Pediat. Res. *3:* 313–319 (1969)

Developmental biochemistry
fetus
glutaminyl proline dipeptidase
glycyl proline dipeptidase
intestine
newborn

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

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Extract

The dipeptidase activity toward L-glutaminyl-L-proline and glycyl-L-proline has been studied in the small intestinal mucosa of human fetuses and newborns between 14 and 36 weeks of fetal age.

The small intestine was removed within 15 minutes of death. It was immediately frozen and used for enzymatic studies within 2 to 30 days. The activity of both enzymes was stable for as long a period as three months at a temperature of −20°. Mucosal scrapings were homogenized in 0.01 M Tris-HCl buffer, pH 7, and then centrifuged at 1500 × g for 10 minutes. The supernatant, which contained more than 95 % of the enzyme activity, was used for assay; it was incubated in 20 mM substrate, at optimum pH, under conditions permitting calculation of initial velocity. Free proline was measured at the end of the incubation period. By the 14th to 16th week of age, levels of activity of both enzymes were comparable with those of the adult (fig. 1).

In order to investigate the distribution of dipeptidase activity, the small bowel from fetuses between 22 and 34 weeks of age was examined. Each sample was homogenized separately before studying. Uniformly high levels of dipeptidase activity were found throughout the proximal six-tenths of the small bowel, while lower values were observed in the terminal ileum (fig. 2). A significant difference in the activity of both enzymes was found between the proximal and the distal third of the small bowel when calculated according to Student's t-test, $p < 0.02$ (table I). The activity ratios were constant throughout the length of the intestine during the entire age span studied.

After centrifugation of the homogenate of intestine at 105,000 × g for one hour, gel filtration on Sephadex G 200 of the supernatant resulted in the partial purification of the enzyme, as indicated by a 4.5- to 6.5-fold increase in specific activity.

The enzymes of the crude and the partially purified extracts were studied to determine the influence on enzyme activity of pH, Co^{++} and Mn^{++} ions, heat treatment, substrate concentrations. Co^{++} and Mn^{++} ions clearly activated hydrolysis of glycyl-L-proline, but did not affect that of L-glutaminyl-L-proline. Heating at 40° in a solution of 0.002 M Mn^{++} resulted in an increase of activity of glycyl-L-proline dipeptidase and the nearly complete disappearance of that of L-glutaminyl-L-proline dipeptidase (fig. 5); however, the activity of both enzymes was not separated by gel filtration, ammonium sulphate precipitation, or DEAE cellulose chromatography. Further studies are necessary to determine if a specific enzyme (s) of the small bowel mucosa, other than glycyl-L-proline dipeptidase (EC.3.4.3.7), causes hydrolysis of L-glutaminyl-L-proline.

Glycyl-L-proline dipeptidase activity did not change during the age period studied; however, L-glutaminyl-L-proline dipeptidase activity of 14- to 24-week-old fetuses displayed a pH dependence and a kinetic versus substrate concentration not observed in fetuses from 26 to 36 weeks of age (fig. 4). These results may suggest that a qualitative change of L-glutaminyl-L-proline dipeptidase had occurred with maturation.

Speculation

This study suggests that in the small intestine of the human fetus, L-glutaminyl-L-proline dipeptidase in early stages of life differs qualitatively from that of older fetuses. Further studies on pure enzymes may elucidate the basic mechanism that underlies qualitative changes of some enzyme molecules during growth.

Introduction

The demonstration that some malabsorption syndromes are due to a congenital deficiency of enzyme activity in intestinal mucosa [19] has focused interest on the development of intestinal enzymatic activities. It has been suggested that gluten-induced enteropathy may also be due to deficiency of a peptidase activity that hydrolyzes one or more peptides containing glutamine and/or proline [6, 9].

Although there have been studies of peptidase activity in the small bowel of human fetuses [5, 7, 8, 11], little attention has been given to enzymes involved in hydrolysis of glutamine- and/or proline-containing peptides. The present study reports properties, development, and distribution of L-glutaminyl-L-proline and glycyl-L-proline dipeptidase activity in the small bowel of the human fetus.

Material and Methods

Sixteen fetuses and newborns were studied. All survived extrauterine life less than 24 hours and were never fed. Fetal age, estimated by measuring the crown-rump length [18], ranged between 14 and 36 weeks.

The small intestine was removed and frozen within 15 minutes after death. Enzyme analyses were performed within 2–30 days. Dipeptidase activity was unaffected by freezing and a single thaw for a period of at least three months.

Following thawing, the intestinal content was removed; the mucosa was scraped off with a glass slide and homogenized for three minutes with 0.01 M Tris-HCl buffer, pH 7 (5 ml/g mucosa) using an Ultra-Turrax homogenizer having an efficient cooling system. The homogenate was then centrifuged in the cold at $1500 \times g$ for ten minutes. The supernatant, which contained more than 95 % of the enzyme activity, was used for enzyme assay and for determination of protein. To study the distribution of enzyme activity, the small intestine was divided into several pieces, and the mucosa from each was homogenized separately.

The reagents, analytical procedures, and definition of units were the same as those reported previously [20], with one exception. In the determination of L-glutaminyl-L-proline dipeptidase activity in fetuses between 14 and 24 weeks of age, the pH of the incubation mixture was 7.2. The method for the enzyme assay was based upon the measure of free proline after incubation of the enzyme solution together with the substrate; the accuracy of this method was ± 1.6 %.

Preliminary experiments revealed that no ammonia or pirrolidone carboxylyl peptides were formed during the incubation of the enzyme solution in the presence of L-glutaminyl-L-proline; no conversion of glutamic acid or glutamine into proline occurred; and proline added to the incubation mixture was quantitatively recovered. Determination of free proline at the end of incubation was considered suitable for measuring the degree of hydrolysis of L-glutaminyl-L-proline into glutamine and proline [20].

For purification and characterization studies, crude homogenates were centrifuged at $105{,}000 \times g$ for 60 minutes. The supernatant, which contained 100 % of each dipeptidase activity, was used for Sephadex G-200 chromatography [20]. Characterization studies, including effects of varying pH, substrate concentration, metal ions, heat inactivation, and ammonium sulphate precipitation were performed under conditions previously reported [20]. For experimental conditions of DEAE cellulose chromatography, see 'Results'.

Results

Dipeptidase Activity in the Developing Small Intestine

Preliminary experiments with crude extracts showed that a pH value of 7.2 was optimum for glycyl-L-proline dipeptidase activity during the entire gestational period studied, while values of 7.2 and 6.3 were optimum for L-glutaminyl-L-proline dipeptidase activity of fetuses 14 to 24 weeks and 26 to 36 weeks of age, respectively (fig. 4). The rate of enzyme activity exceeded 90 % of the maximal velocity at the substrate concentration of 0.020 M during the entire gestational period studied. Zero order kinetics with a linear relation between enzymatic activities and the amount of homogenates were found during incubation less than a period of 20 minutes, and when the activities were lower than 0.06 Units in incubation mixtures. Conditions of enzyme assays were similar to those previously described [20].

The specific activity of intestinal dipeptidases in fetuses of various ages is shown in figure 1. The average values of the entire small intestine of each fetus were compared with values found in surgical biopsies taken from the jejunum of adults (at approximately 10 cm from the ligament of Treitz) [20].

In the 14-week-old fetus, activity of both enzymes was present at levels slightly lower than those in adults; after the 16th week of age, the levels of activity of both enzymes were comparable with those of the adult.

Distribution of Dipeptidase Activity Along the Small Intestine

In nine fetuses 22 to 34 weeks of age, the enzyme activity in the proximal third and in the distal one of the small intestine was measured separately. A significant difference was found between the values in these two tracts (Student's t test: $p < 0.02$). The values obtained in the proximal jejunum of adults did not differ significantly from those obtained in the proximal third of the fetal small intestine (table I).

The small intestines of four fetuses 22, 31, 32, and 34 weeks of age were divided into 9–10 pieces for enzyme assay. The values for specific dipeptidase activity of both enzymes were uniformly high throughout the proximal six-tenths of the small bowel, while lower values were obtained for the terminal ileum (fig. 2). The ratios between the levels of activity of the two enzymes remained the same throughout the entire small intestine and were comparable with those of the adults [20].

Table I. Specific activity of dipeptidases in the small intestine of the fetus and the jejunum of the adult human[1, 2]

Enzyme	Fetal small intestine		Adult jejunum [20] (14)
	Proximal third (9)	Distal third (9)	
L-glutaminyl-L-proline dipeptidase	167±82.3	89.8±24.9	179±50.5
Glycyl-L-proline dipeptidase	128±47.5	62.8±19.4	144±61
Ratio of activities	1.28±0.41	1.45±0.42	1.4±0.39

[1] Units per g protein, mean value ±SD.
[2] Numbers in parentheses refer to number of experiments.

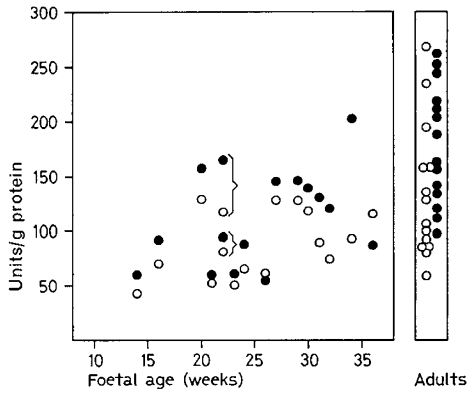

Fig. 1. Dipeptidase activities of the small intestine of human fetus and adult. Symbols: ○, glycyl-L-proline dipeptidase; ●, L-glutaminyl-L-proline dipeptidase. Two 22-week-old fetuses were examined; the activity levels found in each are represented by the symbols linked together. See text for experimental details.

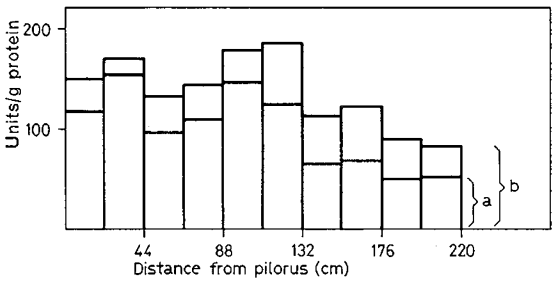

Fig. 2. Distribution of dipeptidase activities along the small intestine of a 22-week-old fetus. Symbols: a, glycyl-L-proline dipeptidase; b, L-glutaminyl-L-proline dipeptidase. See text for experimental details.

Further Studies

Gel filtration, the influence of pH and metal ions, K_m determinations, and heat treatment were performed on pools of mucosa prepared from the entire small bowel of fetuses 14 to 21 weeks of age. For older fetuses, separate pools were prepared for each third of the small intestine. The studies were carried out on both the first and the third segments; essentially similar results were obtained for each.

Gel Filtration

Small bowels from 10 fetuses 14, 16, 22, 24, 26, 29, 31, 32, 34, and 36 weeks of age were used. For the entire age span, a chromatographic pattern identical with that found in the adult intestine [20] was observed (fig. 3). Recoveries of both enzyme activities and prosingle pattern of elution. The percent of activity due to each enzyme in samples taken from the peaks was identical with that found in the original crude homogenate. Recoveries of both enzyme activities and proteins during chromatography were quantitative. The chromatographic fractions containing the enzyme activity were pooled, frozen, and stored. Characterization studies were performed within one week. Enzyme activity was stable. Using the starting homogenate for a reference, specific activities in the pooled fractions were increased 4.5 to 6.5 times for both dipeptidase activities.

Influence of pH

The effect of pH on dipeptidase activity was studied in the same material used for gel filtration. Glycyl-L-proline dipeptidase activity remained the same during the entire age period studied (fig. 4) and was identical with that found in the adult. In contrast, L-glutaminyl-L-proline dipeptidase activity in fetuses from 14 to 24 weeks of age showed a pH-dependence different from that observed in fetuses 26 to 36 weeks of age (fig. 4). During the age period 14–24 weeks, only 60 % of enzyme activity was present at a pH of 6.3, which was the optimum for the older fetuses.

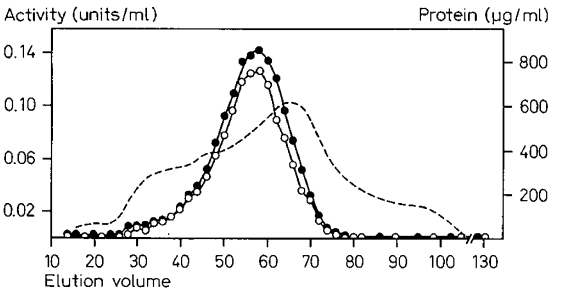

Fig. 3. Gel filtration on Sephadex G-200 of dipeptidases of the small intestine from the human fetus. The enzyme preparation was obtained from the proximal third of the small intestine of a 27-week-old fetus. Symbols: -----, protein; ○, glycyl-L-proline dipeptidase; ●, L-glutaminyl-L-proline dipeptidase.

Effect of Substrate Concentration

In four fetuses 14, 24, 26, and 36 weeks of age, K_m was calculated from the pooled chromatographic fractions. Values for K_m of glycyl-L-proline dipeptidase activity were found to be essentially constant (1.03×10^{-3} to 9.48×10^{-4}) and comparable with those of the adult [20]. Values for K_m of L-glutaminyl-L-proline dipeptidase activity, however, were variable during fetal life, 5.98×10^{-4} for the 14-week-old fetus; 9.43×10^{-4} for the 24-week-old fetus; 8.84×10^{-3} for the 26-week-old fetus, and 7.55×10^{-3} for the 36-week-old fetus. Almost identical values were found when K_m was determined at pH 7.2 or 6.3. In a 24-week-old fetus, K_m was determined separately for the enzymes from the proximal third and the distal one of the small intestine. The results were similar.

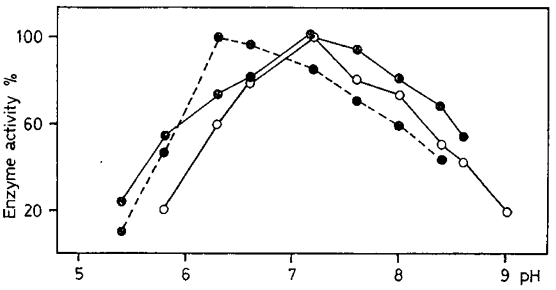

Fig. 4. Dipeptidase activities of the small intestine of the human fetus as a function of pH. A crude extract was used for the experiment; identical pH-activity curves were obtained using pooled Sephadex fractions as the source of enzymes. Enzyme activity is expressed as % of that found at pH optimum. Symbols: ○, glycyl-L-proline dipeptidase activity (covering the age group 14–36 weeks); ●—●, L-glutaminyl-L-proline dipeptidase activity (covering the age group 14–24 weeks); ●--●, L-glutaminyl-L-proline dipeptidase activity (covering the age group 26–36 weeks). The final pH figures of the reaction mixture are given. Buffer used: 0.3 M phosphoric acid/acetic acid/boric acid according to TEORELL and STENHAGEN [22a]. [Author give reference in galley.] (pH 5.4 to 9.2); 0.3 M Tris maleate (pH 5.4 to 8.7); 0.3 M potassium phosphate (pH 5.8 to 7.9). With the three series of buffers, overlapping pH-activity curves were obtained. Other experimental details are given in the text and in ref. 20. Essentially similar curves were observed for the proximal third and the distal one of the small intestine.

Influence of Metal Ions

Crude extracts of the small intestine of fetuses between 14 and 36 weeks of age were used. Co^{++} and Mn^{++} did not affect L-glutaminyl-L-proline dipeptidase activity; however, the rate of hydrolysis of glycyl-L-proline was increased as much as twofold by 5 mM Co^{++} and sixfold by 20 mM Mn^{++}, as in adults [20].

Heat Inactivation

These experiments were performed at 40 and 50° in buffers of pH 6.5 and 8, using crude extracts from the small bowel of fetuses 24, 31, and 34 weeks of age. The results were comparable with results obtained in a study of adult enzymes [20]. Inactivation of the two enzymes was parallel under all conditions of temperature and pH in crude extracts. When the pooled chromatographic fractions were heated in the presence of 0.002 M Mn^{++}, however, glycyl-L-proline dipeptidase activity was increased more than 2.5-fold, while L-glutaminyl-L-proline dipeptidase activity was reduced to less than 25 % of the original level (fig. 5).

Ammonium Sulphate Precipitation and DEAE Cellulose Chromatography

Ammonium sulphate fractionation was performed on the pooled fractions. Activities of both enzymes precipitated between 50 and 70 % of ammonium sulphate saturation, in the same ratio as in the original extract.

Following dialysis against 0.001 M Tris-HCl buffer, pH 8, the ammonium sulphate precipitate, containing 15 mg of protein, was applied on DEAE cellulose [24] 2 × 8 cm columns and was equilibrated with 0.001 M Tris-HCl buffer, pH 8. The enzymatic activities, when eluted with NaCl, either stepwise or through a linear gradient, were found to be associated in a single peak.

Both the entire small intestine of a 22-week-old fetus and the proximal third of the small bowel of a 36-week-old fetus were processed as previously described. Results were identical.

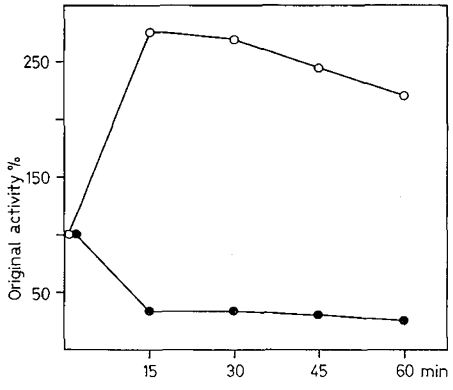

Fig. 5. Inactivation of dipeptidase activities from human fetus at 40° and pH 6.5 in the presence of 0.002 M Mn^{++}. Enzymes partially purified with gel filtration were used for the experiments. The preparation was obtained from the distal third of the small intestine of a 26-week-old fetus. Identical values were obtained for the proximal third and for the age period 14 to 36 weeks.

On the abscissa, the duration of heat treatment is reported. The enzyme activities found after heat treatment are expressed as percentage of original activity. Symbols. ○, glycyl-L-proline dipeptidase; ●, L-glutaminyl-L-proline dipeptidase.

Discussion

There have been few reports on intestinal dipeptidases in the human fetus. Blum *et al.* [2] reported that glycylglycine dipeptidase activity was present in the second month and was higher in the third month of fetal life. Dipeptidase activity related to L-alanyl-L-glutamic acid, L-alanyl-L-proline, glycylglycine, glycyl-L-leucine, and glycyl-L-valine has been found to be well developed at the fetal age of 11 weeks, with no change between the age period of 11 to 23 weeks [11].

The results of the present study indicate that the dipeptidase activity of both L-glutaminyl-L-proline and glycyl-L-proline was present at 14 to 16 weeks of age at levels comparable with those of the adult. Because of the present lack of knowledge concerning number and specificity of dipeptidases in human intestinal mucosa, the significance of our results are difficult to evaluate when compared with those in the literature cited. L-alanyl-L-proline and glycyl-L-proline might be split by the same enzyme(s), the prolidase (EC.3.4.3.7), which hydrolyzes various C-terminal proline-containing dipeptides [22]; however, the effect of Co^{++} and Mn^{++} on the activity of the two enzymes is dissimilar [11].

As has been observed in the adult [20], dipeptidase activity of glycyl-L-proline and glutaminyl-L-proline in the fetuses was affected differently by pH and some metal ions, and was separated by heat inactivation in the presence of Mn^{++}. A number of other procedures, however, including gel filtration, ammonium sulphate precipitation, and DEAE cellulose chromatography were ineffective in separation of the enzymes. Further studies using more purified enzymes are needed to determine whether L-glutaminyl-L-proline is split by an enzyme other than glycyl-L-proline dipeptidase.

The physiological role of dipeptidase activities remains unclear [20]; therefore, the relation between these data and the capability of the premature infant

and the full-term newborn to digest such glutamine- and proline-rich proteins as the gluten ones remains to be established.

In the present study, the levels of dipeptidase activity of both enzymes were higher in the proximal third of the small intestine than in the terminal ileum and were comparable with the levels found in the adults [20] and with those reported by LINDBERG [11].

The results of the present study indicate that a qualitative change in the properties of L-glutaminyl-L-proline dipeptidase activity occurred during the course of fetal life. While a number of properties of L-glutaminyl-L-proline dipeptidase activity, including sensitivity to metal ions and heat treatment, behavior on Sephadex G-200, ammonium sulphate precipitation, and DEAE cellulose chromatography are similar, pH dependence and affinity with substrate appeared to change at the fetal age of 24–26 weeks. Other studies indicate that enzyme molecules may change qualitatively during growth [21]. A number of enzymes, including rat liver galactokinase [3], rat liver galactose-1-phosphate uridyltransferase [1], mouse duodenum alkaline phosphatase [15, 16], mammalian lactic dehydrogenase [4, 12–14], rat, chick, and human malic dehydrogenases [23], and chick brain acid phosphatase [10] have been reported to change physically and/or enzymatically during development. Although a molecular conversion from fetal to adult form of the enzyme molecule has been demonstrated [17], the exact nature and the underlying mechanism of the conversion remain unknown. L-glutaminyl-L-proline dipeptidase may be another instance of a molecular conversion during development. Studies using a purer fetal enzyme might clarify the nature of enzyme conversion.

Summary

Dipeptidase activity using substrates of glycyl-L-proline and L-glutaminyl-L-proline were studied in the small intestine of human fetuses between 14 and 36 weeks of age. Levels of activity present at 14 to 16 weeks of age were comparable with those found in adults. The level of activity of both enzymes was uniformly high in the proximal six-tenths of the small bowel and lower in the terminal ileum.

Activity was partially purified through gel filtration and characterized with regard to the influence of pH, substrate concentration, metal ions, heat treatment, ammonium sulphate precipitation, and DEAE cellulose chromatography. These results suggest that a qualitative variation of L-glutaminyl-L-proline dipeptidase activity occurs in the human fetus during maturation.

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25. The authors are indebted to Mr. Francesco Vollaro for excellent technical assistance.
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