Similarities between a Dipeptide Hydrolase from Brush-Border and Cytosol Fractions of Guinea-Pig Intestinal Mucosa

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A dipeptide hydrolase from the brush border of guinea-pig intestinal mucosa was purified. The enzyme resembles another dipeptide hydrolase isolated from the cytosol fraction of intestinal mucosa. Studies on the binding of cytosol peptide hydrolase to brush-border membranes indicate that the enzyme found in the brush border may be a cytoplasmic contaminant.

Although interest in mammalian peptide digestion has increased recently, the final stages of peptide hydrolysis and transport have not yet been fully elucidated. Peptide hydrolases which probably play a significant role in the terminal stages of protein digestion are located in two regions of the mucosal cells (enterocytes), the brush-border and cytosol fractions (Peters, 1970a). In guinea-pig intestinal mucosa at least three peptide hydrolases, termed α , β and γ on the basis of their electrophoretic mobilities on starch gels, have been detected in both brush-border and cytosol fractions (Donlon & Fottrell, 1972). Some controversy exists about the relationship between peptide hydrolases from these latter fractions of enterocytes. Previous studies from this laboratory showed that the brush-border and cytosol peptide hydrolases had similar electrophoretic mobilities (Donlon & Fottrell, 1972; O'Cuinn & Fottrell, 1975). It was further shown that a guinea-pig intestinal peptide hydrolase which attacked both dipeptides and tripeptides had very similar properties when isolated from either cytosol or brush-border fractions (O'Cuinn *et al.*, 1974). The purpose of the present studies was to compare the properties of another peptide hydrolase (Piggott & Fottrell, 1975) from the cytosol fraction of guinea-pig intestinal mucosa with a peptide hydrolase of similar electrophoretic mobility isolated from brush borders. The present enzyme, termed β_2 peptide hydrolase, differs from the one previously studied (O'Cuinn *et al.*, 1974) in that it preferentially hydrolyses dipeptides, especially those containing glycine.

Materials and Methods

Peptide hydrolase activity was measured as described previously (Donlon & Fottrell, 1971). The unit of peptide hydrolase activity is defined as the

amount of enzyme that releases 1 μ mol of *N*-terminal L-leucine/min. A cytoplasmic peptide hydrolase (termed β_2) was purified from guinea-pig intestinal mucosa as described by Piggott & Fottrell (1975). A brush-border peptide hydrolase of similar electrophoretic mobility was purified as follows. Brush borders were prepared from 63 g of guinea-pig intestinal mucosa by the method of Schmitz *et al.* (1973). The purified brush borders were suspended in 40 ml of 0.1 M-Tris/HCl, pH 8.2, containing 0.1% Triton X-100, and labile enzymes were released by gentle homogenization in a Potter-Elvehjem tube for 30 s. The solubilized enzymes were separated from particulate matter by centrifugation at 22 300 g for 15 min, and a peptide hydrolase electrophoretically similar to the cytosol enzyme (β_2) was precipitated from the resulting supernatant as a 40-65% satd. $(\text{NH}_4)_2\text{SO}_4$ fraction. The $(\text{NH}_4)_2\text{SO}_4$ precipitate was dissolved in 1 ml of 50 mM-sodium borate, pH 8.4, dialysed for 24 h at 4°C against the same buffer and subjected to preparative polyacrylamide-gel electrophoresis at pH 8.1 (Davis, 1964). This procedure yielded a purified β_2 peptide hydrolase which was homogeneous by analytical polyacrylamide-gel electrophoresis.

Brush-border fractions prepared as described by Hübscher *et al.* (1965) were used for studies where the binding of β_2 peptide hydrolase to these fractions was examined.

Several peptides (each tested at 5 mM) were used to compare substrate specificities of the purified brush-border and cytosol peptide hydrolases.

An antiserum was raised in rabbits against the β_2 peptide hydrolase by injection of 100 μ g of purified enzyme in Freund's complete adjuvant at four sites on a rabbit's back as described previously (O'Cuinn *et al.*, 1974). Purified cytoplasmic β_2 peptide hydrolase was iodinated with ^{125}I by using chloramine-T (Hunter, 1970). Radioactivity was measured with an LKB-Wallac 80000 Autogamma counter.

Results and Discussion

Purification and properties of brush-border and cytosol hydrolases

The elution profile obtained after preparative polyacrylamide-gel electrophoresis of the brush-border β_2 peptide hydrolase is shown in Fig. 1. Other peptide hydrolases, previously termed γ and β_1 (Donlon & Fottrell, 1972), are removed by this procedure. A comparative study of the β_2 peptide hydrolase from brush-border and cytosol fractions showed similar pH optima at pH 8.2. The enzymes from the two sources displayed very similar stability patterns: they were stable when frozen (-15°C), but rapidly lost activity at 4°C even in the presence of glycerol or sucrose; they were more stable in Tris/HCl buffer than in barbitone or borate buffers. The substrate specificities of the two preparations were alike (Table 1). The K_m value for L-Leu-L-Leu was the same for both enzymes (0.10 mM). The contribution of the β_2 enzyme to the total dipeptide hydrolase activity is approx. 80% in both the cytosol and brush-border fractions (Donlon & Fottrell, 1972).

Antiserum raised against purified cytosol β_2 peptide hydrolase was incubated with the cytosol enzyme or the brush-border enzyme as described previously (O'Cuinn *et al.*, 1975). In comparison with assays conducted in the presence of rabbit control serum, the antiserum inhibited the cytosol enzyme by 89% and the brush-border enzyme by 50%, thus demonstrating immunochemical similarity between the two hydrolases. The inhibition for the brush-border enzyme was obtained by using the highest available concentration of brush-border β_2

Fig. 1. Preparative polyacrylamide-gel-electrophoretic separation of β_2 peptide hydrolase from the brush-border fraction of guinea-pig intestinal mucosa

Enzyme solution after $(\text{NH}_4)_2\text{SO}_4$ fractionation (1 ml, containing 15 mg of protein) was applied to the column and electrophoresis performed for 16 h at 300 V. The separated proteins were eluted by using 50 mM-sodium tetraborate buffer, pH 8.4. Protein ($\bullet$) was monitored by recording the E_{280} , and peptide hydrolase activity ($\circ$) was measured with L-Leu-L-Leu as substrate. Fractions of volume 3 ml were collected in each case.

Table 1. Substrate specificity of purified β_2 peptide hydrolase from brush-border and cytosol fractions of guinea-pig intestinal mucosa

+, Hydrolysis; —, lack of hydrolysis. For experimental detail, see Piggott & Fottrell (1975).

Substrate	Peptide hydrolase activity	
	Cytosol	Brush border
Gly-L-Tyr	+	+
Gly-L-Phe	+	+
Gly-L-Trp	+	+
Gly-L-Leu	+	+
L-Pro-L-Tyr	+	+
L-Leu-L-Leu	+	+
L-Leu-L-Tyr	+	+
L-Leu-L-Ala	+	+
L-Lys-L-Phe	—	—
L-Leu-NH ₂ *	—	(+)
L-Phe-L-Pro	—	—
L-Pro-L-Ile	—	—

* Very slight hydrolysis by brush-border enzyme was detected.

peptide hydrolase. Unfortunately, there was insufficient material available to carry out the antiserum-inhibition studies at different dilutions.

Studies on the binding of cytosol β_2 peptide hydrolase to brush-border membranes

In view of the similarities in properties between the peptide hydrolases isolated from cytosol and brush-border fractions, the possibility was investigated that the same enzyme was present in both fractions. Because of the relatively high dipeptidase activity in the cytosol fraction (90–95% of the total), a likely possibility was that cytosol enzyme was binding to brush borders during the isolation procedure. The cytosol enzyme was iodinated with ^{125}I and its ability to bind to brush borders was studied. A quantity of this iodinated enzyme was added to a 50 mM-mannitol/2 mM-Tris buffer, pH 7.1, which was then used to homogenize guinea-pig intestinal mucosa. Brush-border membranes, prepared by two different procedures (Schmitz *et al.*, 1973; Hübscher *et al.*, 1965) and cytosol fractions were isolated from the homogenate. Peptide hydrolase activity and radioactivity were measured in the isolated fractions and the results are shown in Table 2. In two separate experiments involving different preparative procedures there was good agreement between the quantity of peptide hydrolase activity and the amount of radioactivity bound to brush-border fractions. The results strongly suggest that the β_2 dipeptidase activity of isolated brush-border fractions is due to cytosol peptide hydrolase, which binds during the preparation of the fractions.

Table 2. Comparison of peptide hydrolase activity and radioactivity in the soluble and brush-border fractions of guinea-pig or rat intestinal mucosa after addition of guinea-pig ^{125}I -peptide hydrolase to the homogenizing buffer

In Expts. A and C, the brush borders were prepared by the method of Schmitz *et al.* (1973). In Expt. B, the brush borders were prepared by the method of Hübscher *et al.* (1965). For details, see the text.

Expt.	Fraction	Peptide hydrolase activity (% of total recovered in both fractions)	Radioactivity (% of total recovered in both fractions)
A	Guinea-pig cytoplasm	97.0	96.5
	Guinea-pig brush border	3.0	3.5
B	Guinea-pig cytoplasm	98.0	99.0
	Guinea-pig brush border	2.0	1.0
C	Rat cytoplasm	92.0	95.0
	Rat brush border	8.0	5.0

These experiments were repeated with rat intestinal mucosa and guinea-pig ^{125}I -labelled peptide hydrolase. Similar results were obtained (Table 2), except that the peptide hydrolase activity and the radioactivity in the rat brush-border fraction were greater than in guinea pig. Others (Wognarowska & Gray, 1975) have reported relatively high dipeptide hydrolase activity in rat brush borders compared with that in guinea pig.

In conclusion, these studies show that there is a strong similarity between the peptide hydrolases in the two subcellular fractions. They also strongly suggest that the relatively small amount (5–10%) of dipeptide hydrolase activity in fractionated guinea-pig intestinal brush borders (Peters, 1970*b*) may be attributable to the binding of cytosol peptide hydrolase to the brush-border fraction during the isolation procedure.

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