IMMUNOLOGICAL RELATIONSHIP BETWEEN THREE INTESTINAL PEPTIDE HYDROLASES AND SIMILAR ENZYMES IN VARIOUS TISSUES OF THE GUINEA-PIG AND OF OTHER ANIMALS

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1. Introduction

Previous studies from this laboratory showed that guinea-pig intestinal mucosa contains at least seven peptide hydrolases distinguishable by their substrate specificities and electrophoretic mobilities [1]. Although these intestinal peptide hydrolases are probably responsible for the final stages of protein digestion the extent of their relationship with peptide hydrolases in other tissues of the guinea-pig is not known. Three of these peptide hydrolases, denoted α, β_2 and γ on the basis of their different electrophoretic mobilities were purified to homogeneity and characterised [2–4]. These three peptide hydrolases which represent over 90% of the total dipeptide hydrolase activity of guinea-pig intestine have several features in common including overlapping substrate specificities although each peptide hydrolase specifically hydrolyses particular peptide linkages. Some relationship between the three hydrolases is suggested by their mol. wts which are 300 000, 170 000 and 112 000 for the α, β_2 and γ peptide hydrolases respectively.

The aim of the present study was to determine the extent to which these peptide hydrolases are immunologically related to, 1) each other, 2) enzymes with similar substrate specificities and electrophoretic mobilities in additional tissues of the guinea-pig, and 3) similar intestinal mucosal enzymes in different species. For this purpose antisera against homogeneous preparations of each of the three intestinal peptide hydrolases were used.

2. Materials and methods

Peptide hydrolases were purified from guinea-pig intestinal mucosa as described previously [2–4]. Each purified peptide hydrolase showed one protein band on analytical polyacrylamide gel electrophoresis. Separate antisera to the 'α', 'β_2' and 'γ' peptide hydrolases were raised by injection of 100 μg of the purified protein in Freund's complete adjuvant. These rabbits were bled 21 days after the last injections.

The antisera were tested for inhibition of peptide hydrolase activity by reacting 0.1 ml aliquots of the respective antiserum with 0.1 ml of each of the purified peptide hydrolases. Control serum (0.1 ml aliquots) from normal rabbits was also reacted with 0.1 ml aliquots of each purified peptide hydrolase. The background level of peptide hydrolase in each rabbit serum was monitored by reacting the serum with the buffer in which each peptide hydrolase was prepared. All reaction tubes were allowed to stand on ice for 30 min before centrifugation at 30 000 *g* for 15 min and aliquots of supernatant were then tested for peptide hydrolase activity [5]. Inhibition of each peptide hydrolase activity by antiserum was expressed as a percentage of the activity found when control serum was used.

The α peptide hydrolase was measured using L-Leu–L-Leu as substrate in 25 mM sodium tetraborate buffer (pH 9.1) with 0.5 mM Mn^{2+}. The β_2 peptide hydrolase was measured using L-Leu–L-Leu as substrate in 25 mM sodium tetraborate buffer (pH 8.2); no metal was added. The γ peptide hydrolase

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(amino acyl proline hydrolase) was assayed with L-Leu–L-Pro as substrate in 25 mM sodium tetraborate pH 8.0 with 0.5 mM Mn^{2+} present.

Immunoelectrophoresis was conducted on glass plates (10 × 8 cm) using 1% agar in 0.15 mM sodium barbitone (pH 8.6) containing 12.5 mM sodium acetate. The bridge buffer was 60 mM sodium barbitone (pH 8.6) containing 50 mM sodium acetate and electrophoresis was carried out at 50 V per plate for 2 hr. Antisera were added to troughs cut between the antigen wells and diffusion was allowed overnight at 30°C in a humid atmosphere. Alternatively, plates were stained to locate peptide hydrolase activities as described previously [1] except that starch (0.5%) was present in the overlay. The α, β_2 and γ peptide hydrolases were located by incorporating L-Leu–L-Leu–L-Leu, L-Leu–L-Leu and L-Leu–L-Pro respectively in the agar overlay.

Immunodiffusion was conducted in 1% agar in a 12.5 mM sodium tetraborate buffer (pH 8.4) containing 0.15 M NaCl. Crude extracts of kidney and brain from guinea-pig and of intestinal mucosa from guinea-pig, rat, pig and cow were prepared by homogenising 1 g of each tissue in 10 ml of 50 mM sodium tetraborate (pH 8.0) containing 0.1 M NaCl. A supernatant fraction following centrifugation at 30 000 *g* for 15 min at 4°C was recovered.

3. Results and discussion

Two immunological procedures were used to compare the peptide hydrolases i.e. inhibition of enzyme activity and immunodiffusion.

3.1. *Inhibition of enzyme activity*

Purified preparations of each intestinal peptide hydrolase were inhibited by the antiserum raised against that homologous peptide hydrolase. No cross-reactivity was detected between any individual intestinal peptide hydrolase and antisera raised against the other hydrolases (table 1).

Aminoacyl proline hydrolase activity in cytoplasmic extracts of guinea-pig intestine, kidney and brain was inhibited by antiserum to the γ peptide hydrolase; the degree of inhibition with a 1:3 dilution of antiserum was 75%, 52% and 40% respectively.

Table 1
Percentage inhibition, relative to controls, of guinea-pig peptide hydrolases by antisera raised against the various enzymes

Peptide hydrolase	Antisera reacted with peptide hydrolases		
	Anti-α	Anti-β_2	Anti-γ
α	38	125	88
β_2	109	11	100
γ	105	100	11

For details see text.

3.2. *Immunodiffusion studies*

Double diffusion tests of each antiserum against individual preparations of purified hydrolases resulted in precipitation only when a given peptide hydrolase diffused towards its specific antiserum. No precipitin lines were observed with antisera of different specificity.

Double diffusion tests of antiserum to intestinal γ peptide hydrolase against cytoplasmic extracts from guinea-pig intestine, brain and kidney in adjacent wells produced one precipitin line in each case and these precipitin lines fused at their contiguous ends. The results indicate that the protein precipitated by the antiserum in each case shared (at least some) antigenic determinants with intestinal aminoacyl proline hydrolase. Immunodiffusion of antisera to either the β_2- or γ - peptide hydrolase of guinea-pig intestinal mucosa against cytoplasmic extracts from intestinal mucosa of guinea-pig, rat, pig and cow resulted in formation of a precipitin line in the case of the guinea-pig preparation only. This result indicates that the β_2 and γ peptide hydrolases of guinea-pig intestinal mucosa are species-specific.

3.3. *Immunoelectrophoresis*

To confirm that the precipitin lines observed in section 3.2. were the result of each peptide hydrolase precipitating its specific antiserum, aliquots of guinea-pig intestinal cytosol were subjected to electrophoresis on two agar plates. After electrophoresis, one plate was stained for peptide hydrolase activity. On the second plate, troughs were cut between the various antigen wells and to each of three troughs a different specific antiserum was added. Values for relative mobilities (related to Bromophenol Blue) of each

peptide hydrolase activity on the first plate corresponded with the position (relative to Bromophenol Blue) of its specific precipitin arc on the second plate. Likewise, in a parallel experiment when extracts of guinea-pig brain and kidney were electrophoresed and subsequently reacted with antiserum to the γ peptide hydrolase, the positions of the γ peptide hydrolase activity and the precipitin line due to the antiserum were identical.

4. Conclusions

These results show that although three peptide hydrolases in guinea-pig intestinal mucosa have several features in common including overlapping substrate specificities, they are immunologically distinct proteins. Each of the three peptide hydrolases is probably the product of a separate gene and the enzymes apparently possess no subunits in common. One of these intestinal mucosal enzymes, the γ peptide hydrolase or amino-acyl proline hydrolase is apparently immunologically identical with the enzyme responsible for hydrolysis of L-Leu–L-Pro in the brain and kidney of guinea-pig. Two of these guinea-pig intestinal mucosa enzymes, the β_2 and the γ, were immunologically distinct from peptide hydrolases present in the intestinal mucosa of rat, pig and cow.

Acknowledgements

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