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fetus intestine

Enzymatic Activity Hydrolyzing γ-Glutamyl-β-Naphthylamide in Human Intestine during Adult and Fetal Life

S. Auricchio[25], F. Ciccimarra, A. Vegnente, G. Andria, and M. Vetrella

II Department of Pediatrics, University of Naples, Naples, Italy

Extract

The enzymatic activity hydrolyzing γ-glutamyl-β-naphthylamide (γ-glutamyltranspeptidase, GGTP) was studied in the small intestine of human adults and fetuses. Surgical biopsies taken from the proximal jejunum approximately 10 cm from the ligament of Treitz served as the source of the enzyme for adult subjects. The entire small bowel was obtained from fetuses of different ages and divided into three thirds for enzymatic assay. All samples were frozen immediately and used within 1–4 months. It was shown that enzymatic activities stored at −20° are stable for that period.

The GGTP activity was enhanced by the presence of glycylglycine in the incubation mixture, the maximal activation being 5.2-fold ± 0.65-fold (mean ± 1 SD) when the dipeptide concentration was 40 mM. The pH optimum for GGTP activity was 9.0 both in the presence and in the absence of glycylglycine. Glycylglycine activation and pH activity curves were similar for the proximal jejunum of adults and of two fetuses (17 and 20 weeks old).

The specific activity of GGTP significantly increased from the proximal to the distal third at every fetal age ($P = 0.01$–0.001). The specific GGTP activity (U/g protein) of the proximal jejunum of the adult was 2.2 ± 0.5 (mean ± 1 SD). It was thus approximately one-eighth that found in the proximal third of the fetal small bowel (18.4 ± 7.7 U/g protein).

In order to investigate the subcellular localization of GGTP activity, isolated brush borders and brush border membranes were prepared from surgical biopsies of adult proximal jejunum. The GGTP- and sucrase (EC. 3.2.1.26)-specific activities increased in a parallel way from the brush border to the membrane fraction. This result indicates that at least part of the GGTP activity is located in this membrane.

Speculation

The physiologic significance of GGTP activity in the intestine is still conjectural. If it is involved in intestinal digestion, the high levels of the enzyme present in the fetus enable the neonate to digest γ-glutamyl peptides.

Introduction

Most intestinal peptidases hydrolyze α-peptide bonds. Peptidase activity able to split γ-glutamyl peptides (γ-glutamyltranspeptidase) has been demonstrated in several animal tissues [8], as well as in human intestine [12].

In this paper the enzymatic activity hydrolyzing γ-glutamyl-β-naphthylamide was studied in human intestine during adult and fetal life.

Materials and Methods

Macroscopically normal pieces of small intestine were obtained from the proximal jejunum of adults, approximately 10 cm from the ligament of Treitz, during surgical operations, and were then frozen at −20° for 1–4 months. Enzymatic activities were stable under these conditions for at least 4 months.

Twenty-six fetuses (13 male, 13 female) were obtained from legal abortions performed because of medical-social indications. Fetal age, estimated by crown-rump length [18], ranged between 13 and 24 weeks. The intestines were immediately removed and frozen. After thawing, the intestinal contents were removed; the mucosa was scraped off with a glass slide and homogenized in distilled water (10 ml/g tissue) with a motor-driven Teflon-glass homogenizer [20]. The supernatant fluid, which contained more than 90% of the enzymatic activity, was used for enzyme assay and for determination of protein.

In order to study the subcellular localization of GGTP activity, human tissue obtained from the proximal jejunum was processed within 30–120 min after sampling without freezing. The scraped mucosa was placed in 5 mM EDTA (25 ml/g tissue), adjusted to pH 8.5 with NaOH, and homogenized in a motor-driven Teflon-glass homogenizer (600 rpm, 40 strokes up and down) for 120 sec in the cold. The homogenate was then processed according to the method of Forstner *et al.* [10] to obtain "crude" brush borders, "purified" brush borders, and brush border membranes. The following modifications of the original method were used: (*1*) the first sediment was homogenized in the same homogenizer, in 5 mM EDTA buffer, pH 7.4, for 30 sec (5 strokes up and down) and then centrifuged (this procedure was repeated three times to obtain the "crude" brush border) and (*2*) the "purified" brush border was maintained in 60 volumes of distilled water for 90 min in order to prepare the brush border membrane.

Morphologic confirmations of brush borders and their membranes were made by electron microscopy [1, 6].

For the assay of GGTP activity, a 1.66 mM solution of γ-glutamyl-β-naphthylamide [21] was freshly prepared before use in 0.05 M sodium carbonate, pH 9.0 (8). Three volumes of this solution were mixed with 1 volume of 0.4 M phosphate/acetate/borate buffer, pH 9.0 [14]. The incubation mixture contained 200 μl substrate buffer solution, 25 μl enzyme solution, and 25 μl distilled water or 0.4 M aqueous glycylglycine solution [22], adjusted to pH 9 with NaOH.

Incubation was carried out at 37° for 30 and 60 min, and the enzymatic reaction was stopped by adding 250 μl 40% trichloroacetic acid (TCA) and 500 μl water. The β-naphthylamine liberated was measured according to the method of Goldbarg *et al.* [11].

"Blank tubes" were run through the procedure with TCA and the enzyme added successively to the substrate-buffer solution incubated at 37°.

Sucrase activity was determined according to the method of Auricchio *et al.* [4]. Protein was estimated by the method of Lowry *et al.* [16] with crystallized bovine albumin [22] used as standard.

One unit of the enzyme activity hydrolyzes 1 μmole substrate per min.

Results

GGTP Activity in the Small Intestine of the Adult

The reaction was linear with respect to both incubation time and protein content when the incubation mixture contained 0.1–0.5 milliunits enzymatic activity.

Liberation of β-naphthylamine from the substrate was strongly activated by the presence of glycylglycine in the incubation mixture (Fig. 1). A 40 mM concentration of the dipeptide in the assay mixture gave the maximal activation of 5.2-fold ± 0.65-fold (mean ± 1 SD) (Table I).

The pH optimum of GGTP activity was 9.0 both in the presence and the absence of glycylglycine (Fig. 2).

The presence of 5 mM EDTA in the incubation mixture did not result in inhibition of GGTP activity.

A study was made in six subjects of the subcellular distribution of GGTP and sucrase activities, the latter being considered a typical marker of the microvillus membrane [9]. Recoveries of sucrase and GGTP activities in the "purified" brush border, as percentages of the activities found in the total homogenate, were 19.0% ± 3.3% and 13.8% ± 2.4% (means ± 1 SD), respectively, with increases in specific activity of 6.4-fold ± 3.4-fold and 4.4-fold ± 2.1-fold (means ± 1 SD), respectively. Further and parallel increases in the specific activity of both enzymatic activities were obtained

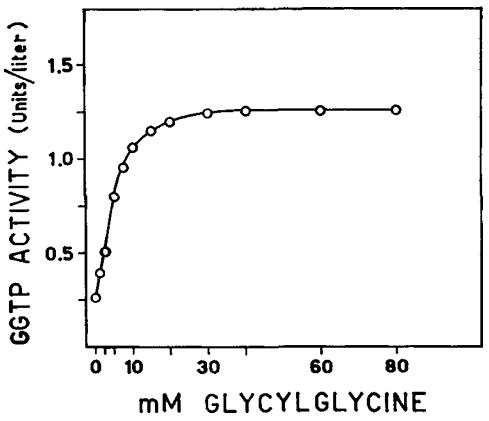

Fig. 1. Effect of glycylglycine concentration on the human adult intestinal γ-glutamyltranspeptidase (*GGTP*) activity. A crude mucosal extract, prepared from the proximal jejunum and containing 3 U of enzymic activity/g protein, was used for the experiment. For concentrations of glycylglycine lower than 5 mM and in the absence of glycylglycine, a 500-μl incubation mixture was used and the enzymatic reaction was stopped by addition of 250 μl 40% trichloroacetic acid and 250 μl water. Other experimental details are given in text. Enzymic activity is expressed as units per liter of incubation mixture. Essentially similar results were observed when experiments were carried out on the human fetal intestine (proximal third).

Table I. Specific activity of sucrase and γ-glutamyltranspeptidase (GGTP) (U/g protein) in the small intestine of 20 fetuses and in the proximal jejunum of 10 human adults[1]

Enzyme activity	Fetal small intestine[2]			Adult jejunum
	Proximal third	Intermediate third	Distal third	
Sucrase				
Mean	136.2	113.0	100.8	85.1
Range	65.4–230.4	52.8–192.1	36.8–202.0	50.0–148.4
1 SD	53.7	40.6	44.7	30.1
GGTP				
Mean	18.4	26.5	30.0	2.2
Range	8.1–32.7	12.7–42.2	11.1–62.0	1.7–3.0
1 SD	7.7	11.2	15.0	0.5
GGTP (in the presence of glycylglycine)				
Mean	85.2	121.6	139.0	11.6
Range	40.1–172.5	42.6–246.8	48.9–361.3	7.2–16.3
1 SD	40.7	62.3	84.0	3.0
Ratio between sucrase and GGTP activities				
Mean	8.4	5.1	3.9	38.1
Range	2.7–22.1	1.8–15.0	1.5–9.2	26.9–62.4
1 SD	4.5	3.1	2.0	11.2

[1] The following differences are statistically significant: (*1*) proximal against distal third of fetal small intestine: sucrase ($P = 0.05$–0.02), GGTP ($P = 0.01$–0.001), ratio between sucrase and GGTP ($P < 0.001$); (*2*) proximal third of fetal small intestine against adult jejunum: sucrase ($P = 0.02$–0.01), GGTP, and ratio between sucrase and GGTP ($P < 0.001$).

[2] In a fetus with a gestational age of 35 weeks, who survived extrauterine life less than 24 hr and was never fed, the following enzymic activities were found for the proximal, intermediate and distal third, respectively: GGTP 11.4, 8.7, 8.2 U/g protein; sucrase 251.4, 130, 75.4 U/g protein.

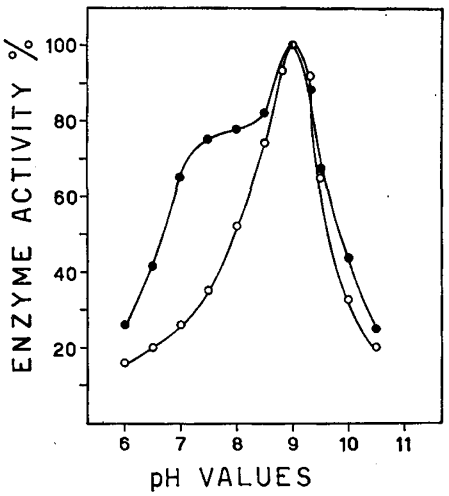

Fig. 2. Effect of pH on the human intestinal γ-glutamyltranspeptidase activity of the adult. A crude mucosal extract, prepared from the proximal jejunum and containing 3.3 U of enzymic activity/g protein, was used for the experiment. A 0.6 mM concentration of substrate in the incubation mixture was used. The enzyme assay was carried out in the absence (○——○) and in the presence (●——●) of glycylglycine. For pH lower than 7.0 and higher than 9.5, a 500-μl incubation mixture was used (see Fig. 1). Enzymic activity is expressed as percentage of that found at the pH optimum. The final pH figures of the reaction mixture are given. Buffer: 0.08 M phosphate-acetate-borate [14]. Other experimental details are given in text. Essentially similar curves were observed in experiments on human fetal intestine (proximal third).

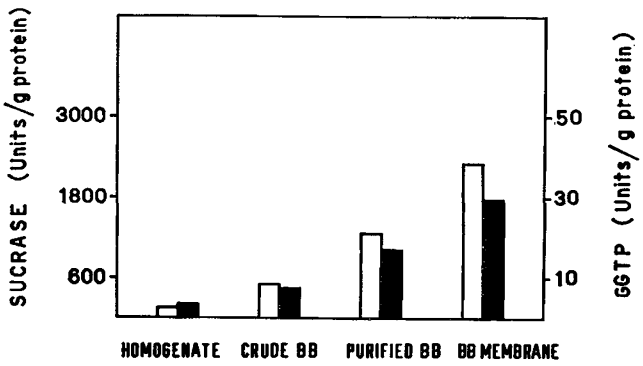

Fig. 3. Subcellular distribution of γ-glutamyltranspeptidase (*GGTP*) (*black*) and sucrase (*white*) activities of the human adult jejunum in the crude brush border (*BB*), purified brush border and brush border membrane. The mucosal homogenate contained 148.4 U sucrase/g protein and 3 U GGTP/g protein, respectively. See text for experimental details.

when the brush border membrane was compared with the intact brush border (Fig. 3).

Essentially similar distribution was observed when GGTP activity was determined in the presence of glycylglycine.

GGTP Activity in the Developing Small Intestine

No difference was found between the GGTP activity of the proximal jejunum of adults and of two fetuses aged 17 and 20 weeks in regard to the activating effect of different concentrations of glycylglycine in the incubation mixture and the pH activity curve in the presence and absence of glycylglycine.

For purposes of statistical analysis, the fetuses studied were arbitrarily divided into three groups, with the age varying from 13 to 15, from 17 to 20, and from 22 to 24 weeks, respectively. The GGTP activity of the proximal and the distal thirds of the small bowel showed no significant variations among the three groups.

The results obtained from study of the GGTP activities in all of the fetuses were therefore combined, and the data are presented in Table I. Specific activity of GGTP was significantly lower in the proximal than in the distal third, whereas the opposite was true of sucrase activity [3].

The small intestines of two fetuses, 17 and 20 weeks of age, and of a full term neonate were divided into nine pieces for enzymatic assay. The results obtained were similar in all of the studied subjects; the specific activity of GGTP increased distally, whereas that of sucrase decreased (Fig. 4).

The GGTP activity of the proximal third of fetal small intestine was approximately 8 times higher than the values found in the surgical biopsies taken from the jejunum of adults approximately 10 cm from the ligament of Treitz (Table I).

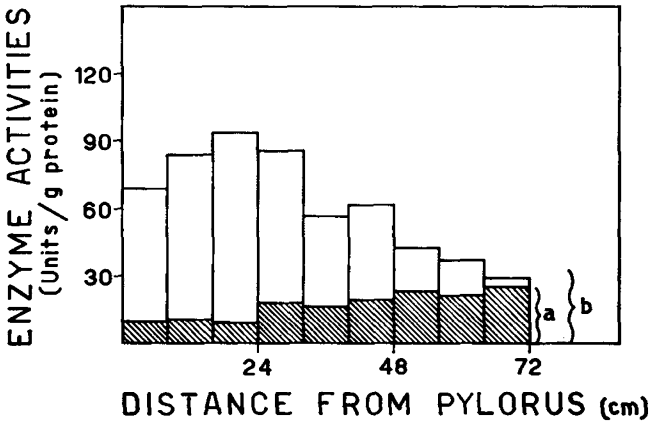

Fig. 4. Distribution of γ-glutamyltranspeptidase (*GGTP*) (*a*) and sucrase (*b*) activities along the small intestine of a 20-week-old fetus. See text for experimental details.

Discussion

It has been suggested that GGTP activity may play a role in the intestinal hydrolysis of peptides and proteins containing γ-glutamyl bonds, such as glutathion, folic acid, collagen, or gluten [8], but the presence of this bond in proteins has been questioned recently [5]. In the kidney, this enzymatic activity has been supposed to be involved in the transport of amino acids across cell membranes [17].

The major location of this enzyme in the intestine of both guinea pig [8] and rat [2] is in the brush border membrane of the enterocyte. Both the recovery of sucrase and GGTP activities in the isolated brush border and the degree of purification from the homogenate to the brush border were lower in man than in rat. Nevertheless, it is evident from the present results that the GGTP activity of human intestine, was purified from the "crude" brush border to the membrane in a way parallel to that of sucrase. This demonstrates that also in man at least some GGTP activity is located in this membrane. Similar results with higher recovery were recently obtained from frozen intestinal biopsies in man by Cerda and Preiser [7] in Crane's laboratory.

GGTP activity showed higher levels in the distal part of human intestine than in the proximal portion, at least during fetal life. The contrary is true for many intestinal peptidases [15, 19] and disaccharidases [3] in man, as well as for other enzymes of the brush border membrane in rat [13] and for GGTP activity itself in guinea pig fetuses [8].

Many peptidase as well as disaccharidase activities are present in man in early periods of fetal life at levels comparable to those found in adults [3, 15, 19]. Intestinal GGTP activity, on the contrary, in man (Table I), as well as in guinea pig [8], is higher during fetal life than after birth.

Summary

The specific activities of γ-glutamyltranspeptidase (GGTP) in human intestine are reported for the proximal, intermediate, and distal thirds of the small bowel of fetuses 13–24 weeks old.

The specific activity of GGTP in the proximal third of fetal small intestine was significantly lower than that in the distal third, and approximately 8 times greater than the specific activity in the proximal jejunum of adults.

The GGTP activity was strongly enhanced by the

presence of glycylglycine in the incubation mixture; it had a pH optimum of 9.0, and appeared to be located, at least in part, in the brush border membrane of the enterocyte after subcellular fractionation.

References and Notes

1. ANDRIA, G., DE MASI, R. V., AND CECIO, A.: Characterization of brush border membranes isolated from human small intestine (manuscript in preparation).
2. AURICCHIO, S., PIERRO, M., ANDRIA, G., AND DE RITIS, G.: Enzymic activities of the brush border membrane of rat intestine hydrolyzing β-naphthylamides of amino acids, leucinamide and dipeptides. Biochim. Biophys. Acta, *274:* 420 (1972).
3. AURICCHIO, S., RUBINO, A., AND MÜRSET, G.: Intestinal glycosidase activities in the human embryo, foetus and newborn. Pediatrics, *35:* 944 (1965).
4. AURICCHIO, S., RUBINO, A., TOSI, R., SEMENZA, G., LANDOLT, M., KISTLER, H. J., AND PRADER, A.: Disaccharidase activities in human intestinal mucosa. Enzymol. Biol. Clin., *3:* 193 (1963).
5. BENSUSAN, H. B.: The glutamyl linkages in collagen. Biochemistry, *8:* 4723 (1969).
6. CECIO, A., DE MASI, R. V., AND ANDRIA, G.: Rilievi submicroscopici comparativi sul "brush border" isolato da intestino di ratto e di uomo. Boll. Soc. It. Biol. Sper., *46:* 1011 (1970).
7. CERDA, J. J., AND PREISER, H.: Personal communication.
8. COHEN, M. I., GARTNER, L. M., BLUMENFELD, O. O., AND ARIAS, I. M. Gamma glutamyl transpeptidase: Measurement and development in guinea pig small intestine. Pediat. Res., *3:* 5 (1969).
9. EICHHOLZ, A.: Structural and functional organisation of the brush border of intestinal epithelial cells. III. Enzyme activities and chemical composition of various fractions of Tris-disrupted brush borders. Biochim. Biophys. Acta, *135:* 475 (1967).
10. FORSTNER, G. G., SABESIN, S. M., AND ISSELBACHER, K. J.: Rat intestinal microvillus membranes. Purification and biochemical characterization. Biochem. J., *106:* 381 (1968).
11. GOLDBARG, J. A., FRIEDMAN, O. M., PINEDA, E. P., SMITH, E. E., CHATTERJI, R., STEIN, E. H., AND RUTENBURG, A. M.: The colorimetric determination of γ-glutamyl transpeptidase with a synthetic substrate. Arch. Biochem. Biophys., *91:* 61 (1960).
12. GREENBERG, E., WOLLAEGER, E. E., FLEISHER, G. A., AND ENGSTROM, G. W.: Demonstration of γ-glutamyl transpeptidase activity in human jejunal mucosa. Clin. Chim. Acta, *16:* 79 (1967).
13. HARRISON, D. D., AND WEBSTER, H. L.: Proximal to distal variations in enzymes of the rat intestine. Biochim. Biophys. Acta, *244:* 432 (1971).
14. HINSBERG, K., AND LANG, K.: Medizinische Chemie für den Klinischen und theoretischen Gebrauch, p. 137. (Urban & Schwarzenberg, Munich, 1957).
15. LINDBERG, T.: Intestinal dipeptidases; characterization, development and distribution of intestinal dipeptidases of the human foetus. Clin. Sci., *30:* 505 (1966).
16. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., AND RANDALL, R. J.: Protein measurements with the folin phenol reagent. J. Biol. Chem., *193:* 265 (1951).
17. ORLOWSKI, M., AND MEISTER, A.: The γ-glutamyl cycle: a possible transport system for amino acids. Proc. Nat. Acad. Sci. U.S.A., *67:* 1248 (1970).
18. PATTEN, B. M.: Human embriology. (McGraw-Hill, New York, 1953).
19. RUBINO, A., PIERRO, M., LA TORRETTA, G., VETRELLA, M., DI MARTINO, D., AND AURICCHIO, S.: Studies on intestinal hydrolysis of peptides. II. Dipeptidase activity toward L-glutaminyl-L-proline and glycyl-L-proline in the small intestine of the human fetus. Pediat. Res., *3:* 313 (1969).
20. Model B, Arthur H. Thomas Co., Philadelphia, Pa.
21. Obtained from Cyclo Chemical Co., Los Angeles, Calif.
22. Obtained from Sigma Chemical Co., St. Louis, Mo.

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25. Requests for reprints should be addressed to: SALVATORE AURICCHIO, M.D., II Department of Pediatrics, University of Naples, Via S. Andrea delle Dame 4, Naples, Italy.

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