 2·5 0·05 M $(NH_4)H_2PO_4$: acetonitrile, 90 : 10, respectively. The former was eluted at 10 min by a flow rate of 1·5 mL min^{-1}, and the latter was eluted at 8 min by a flow rate of 1 mL min^{-1}. Insulin B-Chain was analysed using a C_{18} column and a mobile phase of 0·05% trifluoroacetic acid : acetonitrile, 65 : 35, with a retention time of 6 min at a flow rate of 0·8 mL min^{-1}. Benzyloxycarbonyl-Pro-Ala was analysed using a C_8 column and a mobile phase of 0·05% trifluoroacetic acid : acetonitrile, 75 : 25, and it was eluted at 8 min by a flow rate of 1 mL min^{-1}.

Results and discussion

In both species, activity of an individual peptidase in the homogenate was 1–16% that in the brush-border membrane. It is likely that the enrichment of individual peptidase activity in membrane vesicles is determined by whether each substrate was exclusively degraded in homogenate by a specific brush-border membrane peptidase.

The results for the individual enzymes in the specific segments of intestine are summarized in Tables 1 and 2. In the kidney, the physiological function of angiotensin-converting enzyme is

Table 1. Distribution of brush-border peptidase along the rat intestine.

	Enzyme activity (μmol min^{-1} (g protein)$^{-1}$)				
	Duodenum	Jejunum	Jejuno-ileal junction	Ileum	Caecum
Endopeptidase-24.11[a]	1·2 (0·1)	3·2 (0·2)	1·2 (0·03)	1·1 (0·1)	0·41 (0·07)
Endopeptidase-2	8·9 (1·4)	9·3 (1·0)	9·6 (0·5)	11 (2)	2·7 (1·0)
Angiotensin-converting enzyme[a]	2·1 (0·1)	6.5 (0·5)	1·8 (0·6)	0·83 (0·2)	0·12 (0·02)
Carboxypeptidase P	0·06 (0·01)	0·06 (0·0)	0·07 (0·02)	0·11 (0·02)	0·09 (0·02)
Dipeptidyl peptidase IV[a]	45 (1)	140 (15)	100 (7)	140 (10)	2·7 (0·09)
Aminopeptidase P	20 (4)	19 (3)	37 (8)	31 (6)	23 (6)
Aminopeptidase W	14 (2)	26 (4)	64 (9)	77 (7)	13 (3)

All results were found in a series of experiments, and those highlighted by a superscript [a] had been published previously (Bai 1993b). Results are the mean values of three determinations. This table has been published previously (Bai 1994).

Table 2. Distribution of brush-border enzymes along the rabbit intestine.

	Enzyme activity (μmol min^{-1} (g protein)$^{-1}$)				
	Duodenum	Jejunum	Jejuno-ileal junction	Ileum	Ilio-caecal junction
Endopeptidase-24.11[a]	0·99 (0·09)	1·5 (0·1)	0·79 (0·07)	0·73 (0·12)	0·61 (0·03)
Endopeptidase-2	5·5 (1·6)	7·8 (1)	6·6 (0·1)	13·4 (1·5)	0
Angiotensin-converting enzyme[a]	88 (5)	148 (17)	84 (13)	18 (5)	9·7 (0·6)
Dipeptidyl peptidase IV[a]	9·5 (0·1)	66 (4)	69 (1)	64 (3)	62 (5)
Aminopeptidase P	3·3 (0·2)	11 (0)	15 (2)	26 (3)	0
Aminopeptidase W	8·8 (1·3)	21 (2)	78 (8)	546 (20)	12 (3)

All results were found in a series of experiments, and those highlighted by superscript [a] had been published previously (Bai 1993b). Results are the mean values of three determinations.

Table 3. Comparison of the distribution of peptidase activities along the human, rat, and rabbit intestine.

	Rat	Human	Rabbit	References
Aminopeptidase N	X		X	Auricchio et al (1978); Miura et al (1983)
		X	X	Skovbjerg (1981)
Aminopeptidase A	X	X	X	Auricchio et al (1978); Skovbjerg (1981); Sterchi (1981); Song et al (1986)
Endopeptidase-24.11	X		X	Bai (1993a,b)
Endopeptidase-2	X		X	
Angiotensin-converting enzyme	X		X	Bai (1993a,b)
Carboxypeptidase P	X	X	X	Skovbjerg (1981); Miura et al (1983)
Dipeptidyl peptidase IV	X	X	X	Skovbjerg (1981); Bai (1993a,b)
Aminopeptidase W	X		X	
Aminopeptidase P	X			

X denotes similarity.

involved in converting angiotensin I to angiotensin II. In the intestine, it is unknown whether there is any other significant physiological function of high activity of angiotensin-converting enzyme except contributing to the intestinal digestion of polypeptides. To compare any similarity in the enzyme distribution in rabbit, rat, and human intestine, the activity profiles along the intestine of the peptidases reported by different laboratories were compared and are summarized together with the current results in Table 3. The rabbit, rat, and human intestine seem to have similar distribution profiles for aminopeptidase N and aminopeptidase A, carboxypeptidase, and dipeptidyl peptidase IV, while the rat and rabbit intestine have similar distribution patterns for each of seven brush-border peptidases. Since peptidases have varying distribution profiles, targeting peptide drugs to a specific intestinal site with lower proteolytic activity, or targeting polypeptide formulations and peptide prodrugs to a specific site with optimal proteolytic activities will depend on individual need and distribution of specific peptidases.

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