BBA 66000

STUDIES ON INTESTINAL HYDROLYSIS OF PEPTIDES

I. L-GLUTAMINYL–L-PROLINE DIPEPTIDE HYDROLASE ACTIVITY IN THE HUMAN SMALL INTESTINE

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(Received July 3rd, 1969)

SUMMARY

The activities of L-glutaminyl–L-proline dipeptide hydrolase and glycyl–L-proline dipeptide hydrolase (EC 3.4.3.7) have been studied in the human intestinal mucosa. Both jejunum and ileum demonstrate marked activities, the jejunum being more efficient than the ileum. Characterization studies have been performed on partially purified enzymes obtained by gel filtration. L-Glutaminyl–L-proline dipeptide hydrolase and glycyl–L-proline dipeptide hydrolase are different with respect to pH and ion dependence. The two enzyme activities can be separated with heat treatment in the presence of Mn^{2+}.

INTRODUCTION

Among the proteins of nutritional significance, the gliadins represent a peculiar group in respect to their amino acid composition, glutamine and proline accounting for about 55% of the amino acid content[1–3]. While it is commonly accepted that mucosal peptidases are responsible for the terminal exogenous protein digestion[4], scanty attention has been paid to peptidases hydrolyzing glutamine-containing peptides.

The present report describes an assay method for and some preliminary studies on the L-glutaminyl–L-proline dipeptide hydrolase activity (Gln–Pro dipeptidase) in the human intestinal mucosa. Glycyl–L-proline dipeptide hydrolase (EC 3.4.3.7) activity (Gly–Pro dipeptidase) is also included in the study.

MATERIAL AND METHODS

L-Gln–L–Pro was prepared by YEDA (Rehovot, Israel) and was found to be chromatographically pure. Gly–L-Pro was obtained from Nutritional Biochemical

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Corporation, Sephadex G-200 from Pharmacia (Uppsala, Sweden). All other products were reagent grade.

Histologically normal samples obtained surgically from 19 human subjects (between 26 and 73 years of age) were immediately frozen and analyzed within 30 days. Jejunal samples were taken from 10-cm distal to the ligament of Treitz, ileal samples from about 40 cm above the ileocecal valve. Preliminary experiments revealed that freezing for 30 days followed by a single thawing did not affect the enzyme activities.

Mucosal scrapings were homogenized for 3 min in 0.01 M Tris–HCl buffer, pH 7 (5 ml per g mucosa) with an Ultra-Turrax homogenizer. After centrifugation at $1500 \times g$ for 15 min, the supernatant (found to contain more than 90% of the enzyme activities) was used for the assays. For the enzyme assay, the enzyme solution (containing 20–100 μg of protein) was incubated at 37° for 5–10 min, in the presence of 8 μmoles of substrate and 120 μmoles of Tris–maleate buffer (pH 6.3, for Gln–Pro dipeptidase assay; pH 7.2, for the Gly–Pro dipeptidase assay), in a total volume of 0.4 ml. As a measure of the enzyme activity, after the incubation the free proline was determined by the method of WREN AND WIGGALL[5] slightly modified to render negligible the interference due to the dipeptides: the acid strength of the reaction mixture was reduced by using acetic acid–6 M phosphoric acid–water (2.8:3.2:4, by vol.), and the reaction was developed at 70° for 60 min. The acid reagent was added directly to the incubation mixture and served to terminate the incubation.

In preliminary experiments, aliquots of the incubation mixtures with L-glutaminyl–L-proline as a substrate were used for NH_3 determination according to SELIGSON AND HIRAHARA[6] and for descending paper chromatography (solvent systems: *n*-butanol–acetic acid–water 4:1:1, by vol.; and *n*-propanol–water 4:1, v/v; paper Whatman No. 1) 0.2% ninhydrin in acetone, and starch iodide following chlorination[7] were used for the staining. No ninhydrin unreactive starch iodide reactive compounds and no NH_3 were found to be formed during the incubation, indicating that L-Gln–L-Pro was neither converted to Glu–Pro nor to pyrrolidone carboxylylproline under our experimental conditions.

Zero-order kinetics were always obtained in the enzyme assays, while the observed velocities were higher than 84% of the v_{max} (calculated by standard procedure)[8,9]. The accuracy of the enzyme assay method was $\pm$ 1.6%.

One unit of enzyme activity is the quantity of enzyme splitting 1 μmole of dipeptide per min.

Protein determination was performed by the method of LOWRY *et al.*[10] as modified by EGGSTEIN AND KREUTZ[11]. Human serum albumin purchased from Behringwerke was used for the standard curve.

RESULTS

The activities of the jejunal enzymes (units per g protein) were 179 $\pm$ 50.5 and 144 $\pm$ 61 (mean $\pm$ S.D., $n = 14$) for Gln–Pro dipeptidase and Gly–Pro dipeptidase, respectively. The corresponding values for ileum ($n = 5$) were 95 $\pm$ 37.2 and 70 $\pm$ 30. Both enzyme activities were eluted from Sephadex G-200 in a single peak, and the ratio between them was found to be constant in the various fractions (Fig. 1). The specific activities increased 5–7-fold when compared to the homogenate applied to the column; the enzyme-containing fractions were pooled and used for characterization studies.

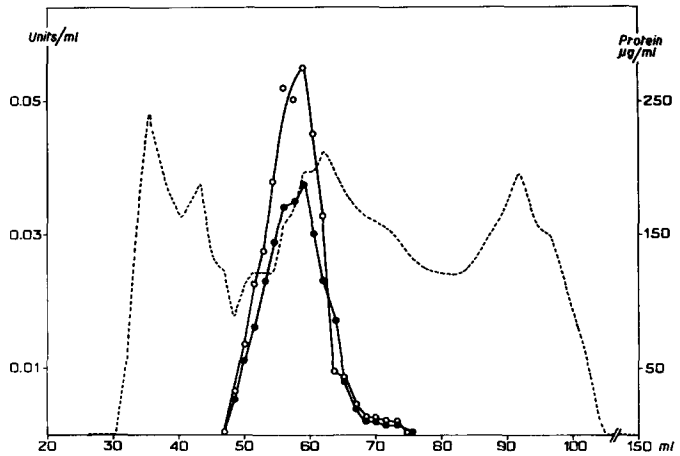

Fig. 1. Gel filtration on Sephadex G-200 of dipeptide hydrolases from human ileum. Sephadex was allowed to swell and was packed according to standard procedures[12]. The homogenate was centrifuged at 105 000 × *g* for 90 min and the supernatant (20–30 mg protein with more than 90% of total original enzyme activity) was applied on columns (1.4 cm × 52 cm) and eluted with 0.01 M Tris–HCl buffer (pH 7) at a flow rate of 4 ml/h. The elution volumes are reported on the abscissa. ·····, protein; ●—●, Gly–Pro dipeptidase; ○—○, Gln–Pro dipeptidase. The recoveries of enzyme activities and protein were essentially quantitative. Similar results were obtained from human jejunum.

As shown in Fig. 2, pH affects the two enzyme activities differently. At the pH optimum for Gln–Pro dipeptidase, there is only 40% of the Gly–Pro dipeptidase activity. The K_m values of Gln–Pro dipeptidase were 2.93–6.66 mM for the jejunum and 4.02–5.27 mM for the ileum. For Gly–Pro dipeptidase a K_m of 2.10 mM was observed in the jejunum, 1.92 mM in the ileum.

The effect of divalent metal ions on enzyme activities is shown in Table I. While the Gln–Pro dipeptidase was not significantly affected by Co^{2+} and Mn^{2+}, the Gly–Pro dipeptidase was activated 2-fold by Co^{2+} and up to 6-fold by Mn^{2+}.

The course of the heat inactivation ran closely parallel for the two enzyme activities in the pH range 4.8–8. However, when the enzyme solution was heated in the presence of 2 mM $MnCl_2$ (in 25 mM Tris–maleate buffer, pH 6.5), the Gln–Pro dipeptidase was completely destroyed after 1 h at 40°, while the Gly–Pro dipeptidase was still activated.

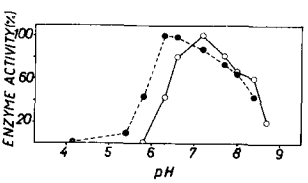

Fig. 2. Dipeptide hydrolase activities from human jejunal mucosa as a function of pH. The enzyme activities are expressed as percent of those at the optimum pH. ●·····●, Gln–Pro dipeptidase; ○—○, Gly–Pro dipeptidase. The final pH figures of the incubation mixtures are given on the abscissa. Buffer used: 0.3 M phosphoric acid–acetic acid–boric acid[13] (pH 5.4–9.2); 0.3 M Tris–maleate (pH 5.4–8.7); 0.3 M potassium phosphate (pH 5.8–7.9). With the three series of buffers overlapping pH–activity curves were obtained. Similar results were observed for ileum and using both crude extracts and pooled chromatographic fractions as source of enzymes.

TABLE I

EFFECT OF DIVALENT METAL IONS ON THE DIPEPTIDE HYDROLASE ACTIVITIES OF HUMAN JEJUNUM

For studies with Mn^{2+}, the enzyme solution was preincubated at 37° for 30 min in 0.01 M Tris–HCl buffer (pH 7) in the presence of 1–40 mM Mn^{2+}. After preincubation the enzyme activities were determined according to the standard procedure in the presence of 0.5–20 mM Mn^{2+}. For studies with the other metals ions, the enzyme solution was directly incubated according to the standard procedure in the presence of the ion. All the metals were added as chloride salts. In the table, the final concentrations of the metals in the incubation mixtures are reported. The rate of hydrolysis in the absence of metal ions is arbitrarily taken as 100. Similar results were obtained from human ileum.

Ion added	*Concn. (mM)*	*Relative rate of hydrolysis*	
		Gln–Pro dipeptidase	*Gly–Pro dipeptidase*
None		100	100
Co^{2+}	2.5	103	209
	5	111	211
Cd^{2+}	5	10	8
Zn^{2+}	5	15	18
Mg^{2+}	5	150	164
Cu^{2+}	5	0	0
Mn^{2+}	0.5	104	245
	1	110	305
	2	125	375
	10	93	425
	20	98	615

Both activities precipitated between 50 and 70% $(NH_4)_2SO_4$ saturation and between 45 and 65% ethanol, without any change in the ratio between them.

DISCUSSION

The present results indicate that human intestinal mucosa actively hydrolyzes L-Gln–L-Pro. The enzyme activity in the proximal jejunum is higher than in ileum in accordance with the distribution observed in human fetuses for the same activities[14] and in pig and rat for other dipeptidases[15–17]. Although the physiological role of the L-Gln–L-Pro dipeptide hydrolase cannot be established at present, these observations might help to clarify the mechanism of the terminal digestion of glutamine- and proline-rich proteins such as the gliadins.

Gly–L-Pro dipeptide hydrolase is known to hydrolyze C-terminal proline containing dipeptides. It has been studied in many animal and human tissues[18–23], including human small intestinal mucosa[24,25]. Whether the activity hydrolyzing L-Gln–L-Pro is due to the same enzyme remains to be established. Our results indicate that the two enzyme activities differ from each other as far as the effects of pH and metal ions are concerned. While a difference in the pH–activity curves is not unusual, even if both substrates are acted upon by the same enzyme, the difference in the effects of Mn^{2+} and Co^{2+} and the selective protective effect of Mn^{2+} on the Gly–Pro dipeptidase may suggest that a specific enzyme hydrolyzes Gln–Pro in the human intestinal mucosa. A final conclusion must await more extensive purification of the enzyme activities.

ACKNOWLEDGMENTS

The authors are indebted to Professor Edoardo Scarano for many fruitful discussions; to Professor Francesco Salvatore and Dr. Concetta Pietropaolo for determination of NH_3; and to Mr. Francesco Vollaro for excellent technical assistance.

REFERENCES

1 J. H. Woycgik, J. A. Boundy and R. J. Dimler, *J. Agr. Food Chem.*, 9 (1961) 307.
2 J. Mosse', *Federation Proc.*, 25 (1966) 1663.
3 J. A. D. Ewart, *J. Sci. Food Agr.*, 18 (1967) 111.
4 J. B. Rhodes, in C. F. Code, *Handbook of Physiology*, Section 6, Vol. 5, Am. Physiol. Soc., 1968, p. 2589.
5 J. J. Wren and P. H. Wiggall, *Biochem. J.*, 94 (1965) 216.
6 D. Seligson and H. Kirahara, *J. Lab. Clin. Med.*, 49 (1957) 962.
7 S. C. Pan and J. D. Dutcher, *Anal. Chem.*, 28 (1956) 836.
8 H. Lineweaver and D. Burk, *J. Am. Chem. Soc.*, 56 (1934) 658.
9 M. Dixon, *Biochem. J.*, 55 (1953) 170.
10 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, 193 (1951) 265.
11 M. Eggstein and F. H. Kreutz, *Klin. Wochschr.*, 33 (1955) 879.
12 P. Flodin, *Dissertation*, Uppsala, 1962.
13 T. Teorell and E. Stenhagen, *Biochem. Z.*, 299 (1938) 416.
14 A. Rubino, M. Pierro, G. La Torretta, M. Vetrella, D. Di Martino and S. Auricchio, *Pediat. Res.*, 3 (1969) 313.
15 L. Josefsson and T. Lindberg, *Acta Physiol. Scand.*, 66 (1966) 410.
16 L. Josefsson and T. Lindberg, *Biochim. Biophys. Acta*, 105 (1965) 162.
17 G. B. Robinson and B. Shaw, *Biochem. J.*, 77 (1960) 351.
18 E. L. Smith and M. Bergmann, *J. Biol. Chem.*, 153 (1944) 627.
19 E. Adams and E. L. Smith, *J. Biol. Chem.*, 198 (1952) 671.
20 E. Adams, N. C. Davis and E. L. Smith, *J. Biol. Chem.*, 208 (1954) 573.
21 N. C. Davis and E. L. Smith, *J. Biol. Chem.*, 224 (1957) 261.
22 H. P. Chase and V. Kumar, *Abstr. 39th Meeting Am. Soc. Pediat. Res., Atlantic City, 1969*, p. 80.
23 C. Schwabe, *Biochemistry*, 8 (1969) 771.
24 M. Messer, Ch. M. Anderson and R. R. W. Townley, *Clin. Chim. Acta*, 6 (1961) 768.
25 W. D. Heizer and L. Laster, *J. Clin. Invest.*, 48 (1969) 210.