Glycyl-L-Leucine Hydrolase, a Versatile 'Master' Dipeptidase from Monkey Small Intestine

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1. A highly active and electrophoretically homogeneous dipeptidase was purified from the soluble extracts of monkey small-intestinal mucosa. 2. By gel-filtration studies the molecular weight of the enzyme was found to be 107000. It is composed of two identical, subunits of molecular weight 54000. 3. A paper-chromatographic method of dipeptidase assay was developed to overcome some of the difficulties encountered in the generally used spectrophotometric procedure. By using this method, the K_m and k_o values of a few substrates were determined. 4. The substrate specificity of the enzyme was investigated in great detail with substrates of a wide range of possible structural types. The enzyme hydrolyses a very large proportion of the range of dipeptides tested. This enzyme, which exhibits such a wide range of action, has been termed the 'master' dipeptidase of the intestine. Glycylglycine, glycyl-L-proline, glycyl-L-histidine, L-prolylglycine and some of the arginine- and aspartic acid-containing dipeptides were not substrates and are possibly hydrolysed by other peptidases. These results therefore suggest that in the intestine the number of dipeptidases is rather limited. 5. In the light of these findings, the implications on the role of dipeptidases in intestinal peptide transport are discussed.

The substrate specificity of a partially purified soluble dipeptidase (glycyl-L-leucine hydrolase) from monkey small intestine was described by Das & Radhakrishnan (1972). By these studies with a limited number of dipeptides, we were led to believe that the enzyme may have a narrow range of specificity. However, subsequent studies with an electrophoretically homogeneous enzyme preparation showed that the enzyme catalysed the hydrolysis of a much wider variety of dipeptides than reported by Das & Radhakrishnan (1972). A systematic study was then made with dipeptides of a wide range of possible structural types. From these results the view emerged that in the monkey small intestine there exists a highly active 'master' dipeptidase with a remarkably wide range of action. These results and other properties of the enzyme are reported in the present paper. This work in part was presented at a recent meeting in San Juan (Das & Radhakrishnan, 1973).

Experimental

Chemicals

Peptides. The peptides L-phenylalanyl-L-methionine, L-methionyl-L-valine, L-methionyl-L-threonine, L-methionyl-L-phenylalanine, L-methionyl-L-tyrosine and L-glutamyl-L-glutamine were kindly given by Professor Alton Meister (Cornell University, New York, N.Y., U.S.A.). Professor D. M. Matthews (Westminster Hospital, London S.W.1, U.K.) kindly supplied the following peptides: L-seryl-L-serine, L-glutamylglycine, L-glutamyl-L-tyrosine,

L-glutamyl-L-aspartic acid, L-glutamyl-L-glutamic acid, L-glutamyl-L-lysine, L-aspartyl-L-lysine, L-alanyl-L-arginine, L-arginylglycine, L-arginyl-L-alanine, L-arginyl-L-arginine, L-arginyl-L-lysine and γ -L-glutamyl-L-methionine. L-Threonyl-L-methionine, L-serylglycine and L-tyrosyl-L-lysine were from Schwarz-Mann, Orangeburg, N.Y., U.S.A., and all the other dipeptides were purchased from Sigma Chemical Co. St. Louis, Mo., U.S.A.

The peptides were all chromatographically pure, except L-glutamylglycine, which showed a ninhydrin-positive contamination.

Protein markers. Malate dehydrogenase (E.C.1.1.1.37) was from Sigma Chemical Co. and all other protein markers were purchased from Schwarz-Mann.

Other chemicals. These were commercially obtained as indicated. Tris (ultrapure grade) and $(\text{NH}_4)_2\text{SO}_4$ (enzyme grade) were from Schwarz-Mann; spectrophotometric-grade ethanol was prepared from rectified spirits; chemicals for disc electrophoresis were from Eastman, Rochester, N.Y., U.S.A. All other chemicals were of the analytical or the best available grade.

Enzyme assay

The standard assay mixture contained the dipeptide (0.5–20mM), the buffer and the enzyme, in a total volume of 0.05ml. The buffers used in the assay were (1) 0.2M-sodium phosphate, pH 7.8 (0.017M- NaH_2PO_4 , 0.183M- Na_2HPO_4), (2) 0.1M-Tris-HCl,

pH 7.8 (0.1 M-Tris, pH adjusted with HCl) and (3) Michaelis buffer (0.015 M-sodium acetate, 0.015 M-sodium diethylbarbiturate, 0.062 M-NaCl, pH adjusted with HCl). In assays with phosphate and Michaelis buffers, the reaction mixture contained 0.1 mM-ZnSO₄. No metal ion was added in assays with Tris buffer.

The spectrophotometric assay method of Josefsson & Lindberg (1965), with some modifications (Das & Radhakrishnan, 1972), was used during the enzyme purification, with glycyl-L-leucine (12 mM) as substrate. In earlier studies these assays were done in the presence of phosphate buffer and Zn²⁺. In later studies, Tris buffer replaced the phosphate buffer. Since saturating substrate concentrations were not used, the activities measured during the purification did not represent the maximal velocities. One unit of enzyme activity is the amount required to catalyse the hydrolysis of 1 μ mol of glycyl-L-leucine/min at 37°C at 12 mM substrate concentration.

A paper-chromatographic method of assay was developed to overcome certain difficulties encountered in the spectrophotometric procedure, in the determination of K_m values or in a study of competition among substrates. It involved a modification of an earlier copper-ninhydrin method of amino acid determination (Giri *et al.*, 1952). In this procedure the standard assay mixture was used. After incubation for 5 min the reaction was stopped by placing in a boiling-water bath for 30 s. This was effective in inactivating the enzyme instantaneously as judged by no detectable hydrolysis when similar heating was carried out at zero time. Portions (0.02 ml) of the assay mixtures were spotted on Whatman no. 1 paper, and the chromatograms (descending) were developed with a suitable solvent, usually butan-1-ol-acetic acid-water (4:1:1, by vol.). After spraying of the paper with 0.5% ninhydrin in acetone-water (19:1, v/v), the spot containing one of the reaction products that was well separated was cut out and eluted with 4 ml of ethanol-water (3:1, v/v) containing CuSO₄. The colour was read at 510 nm. The size of the paper cut out for elution was kept the same for a particular amino acid. The amount of CuSO₄ varied according to the amino acid and the buffer used in the assay. Generally, the amount of CuSO₄·5H₂O used was 1.2 mg/assay. When glycine was measured, with phosphate buffer present in the assay mixture, excess of CuSO₄·5H₂O (4 mg) was required to overcome the effect of phosphate appearing in the same position as glycine in the chromatograms. By this method, glycine and alanine, in the range 0.01–0.1 μ mol, could be measured conveniently. The K_m and k_0 values for glycyl-L-leucine, glycyl-L-isoleucine, glycyl-L-valine, glycyl-L-phenylalanine and L-alanyl-L-leucine were determined by the chromatographic method. For the four glycyl peptides glycine was measured (with substrates at six

different concentrations in the range 1–20 mM), and with L-alanyl-L-leucine alanine was measured (with substrate at seven different concentrations in the range 0.5–20 mM). The linearity of the progress of hydrolysis of the five peptides tested in Table 1 was checked at three peptide concentrations, 1, 2 and 20 mM (and also at 0.5 mM for L-alanyl-L-leucine), and it was found that the reactions were linear with respect to time (5 min) and to enzyme concentration. The extent of hydrolysis in the different experiments did not exceed 20% of the initial substrate concentration. The K_i value for L-alanyl-L-leucine was obtained by determining the apparent K_m values of the four glycyl peptides in the presence of 1 mM- and 2.5 mM-L-alanyl-L-leucine. The paper-chromatographic method has general applicability and can be used with any peptide with at least one constituent amino acid that separates well on chromatograms.

For studying the variation of K_m for glycyl-L-leucine over a wide range of pH, Michaelis buffer was used, though it gave lower activity than with Tris-HCl buffer. As Michaelis buffer interfered with the spectrophotometric assay, the reaction was followed by the paper-chromatographic measurement of glycine.

Enzyme purification procedure

The enzyme was purified by the procedure given earlier (Das & Radhakrishnan, 1972), with the inclusion of a second DEAE-Sephadex fractionation as the last step, to eliminate minor contaminating protein. The enzyme fraction from the cellulose-hydroxyapatite step (1.8 mg of protein) was dialysed against 0.025 M-potassium phosphate buffer, pH 6.5 (17.1 mM-KH₂PO₄, 7.9 mM-K₂HPO₄) and then applied to a DEAE-Sephadex column (5.5 cm × 0.6 cm) equilibrated with the same buffer. The enzyme was eluted with a linear gradient (0.025–0.1 M-potassium phosphate buffer, pH 6.5) by using a Varigrad device (Technicon). The peak-activity fractions were pooled and the enzyme was concentrated by precipitation with 90% satd. (NH₄)₂SO₄.

Substrate-specificity studies

A paper-chromatographic method was mainly used for these studies. A standard assay mixture was used, except for a large excess of the enzyme (3–4 units) and a longer incubation time (30 min), which together represent about a 600-fold increase in the extent of hydrolysis. For most peptides the developing solvent system used for chromatography was butan-1-ol-acetic acid-water (4:1:1, by vol.). For the peptides L-glutamylglycine, L-glutamyl-L-tyrosine, L-seryl-L-serine, L-arginyl-L-lysine, L-arginylglycine, L-lysylglycine, glycyl-L-glutamic acid, glycyl-L-lysine, glycyl-L-threonine, L-lysyl-L-aspartic acid, L-lysyl-L-lysine and L-arginyl-L-aspartic acid the

solvent system used was butan-1-ol-acetic acid-0.2M-KCl-HCl, pH1.0 (4:1:1, by vol.). For the peptides L-glutamyl-L-aspartic acid and L-glutamyl-L-glutamic acid the solvent system was phenol-0.2M-KCl-HCl, pH1.0 (50:7, v/v). For L-aspartylglycine and L-serylglycine the spectrophotometric method of following the disappearance of peptide-bond absorbance at 210nm was used for detection of hydrolysis, since the separation on chromatograms was poor.

General methods

Disc electrophoresis. Polyacrylamide-gel disc electrophoresis was carried out by the method of Davis (1964) by using a Canalco instrument, with 0.025M-Tris-0.192M-glycine, pH8.3, as electrode buffer. The separating gel contained 7% (w/v) acrylamide, and 0.18% *NN'*-methylenebisacrylamide. The gels (0.45cm × 5cm) were run at 3mA constant current per gel tube for 1h. The gels were stained for protein with Coomassie Blue by the method of Chrambach *et al.* (1967). Sodium dodecyl sulphate gel electrophoresis was carried out by the method of Weber & Osborn (1969) in gels containing 10% (w/v) acrylamide and 0.3% *NN'*-methylenebisacrylamide. The gels (0.45cm × 5.5cm) were run at 8mA per gel for 3h.

Determination of molecular weight. The molecular weight of the enzyme was determined by the procedure of Andrews (1965) by using a column of Sephadex G-200 (bed. vol. 200ml) calibrated with the following marker proteins: γ -globulin (mol.wt. 160000), bovine serum albumin (mol.wt. 68000), malate dehydrogenase (mol.wt. 63000) and myoglobin (mol.wt. 17800). The experiments were performed with columns equilibrated with 0.05M-Tris-HCl buffer, pH7.5 (0.05M-Tris, pH adjusted with HCl), containing 0.1M-KCl. The molecular weight of the subunit was obtained by the electrophoretic method of Weber & Osborn (1969) by using 10% acrylamide gel. The protein standards used for this purpose were γ -globulin (H chain, mol.wt. 50000, and L chain, mol.wt. 23500), bovine serum albumin (mol.wt. 68000), ovalbumin (mol.wt. 43000) and myoglobin (mol.wt. 17800). The electrode buffer was 0.1M-sodium phosphate, pH7.1 (33mm-NaH₂PO₄, 67mm-Na₂HPO₄), containing 0.1% sodium dodecyl sulphate.

Determination of protein. Protein was determined by the procedure of Lowry *et al.* (1951) by using crystalline dry bovine serum albumin as standard.

Results

Purification

During the purification enzyme activity was measured with both Tris and phosphate buffers. In the crude extract the activity with both the buffers

was the same, but the activity with Tris buffer was almost twice that with phosphate buffer in all the subsequent stages of purification. The last step, which is a repetition of the DEAE-Sephadex fractionation, removed some trace impurities revealed in disc electrophoresis and gave a homogeneous preparation. A single band was also obtained when electrophoresis was done in the presence of sodium dodecyl sulphate and 2-mercaptoethanol. The specific activity (enzyme units/mg of protein) of the final purified enzyme was 4500 in phosphate buffer and 9316 in Tris buffer.

The enzyme became extremely labile after the first DEAE-Sephadex step, and the final preparation could be stabilized to a certain extent if kept at a protein concentration above 5mg/ml and in 50%-satd. (NH₄)₂SO₄ solution containing 0.1mM-dithiothreitol. The overall recoveries were generally between 2 and 5%. A similar degree of purification has also been achieved with batch sizes of mucosa from about 25 monkeys.

Molecular weight

The molecular weight of the enzyme obtained by the gel-filtration method was 107000. The electrophoretic method of molecular-weight determination in the presence of sodium dodecyl sulphate and 2-mercaptoethanol gave a value of 54000. Thus it can be tentatively concluded that there are two weight-identical subunits per molecule of active enzyme.

Kinetic studies

The K_m and k_0 values for a few peptides are shown in Table 1. These values were obtained by Lineweaver-Burk plots of the rate data. The values vary according to the buffer used in the assays. Compared with the values in phosphate buffer, the use of Tris buffer gave lower K_m and higher k_0 values for all the five dipeptides tested. Thus phosphate buffer inhibits the enzyme by affecting both the catalytic rate and the affinity for the substrate. However, irrespective of the buffer used in the assay system, the four glycyl peptides glycyl-L-leucine, glycyl-L-isoleucine, glycyl-L-valine and glycyl-L-phenylalanine belong to one type, having very similar K_m and k_0 values, both significantly higher than those for L-alanyl-L-leucine. The ratio k_0/K_m in phosphate buffer is comparable for all the peptides, including L-alanyl-L-leucine. In Tris buffer the ratio was higher, with the value for L-alanyl-L-leucine being much higher than that for the glycyl peptides. L-Alanyl-L-leucine is a competitive inhibitor for all the four glycyl peptides (affecting the K_m but not the k_0 values), and the K_i values in both the buffers are given. The K_m values for glycyl-L-leucine, glycyl-L-isoleucine and glycyl-L-valine reported earlier (Das

Table 1. *Kinetic constants of the dipeptidase*

For experimental details see the text. k_0 (s^{-1}) is defined as mol of substrate hydrolysed/s per mol of dimer enzyme. The K_i for Ala-Leu was $0.53 \pm (s.d.) 0.07$ mM in phosphate buffer and $0.22 \pm (s.d.) 0.05$ mM in Tris buffer. These values were obtained from the data with each of the peptides. Average values of K_m and k_0 given are obtained from duplicate or triplicate determinations, which were within 5% of the average values.

Substrate	K_m (mM)		k_0 (s^{-1})		k_0/K_m ($s^{-1} \cdot mM^{-1}$)	
	Phosphate	Tris	Phosphate	Tris	Phosphate	Tris
Gly-Leu	10	2.74	10890	23140	1090	8440
Gly-Ile	10	4.4	10680	22960	1070	5340
Gly-Val	8	3.6	7450	21360	930	5923
Gly-Phe	10	2.4	8190	20360	820	8540
Ala-Leu	1	0.44	1190	6510	1193	14770

Fig. 1. Variation of k_0 and K_m values with pH

Standard assay conditions (see the Experimental section) were used, with glycyl-L-leucine as substrate (1–20 mM) and with pH varied. (a) $\log k_0$ plotted against pH; k_0 is expressed as mol of substrate hydrolysed/s per mol of dimer enzyme. (b) pK_m ($-\log K_m$) plotted against pH; K_m is expressed as M.

& Radhakrishnan, 1972) were determined by the spectrophotometric method with phosphate buffer in the assays. Those values differ somewhat from the ones obtained by the more reliable paper-chromatographic procedure reported here.

Fig. 1 shows the variation of K_m and k_0 for glycyl-L-leucine over the range pH 5–9. The pK_m curve shows a sharp break at about pH 6.4. This indicates a group with pK of 6.4 present in the free

enzyme, free substrate or enzyme-substrate complex influencing enzyme activity. The $\log k_0$ curve shows a smooth gradual change with pH and there is no sharp break in it.

The observed differences in the activity of the enzyme with phosphate, Tris and Michaelis buffers are not readily explained. As reported earlier (Das & Radhakrishnan, 1972), a slight increase (about 35%) in activity was observed on the addition of Zn^{2+} (and not by many other bivalent metal ions) in assays with phosphate buffer, and this was also true with Michaelis buffer. However, with Tris buffer, which gave high activity even in the absence of Zn^{2+} , no further increase was observed on addition of Zn^{2+} . Although EDTA (5 mM) and *o*-phenanthroline (1 mM) completely inhibited the enzyme activity when present in the assay mixture, dialysis of the enzyme against the chelating agents did not diminish the activity. (In the dialysis experiments, the enzyme was dialysed against 0.05 M-sodium phosphate buffer, pH 7.0, containing 10 mM-EDTA for 12 h with two changes of the buffer, and was then further dialysed against the same buffer, but without EDTA, for 12 h with four changes of the buffer.) The effect of low concentrations of chelating agents and the specific activation by Zn^{2+} may be taken as presumptive evidence for the requirement for Zn^{2+} . Perhaps the lowered activity in phosphate and Michaelis buffer may be related to this. More work is obviously needed to elucidate the involvement of Zn^{2+} in the dipeptidase activity.

Substrate-specificity studies

Table 2, in which some of the substrates tested in our earlier study (Das & Radhakrishnan, 1972) are also included, shows the latest picture on the substrate specificity of the enzyme. All the theoretically possible dipeptides (except peptides containing L-proline,

Table 2. *Substrate specificity of the dipeptidase*

All the peptides contained amino acids of the L-configuration. The peptides that were inactive as substrates are shown in **bold type**.

C-Terminal	...	Gly	Ala	Ile, Leu Val	Ser, Thr	Phe, Tyr, Trp, His	Met	Asp, Glu	Lys, Arg
N-Terminal									
Gly		Gly-Gly	Gly-Ala	Gly-Ile Gly-Leu Gly-Val	Gly-Thr	Gly-Trp Gly-Phe Gly-His	Gly-Met	Gly-Glu	Gly-Lys
Ala		Ala-Gly	Ala-Ala	Ala-Leu Ala-Val	Ala-Ser	Ala-Phe	Ala-Met	Ala-Asp	Ala-Arg Ala-Lys
Ile, Leu, Val		Leu-Gly	Leu-Ala	Leu-Leu Val-Leu	Leu-Ser	Leu-Phe	Leu-Met		
Ser, Thr		Ser-Gly	Ser-Ala	Ser-Leu His-Leu	Ser-Ser	Ser-Phe	Thr-Met		
Phe, Tyr Trp, His		Phe-Gly	Phe-Ala	Phe-Leu Tyr-Leu Trp-Leu		Phe-Phe	Phe-Met	Tyr-Glu	Tyr-Lys
Met		Met-Gly	Met-Ala	Met-Leu Met-Val	Met-Ser Met-Thr	Met-Phe Met-Tyr	Met-Met	Met-Glu	
Asp, Glu		Glu-Gly Asp-Gly	Glu-Ala	Glu-Val		Glu-Tyr		Glu-Glu Glu-Asp	Glu-Lys Asp-Lys
Lys, Arg		Lys-Gly Arg-Gly	Lys-Ala Arg-Ala	Lys-Leu		Lys-Phe		Lys-Asp Arg-Asp	Lys-Lys Arg-Arg Arg-Lys

L-hydroxyproline, L-glutamine, L-asparagine and L-cysteine) have been grouped into 64 types based on the structure of the constituent amino acids. Of 75 dipeptides checked for activity, representing 54 combinations of different amino acid groups, 65 were active substrates. The following dipeptides (in bold type in Table 2) were not hydrolysed by the enzyme: glycylglycine, glycyl-L-histidine, L-aspartylglycine, L-aspartyl-L-lysine, L-glutamyl-L-aspartic acid, L-arginyl-L-aspartic acid, L-arginylglycine, L-arginyl-L-lysine, L-arginyl-L-arginine and L-arginyl-L-alanine. The mode of presentation in Table 2 has the additional advantage of showing that a large number of peptides, in which the sequence of amino acid residues is reversed, are also substrates for the enzyme. In Table 2, the ring amino acids L-phenylalanine, L-tyrosine, L-tryptophan and L-histidine are grouped together even though they are not entirely similar in structure. Further work is necessary to check the susceptibility of other combinations of peptides containing tryptophan and histidine, since a reasonable number of peptides containing phenylalanine and tyrosine have been checked for activity.

Not included in the table are L-glutamyl-L-glutamine, which is an active substrate, and glycyl-L-proline and L-prolylglycine, which are inactive. A γ -glutamyl dipeptide (γ -L-glutamyl-L-methionine) and a tripeptide, glutathione (γ -L-glutamyl-L-cysteinylglycine), were not substrates.

Discussion

The work reported here was initiated with a view to finding out whether in the intestine there is a large family of dipeptidases with narrow specificity or whether there is a limited number with broad specificity. The available data strongly suggest that most of the theoretically possible 400 and more dipeptides would be hydrolysed by the 'master' dipeptidase. Of the peptides that are not hydrolysed by the enzyme, the peptides with imino acids (proline and hydroxyproline) at the C-terminus are probably hydrolysed by an enzyme easily separable from others (K. Bakshi & A. N. Radhakrishnan, unpublished work). It is not yet established whether glycylglycine, glycyl-L-histidine and L-prolylglycine (and related peptides) are hydrolysed by one or more enzymes. These results strongly suggest that in the intestinal cells there is a 'master' dipeptidase and possibly four or five other dipeptidases, including the imino and imido dipeptidases. It should be noted that in Table 2 there are a few peptides, such as the arginyl peptides, which are not hydrolysed by the 'master' dipeptidase but which are unlikely to be present under physiological conditions, in view of the known specificity of cleavage of the proteolytic enzymes in the intestinal lumen.

Although the glycyl-L-leucine hydrolase shows a wide range of action with no obvious discrimination in relation to the chemical structure of the amino

acid residues, the following general observations can be made. Thus it was found that all the neutral dipeptides tried, except for glycylglycine, were substrates for the enzyme. Among peptides containing acidic and basic amino acids a few were not hydrolysed, and in this group the peptides contained aspartic acid, arginine or histidine. Although all the glutamic acid peptides (except L-glutamyl-L-aspartic acid) were hydrolysed irrespective of its position at the *N*- or *C*-terminus, of the five aspartic acid peptides tested only two (L-alanyl-L-aspartic acid and L-lysyl-L-aspartic acid) were substrates whereas the other three (L-aspartylglycine, L-aspartyl-L-lysine and L-glutamyl-L-aspartic acid) were not hydrolysed. L-Histidyl-L-leucine was an active substrate but glycyl-L-histidine was inactive. Except for L-alanyl-L-arginine, all the arginine-containing peptides were inactive. On the other hand, lysine-containing peptides (except for L-aspartyl-L-lysine), where the charged ϵ -amino group is located further from the peptide bond than the guanidino group of arginine, were hydrolysed. These results suggest lack of hydrolysis of peptides with a charged group near the peptide bond. The observed influence of charged groups in close vicinity of the site of hydrolysis, the peptide bond, and the earlier demonstration of the requirement for free α -amino and α -carboxyl groups (Das & Radhakrishnan, 1972), would suggest a three-point attachment of the substrate to the enzyme. More refined analysis with available data may throw further light on the dimensions of the active site of the enzyme.

In contrast with the disaccharidases, which are localized in the brush-border region of the intestinal cells, the dipeptidase activities are present predominantly (over 95%) inside the cell (Peters, 1970). Although it is easier with disaccharides to conceive of hydrolysis followed by active transport of sugar monosaccharides, with dipeptides the hydrolysis can occur only after the peptide is transported into the cell. Matthews (1971) and Adibi (1971) have suggested that peptide transport may represent a second major mode of transport of the terminal products of protein digestion, in addition to the well-known amino acid transport. This idea is especially borne out by studies in Hartnup disease (Asatoor *et al.*, 1970) and cystinuria (Hellier *et al.*, 1971). The nature of the initial uptake and transport processes of dipeptides is not yet clear. Dipeptides may be transported by the ' γ -glutamyl cycle' (Orlowski & Meister, 1970*a*), which could operate for peptides as well as amino acids, as suggested by Orlowski & Meister (1970*b*). In view of the very highly active intracellular dipeptidases, it is conceivable that a steep chemical gradient would be instantly established between the intestinal lumen and the inside of the cell, and consequently an efficient transport of dipeptides would take place, whether

or not the initial uptake is passive or a weakly active process. In this scheme the 'master' dipeptidase would have a major role, facilitating the uptake of a wide variety of dipeptides at a rapid rate. The total amount of the glycyl-L-leucine hydrolase present in the monkey intestine ranges between 0.03 and 0.05 μ mol, and on this basis it can catalyse the hydrolysis of 30–50 mmol of glycyl-L-leucine/min, and it is therefore capable of efficiently handling the major dipeptide load arising from the dietary proteins.

Kinetic studies with the four glycyl peptides (Table 1) showed that the K_m , k_0 and k_0/K_m values were of the same order in both phosphate and Tris buffers. When the *N*-terminal glycine was replaced by L-alanine there was a drastic decrease in k_0 and also in K_m , resulting in a very much higher k_0/K_m ratio in Tris buffer. Since the k_0/K_m ratio is a better index of enzyme activity, L-alanyl-L-leucine appears to be as reactive as the glycyl peptides. The variation of K_m (for glycyl-L-leucine) with pH is indicative of a histidine residue playing a part in the substrate-binding process, but this finding requires further substantiation.

The presence of a 'master' dipeptidase in the monkey intestine would call for a re-evaluation of earlier reports that in the human several dipeptidases are deficient in pathological states, assuming that the enzyme in the human intestine is similar to that in the monkey. Thus the substrates used in the reports by Berg *et al.* (1970) and Heizer & Laster (1969), other than the peptides with imino acids, are all substrates for the same enzyme and the data may simply reflect different rates of activity.

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References

- Adibi, S. (1971) *J. Clin. Invest.* **50**, 2266–2275
- Andrews, P. (1965) *Biochem. J.* **96**, 595–606
- Asatoor, A. M., Cheng, B., Edwards, K. D. G., Lant, A. F., Matthews, D. M., Milne, M. D., Navab, F. & Richards, A. J. (1970) *Gut* **11**, 380–387
- Berg, N. O., Dahlqvist, A., Lindberg, T. & Norden, A. (1970) *Gastroenterology* **59**, 575–582
- Chrambach, A., Reisfield, R. A., Wyckoff, M. & Zaccari, J. (1967) *Anal. Biochem.* **20**, 150–154
- Das, M. & Radhakrishnan, A. N. (1972) *Biochem. J.* **128**, 463–465

- Das, M. & Radhakrishnan, A. N. (1973) *Abstr. Conf. Intestinal Absorption Allied Disorders 20th Anniv.*
- Davis, B. J. (1964) *Ann. N.Y. Acad. Sci.* **121**, 404-427
- Giri, K. V. Radhakrishnan, A. N. & Vaidyanathan, C. S. (1952) *Anal. Chem.* **24**, 1677-1678
- Heizer, W. D. & Laster, L. (1969) *J. Clin. Invest.* **48**, 210-228
- Hellier, M. D., Perrett, D., Holdsworth, L. D. & Thirumalai, C. (1971) *Gut* **12**, 496-497
- Josefsson, L. & Lindberg, T. (1965) *Biochim. Biophys. Acta* **105**, 149-161
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Matthews, D. M. (1971) *J. Clin. Pathol.* **24**, Suppl. (Roy. Coll. Pathol.) **5**, 29-40
- Orlowski, M. & Meister, A. (1970a) *Proc. Nat. Acad. Sci. U.S.* **67**, 1248-1255
- Orlowski, M. & Meister, A. (1970b) *Methods Enzymol.* **17A**, 883-889
- Peters, T. J. (1970) *Biochem. J.* **120**, 195-203
- Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406-4412