SIMILARITIES BETWEEN ONE OF THE MULTIPLE FORMS OF PEPTIDE HYDROLASE PURIFIED FROM BRUSH-BORDER AND CYTOSOL FRACTIONS OF GUINEA PIG INTESTINAL MUCOSA

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1. Introduction

The final stages of mammalian protein digestion are mediated by peptide hydrolases present in the mucosal cells (enterocytes) of the small intestine. These intestinal hydrolases, which occur in multiple forms with broad substrate specificity, are present in brush-border and cytosol fractions of enterocytes [1]. A topic of current interest in intestinal enzymology is whether peptide hydrolases, from brush-border and cytosol fractions of enterocytes are different. Further information on this topic, about which some confusion exists at present, is essential to provide a better understanding of protein digestion and may also help in defining the aetiology of protein malabsorption conditions such as coeliac disease (gluten-induced enteropathy) [2].

Previous studies showed that many brush border and cytosol peptide hydrolases had similar electrophoretic mobilities [1]. The purpose of the present studies was to compare the properties of one of the multiple forms of peptide hydrolase recently purified and characterized from the cytosol of the enterocytes [3], with an enzyme of similar electrophoretic mobility, purified from brush-border fractions.

2. Materials and methods

Cytoplasmic 'α' peptide hydrolase (so called on the basis of its electrophoretic mobility) [1] was purified as described by Donlon and Fottrell [3]. Brush-borders were prepared [4] and after two washes with 0.3 M sucrose pH 7.4 were resuspended in 0.3 M sucrose pH 7.4 and sonicated with an M.S.E. disintegrator at 1.3 A for 20 sec. Enzymes released from brush-borders were separated from particulate matter by centrifugation at 22 300 *g* for 15 min and the resulting supernatant is referred to as the brush-border enzyme preparation. Sonication was adopted to release peptidase activity from brush border preparations because this method routinely gave greater than 80% release of enzyme activity. Brush-border 'α' peptidase hydrolase was purified from this latter supernatant by the method previously cited for purification of the cytoplasmic 'α' peptide hydrolase [3]. The purified brush border 'α' peptide hydrolase represented 18% of the total solubilised brush-border peptidase activity. Peptidases were separated by starch-gel electrophoresis and located in situ on the gels with an agar overlay [1]. Peptidase activity was measured using L-Leu-L-Leu as described previously [5] with the exception that 25 mM sodium tetraborate pH 9.1 containing 0.1 M NaCl was used.

Several substrates at a concentration of 5 mM were used to compare substrate specificities of purified brush border and cytosol peptide hydrolase. An antibody to the cytoplasmic 'α' peptide hydrolase was raised in rabbits by the method of Goudie et al. [6]. γ-Globulins were prepared from the specific antiserum and from control serum by batch adsorption with DEAE-cellulose and precipitation with 40% saturation of ammonium sulphate.

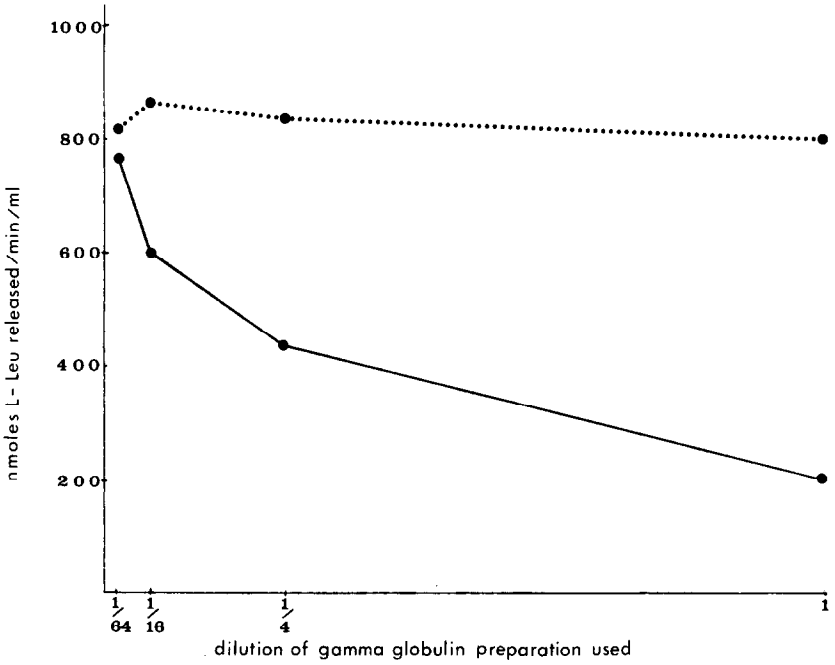

Fig. 1. Brush-border 'α' peptide hydrolase activity after treatment with various dilutions of a) rabbit anti-guinea-pig cytosol 'α' peptide hydrolase gamma globulin (o—o—o) and of b) normal rabbit gamma globulin (o···o···o). In both cases the supernatants were tested for activity following centrifugation at 30 000 *g* for 15 min.

3. Results

3.1. *Immunological studies*

When aliquots of the purified brush border 'α' peptide hydrolase were incubated with equal volumes of various dilutions of anti-cytosol 'α' peptide hydrolase antibody, the resultant complex was precipitated by centrifugation at 30 000 *g* for 15 min (fig. 1). When a 'γ' globulin preparation from control rabbit serum was substituted for the aformentioned antiserum it was found that peptide hydrolase activity was not precipitated but remained in the supernatant after centrifugation. Moreover when cytosol and brush border enzyme preparations were preincubated with antiserum and control serum and the mixtures run on starch-gel electrophoresis, the resultant zymograms indicated that the antiserum reacted only with the 'α' peptide hydrolase of both brush-borders and cytosol (fig. 2). This antiserum did not appear to react with three of the other multiple forms of peptide hydrolase from guinea-pig mucosal cytoplasm, i.e., β, γ and *a*.

3.2. *Substrate specificities*

Thirteen dipeptides and four tripeptides were used to compare the substrate specificities of the cytosol and brush border enzymes. As shown in table 1 both enzymes hydrolysed the same substrates and had no effect on peptides containing proline or acidic amino acid residues with the exception of L-Glu-L-Tyr. K_M values for both enzymes with eight substrates are compared in table 2. The three tripeptides and three of the five dipeptides gave similar K_M values. In contrast, L-Leu-L-Ala and L-Leu-Gly gave significantly different K_M values, the K_M for the brush-border enzyme being lower with both substrates (table 2).

3.3. *Divalent metals*

Various divalent cations were tested for their ability to stimulate hydrolysis of L-Leu-L-Leu by the brush-border hydrolase (table 3). Both manganese (0.5 mM) and magnesium (0.5 mM) promoted hydrolysis, the manganese ion being more efficient in this regard. Zinc

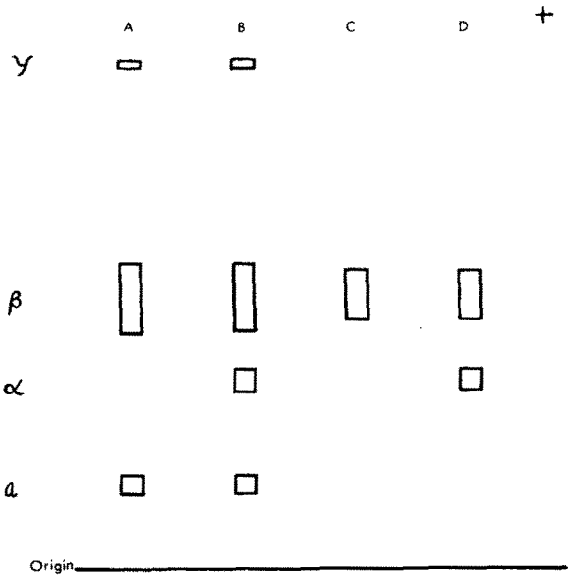

Fig. 2. Zymograms of mixtures of A) guinea-pig cytosol with rabbit anti-cytosol 'α' peptide hydrolase serum; B) guinea-pig cytosol with normal rabbit serum; C) guinea-pig brush-border enzymes with rabbit anti-guinea-pig cytosol 'α' peptide hydrolase serum and D) guinea-pig brush-border enzymes with normal rabbit serum. The nomenclature used to designate the various peptide hydrolase activities was as described in a previous publication [1]. The agar overlay used to visualise peptide hydrolase activities contained both L-Leu-L-Leu and L-Leu-L-Leu-L-Leu.

and cobalt (0.5 mM) inhibited hydrolysis while calcium at the same concentration had little effect. These results correlate well with previous findings on the cytosol peptide hydrolase [7].

4. Discussion

On the basis of these results and previous studies from this laboratory [3] we conclude that at least one of the multiple forms of peptide hydrolase i.e., 'α' peptide hydrolase has very similar properties when isolated from either the cytosol or brush-border fractions of guinea-pig intestinal mucosa. Some small differences may exist between the 'α' peptide hydrolase from the two aforementioned fractions reflected for instance in the different K_M values for L-Leu-L-Ala and L-Leu-Gly.

Others [8, 9] have used electrophoretic properties and a number of other criteria such as sensitivity to

Table 1

Substrates hydrolysed (+) by the 'α' peptide hydrolase from cytosol and brush-border fractions of guinea-pig intestine.

Peptides	Cytosol hydrolase	Brush-border hydrolase
L-Pro-L-Leu	–	–
L-Pro-L-Tyr	–	–
L-Pro-L-Trp	–	–
Gly-L-Leu	–	–
γ-L-Glu-L-Leu	–	–
α-L-Glu-L-Trp	–	–
α-L-Glu-L-Tyr	+	+
α-L-Glu-L-Val	–	–
L-Leu-L-Leu	+	+
L-Leu-L-Tyr	+	+
L-Leu-Gly	+	+
L-Leu-L-Ala	+	+
L-Val-L-Leu	+	+
L-Leu-L-Leu-L-Leu	+	+
L-Leu-Gly-Gly	+	+
L-Met-Gly-Gly	+	+
L-Met-L-Met-L-Ala	+	+

Table 2

K_M values (mM) with different substrates for 'α' peptide hydrolases from cytoplasms and brush-border fractions.

Substrate	Cytosol α peptide hydrolase	Brush-border α peptide hydrolase
L-Leu-L-Leu	0.47	0.58
L-Val-L-Leu	0.25	0.37
L-Leu-L-Ala	7.1	1.1
L-Leu-Gly	2.6	1.43
L-Leu-L-Tyr	0.52	0.71
L-Leu-Gly-Gly	2.8	2.5
L-Met-Gly-Gly	3.8	3.8
L-Met-L-Met-L-Ala	0.5	0.66

Table 3

The effect of different divalent cations on brush-border and cytosol 'α' peptide hydrolases.

Divalent cation	nmoles of L-Leu released/min per ml	Percentage of control*
–	27	100 –
$MnCl_2$	62	230 (300)
$ZnCl_2$	20	74 (83)
$CoCl_2$	21	78 (103)
$MgCl_2$	39	142 (103)
$CaCl_2$	29	107 –

* Figures for the cytosol enzyme, given in brackets, are taken from previous studies in which metal sulphates were used [7]. Metal salts were included in the assay mixture and compared with controls to which no additions were made. Substrate, L-Leu-L-Leu.

heat and certain inhibitors to compare crude extracts of rat border and cytosol peptide hydrolases. Kim et al. [9], for instance, proposed that differences exist between rat brush-border and cytosol peptide hydrolases based solely on electrophoretic criteria, following solubilization of brush-border enzymes with papain. Such studies, in our opinion, while suggesting that possible differences may exist betweeen rat brush-border and cytosol peptide hydrolases must nevertheless be interpreted cautiously until reproduced with pure preparations of enzymes.

Acknowledgements

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