Intestinal Assimilation of a Proline-containing Tetrapeptide

ROLE OF A BRUSH BORDER MEMBRANE POSTPROLINE DIPEPTIDYL AMINOPEPTIDASE IV

AKIRA MORITA, YOON-CHUL CHUNG, HUGH J. FREEMAN, ROGER H. ERICKSON, MARVIN H. SLEISENGER, and YOUNG S. KIM, *Gastrointestinal Research Laboratory (151M2), Veterans Administration Medical Center, San Francisco, California 94121; University of California, Department of Medicine, San Francisco, California 94143*

ABSTRACT The mechanism of hydrolysis and absorption of a proline-containing tetrapeptide, Leu-Pro-Gly-Gly (10 mM) by rat intestine was examined in vivo by using jejunal perfusion methods. The peptide substrate and hydrolysis products were analyzed by use of an automated amino acid analyzer. Leucine, proline, and glycine were absorbed by the intestine at a significantly higher rate from the tetrapeptide than from an equivalent amino acid mixture. The analysis of the hydrolytic products in the lumen during in vivo perfusion of the tetrapeptide showed that two dipeptides, Leu-Pro and Gly-Gly, were the major products. These two dipeptides were also the major hydrolytic products when a purified rat intestinal brush border membrane preparation was incubated with Leu-Pro-Gly-Gly. The rate of hydrolysis of the tetrapeptide was much higher than that for several other proline-containing peptides (Leu-Pro, Pro-Leu, and Pro-Gly-Gly) that were tested. Studies using Gly-Pro-β-naphthylamide, a specific substrate for postproline dipeptidyl aminopeptidase IV, showed that this enzyme is mainly localized to the brush border membrane and is responsible for the hydrolysis of the tetrapeptide into the two dipeptides Leu-Pro and Gly-Gly. Thus, brush border membrane dipeptidyl aminopeptidase IV very likely plays an important role at the intestinal mucosal cell surface in the final stages of digestion of proline-containing peptides.

Address correspondence to Dr. Kim, Veterans Administration Medical Center.

Received for publication 2 November 1982 and in revised form 4 April 1983.

INTRODUCTION

The final protein digestion products presented to the small intestine are free amino acids and small peptides consisting of two to six amino acids (1–4). The processes involved in the absorption of these final products after luminal proteolysis by pancreatic endo- and exopeptidases are complex, but in most cases additional hydrolysis of these oligopeptides by brush border membrane peptidases is necessary before absorption by the intestinal enterocyte (5–9). Even though a variety of peptidases have been described as being localized to the intestinal brush border membrane (10–20), only a few have been studied in any great detail (21, 22). Therefore, many of the biochemical and physiological events that occur during the final stages of digestion and absorption of peptides at the intestinal mucosal cell surface remain to be elucidated.

Proline is found in a wide variety of dietary proteins, and the collagen molecule, for example, contains a significant amount of Gly-Pro in its amino acid sequence (23). In general, proline-containing peptides are resistant to hydrolysis by many of the known pancreatic and brush border membrane proteases (10, 22). Thus, the means by which these types of oligopeptides are hydrolyzed and assimilated by the intestinal enterocyte is of nutritional and physiological importance. In this report, the peptide Leu-Pro-Gly-Gly was used in order to further elucidate the mechanisms involved in the assimilation of proline-containing peptides by the intestine. With the use of an in vivo perfusion technique to closely approximate physiological conditions, we were able to demonstrate that intestinal

 J. Clin. Invest. © The American Society for Clinical Investigation, Inc. · 0021-9738/83/08/0610/07 $1.00
Volume 72 August 1983 610–616

brush border membrane postproline dipeptidyl aminopeptidase IV (DAP IV) is of primary importance in the terminal digestion of Leu-Pro-Gly-Gly and probably plays a similar role in the digestion of other proline-containing peptides.

METHODS

Chemicals. All amino acids, peptides, and derivatives used in this study were purchased from Bachem Inc., (Torrance, CA). Bovine serum albumin, *p*-hydroxymercuribenzoate, and *p*-nitrophenyl phosphate were obtained from Sigma Chemical Co., (St. Louis, MO). [^{14}C]polyethylene glycol was obtained from New England Nuclear (Boston, MA). All other chemicals were of reagent grade quality.

Animals. Female rats (Wistar strain, Simonsen Laboratories, Gilroy, CA) weighing ~250 g and maintained on a laboratory chow diet (Ralston Purina Co., St. Louis, MO) were used throughout the study. All experiments were carried out using fed animals.

Perfusion study. A steady state perfusion technique described in detail in a previous communication (9) was used. Test solutions contained the peptide, Leu-Pro-Gly-Gly, at a concentration of 10 mM, or an equivalent concentration of the constituent-free amino acids. [^{14}C]PEG was added to the test solutions as a nonabsorbable volume marker. The test solutions were made isoosmotic (300 mosM) by addition of sodium chloride and the pH was adjusted to 7 by titration with sodium hydroxide. The solutions were maintained at 37°C and were perfused into a 20-cm long jejunal segment beginning 5 cm distal to the ligament of Treitz. A flow rate of 19 ml/h was maintained with a Harvard model 2681 infusion apparatus (Harvard Apparatus Co., Mills, MA). After an equilibration period of 30 min to achieve steady state conditions the perfusate was collected for two consecutive 10-min periods and immediately frozen in a plastic tube maintained in a mixture of solid carbon dioxide in ethanol. Each animal was perfused with two test solutions, one containing peptide and the other its equivalent amino acid mixture. The sequence of perfusion of the test solutions was randomized. At the end of each experiment, the perfused intestinal segment was removed and the mucosa was scraped and weighed.

The [^{14}C]PEG content of test solutions and perfusates was measured by a previously reported method (9). The absorption rate of the amino acids during perfusion was calculated by using the formula described previously (9).

Preparation of brush border membranes. Fed animals were killed by decapitation and the small intestine distal to the ligament of Treitz was removed. After a wash with ice-cold saline, the mucosa was scraped from the proximal half of the small intestine using a glass slide. Brush border membranes were prepared from the mucosal scrapings by the method described by Kessler et al. (24). Isolated brush border membranes were suspended in 4 mM Tris-HCl buffer, pH 7.5, stored at −20°C, and used within 3 d.

Enzyme and protein assays. Postproline dipeptidyl aminopeptidase IV was assayed in 50 mM Tris-HCl buffer, pH 8.4, with 2 mM Gly-Pro-β-naphthylamide as substrate. Aminopeptidase activity was measured in 50 mM phosphate buffer, pH 7.0, containing 0.5 mM *p*-hydroxymercuribenzoate, with 2 mM Leu-β-naphthylamide as substrate. The total volume of the incubation mixture was 400 μl in both assays. The reactions were initiated by the addition of substrate, incubated for 30 min at 37°C and terminated by the addition of 300 μl of 32% TCA. A colorimetric assay of the released β-naphthylamine was used using the Branton-Marshal reaction (25, 26). Addition of the following reagents to the assay mixture was done in a sequence: (*a*) 100 μl of 0.3% sodium nitrite; (*b*) 100 μl of 1.5% ammonium sulfamate; (*c*) 300 μl of 0.1% *N*-1-naphthylethylenediamine dihydrochloride in 95% ethanol. The absorbance was read at 560 nm. Postproline endopeptidase activity was assayed using benzoyloxycarbonyl Gly-Pro-β-naphthylamide as a substrate (27) and aminopeptidase activity was measured as described previously (15) using Leu-β-naphthylamide as a substrate. Alkaline phosphatase activity was measured by the method of Fujita et al. (28). Protein concentration was determined by the method of Lowry et al. (29).

Brush border membrane hydrolysis of Leu-Pro, Pro-Leu, Gly-Gly, Pro-Gly-Gly, Leu-Gly-Gly, and Leu-Pro-Gly-Gly was examined by incubating the membranes with 5 mM of each peptide in 50 mM Tris-HCl buffer pH 7.0, for 60 min at 37°C in a total volume of 2 ml. Control incubations, consisting of either enzyme alone or substrate alone, were also carried out. The reaction was terminated by the addition of 1 ml of 6% sulphosalicylic acid. The acidified reaction mixture was stored at −20°C until amino acid analysis. When the time course of hydrolysis of Leu-Pro-Gly-Gly (5 mM) by the brush border membranes was examined, an aliquot of the reaction mixture was removed for periods of up to 3 h.

Amino acid analysis. The amounts of free amino acids and peptides in the intestinal perfusion sample and brush border membrane hydrolysis sample were determined by ion-exchange chromatography with a 119CL amino acid analyzer (Beckman Instruments Inc., Palo Alto, CA). Samples were applied to a W-3 column and eluted successively with sodium citrate buffer as follows: (*a*) 0.2 M Na citrate pH 3.25, 12.5 min; (*b*) 0.4 M Na citrate pH 3.85, 24.5 min; (*c*) 1.0 M Na citrate pH 4.50, 28 min. In addition the temperature of the column was linearly increased from 50 to 65°C during the first 20 min of the run. Using this protocol

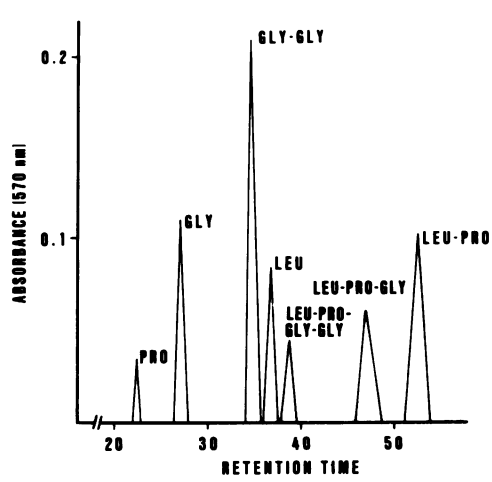

FIGURE 1 Elution profile of Leu-Pro-Gly-Gly and its possible products of hydrolysis. A Beckman model 119CL amino acid analyzer was used for the analysis. The elution time (in minutes) of Pro-Gly-Gly and Pro-Gly was the same as that of Leu-Pro-Gly-Gly.

a satisfactory separation of proline, glycine, Gly-Gly, leucine, Leu-Pro-Gly-Gly, and Leu-Pro was achieved (Fig. 1).

RESULTS

Perfusion study. When the absorption rates of leucine, proline, and glycine from Leu-Pro-Gly-Gly and an equivalent free amino acid mixture were compared, all three amino acids were absorbed by the rat jejunum at a significantly higher rate from the tetrapeptide than from the amino acid mixture as shown in Fig. 2. The rate of luminal appearance of the hydrolytic products during in vivo perfusion of Leu-Pro-Gly-Gly is shown in Fig. 3. The three constituent amino acids and two dipeptides, Leu-Pro and Gly-Gly, were detected as the major hydrolytic products; however, the rate of appearance of the two dipeptides was approximately three times higher when compared with that for the amino acids. No Leu-Pro-Gly or Pro-Gly-Gly peptide products were detected.

Intact transport of Leu-Pro-Gly-Gly was not monitored during this study. The existence of a distinct tetrapeptide transport system has been a subject of controversy in the recent literature (8, 9, 30). Therefore, additional studies in this area are warranted in order to be able to adequately assess the possible physiological importance of intact tetrapeptide transport.

Hydrolysis of Leu-Pro-Gly-Gly by the brush border membranes. When a purified brush border membrane preparation was incubated with Leu-Pro-Gly-Gly for various time periods, the major hydrolytic products were Leu-Pro and Gly-Gly for all periods examined (Fig. 4). In addition, the rate of appearance of these two dipeptides was linear with time and equimolar. Only small amounts of free leucine, proline, and glycine were released from Leu-Pro-Gly-Gly by the brush border membranes. These data indicate that the rat intestinal brush border membranes contain an enzyme capable of releasing the dipeptides Leu-Pro and Gly-Gly from Leu-Pro-Gly-Gly.

Detection of dipeptidyl aminopeptidase IV on the brush border membranes. The data described above

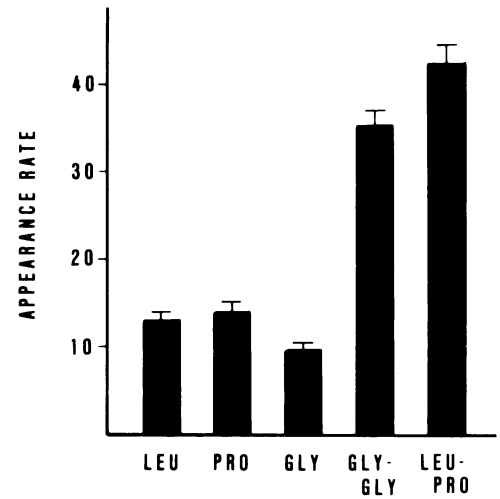

FIGURE 3 Net luminal appearance rates (in micromoles per hour per gram of mucosa) of leucine (*LEU*), proline (*PRO*), glycine (*GLY*), glycylglycine (*GLY-GLY*), and leucylproline (*LEU-PRO*) during intestinal perfusion of Leu-Pro-Gly-Gly. Each value represents the mean appearance rate±SEM obtained from six experiments.

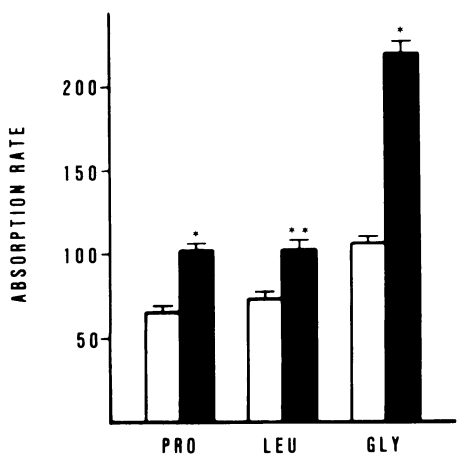

FIGURE 2 Absorption rates (micromoles per hour per gram of mucosa) of proline (*PRO*), leucine (*LEU*), and glycine (*GLY*) during intestinal perfusion. Leu-Pro-Gly-Gly (10 mmol/liter; filled boxes) or a mixture of proline and leucine each at 10 mmol/liter and glycine at 20 mmol/liter; (empty boxes) were perfused. Mean of six experiments and SEM are shown. $^{*}P < 0.05$; $^{**}P < 0.001$.

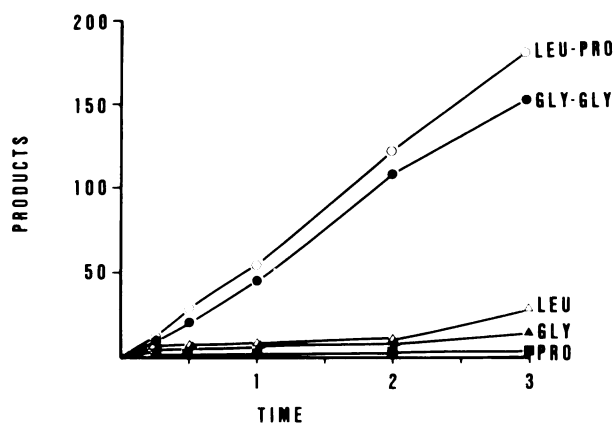

FIGURE 4 Hydrolysis of Leu-Pro-Gly-Gly by rat brush border membranes. The time represents the numbers of hours the reaction mixture was incubated at 37°C. Products are given in micromoles per hour per milligram of protein.

TABLE I
Activities of Rat Intestinal Brush Border Membrane Enzymes

	Enzyme activity			
	Dipeptidyl aminopeptidase IV	Postproline endopeptidase	Aminopeptidase	Alkaline phosphatase
	μmol/min/mg protein			
Homogenate	0.049±0.001	0.008±0.001	0.060±0.003	0.893±0.042
Brush border membrane	0.745±0.059	0.007±0.001	0.920±0.048	13.168±1.294
Purification factor	15.3±1.3	0.9±0.1	15.3±1.6	14.8±1.0

Values are mean±SD. Four animals were used in this experiment.

strongly indicate that the rat intestinal brush border membranes contain a postproline dipeptidyl aminopeptidase IV type enzyme. Therefore, we next sought to examine if this enzyme would co-purify with other well established brush border membrane marker enzymes. For this experiment, Gly-Pro-β-naphthylamide, benzyloxycarbonyl-Gly-Pro-β-naphthylamide, Leu-β-naphthylamide, and *p*-nitrophenylphosphate were used as substrates for postproline dipeptidyl aminopeptidase IV, postproline endopeptidase, aminopeptidase and alkaline phosphatase, respectively. Gly-Pro-β-naphthylamide has been most widely used as a substrate in the assay for dipeptidyl aminopeptidase IV activity (25, 26). As shown in Table I, the purification factor for brush border membrane dipeptidyl aminopeptidase IV was comparable with that for aminopeptidase and alkaline phosphatase. Only a negligible amount of postproline cleaving enzyme activity could be detected in the homogenate and the brush border membranes.

When Leu-Pro-Gly-Gly was added to the standard assay mixture for dipeptidyl aminopeptidase IV, hydrolysis of Gly-Pro-β-naphthylamide by the brush border membranes was significantly inhibited as shown in Fig. 5. No effect was observed by this peptide on the activity of brush border membrane aminopeptidase. Therefore the data suggest that dipeptidyl aminopeptidase IV is associated with the rat intestinal brush border membranes and that Leu-Pro-Gly-Gly is a substrate for this enzyme.

Hydrolysis of peptides by the brush border membranes. To study the mechanisms involved in the intestinal assimilation of Leu-Pro-Gly-Gly, the hydrolysis of various peptide substrates by the brush border membranes was examined. All peptides were incubated with brush border membranes at a concentration of 5 mM. As shown in Table II, the hydrolytic rates of three proline-containing peptides, Leu-Pro, Pro-Leu, and Pro-Gly-Gly were very low when compared with that of Leu-Gly-Gly, which has been shown to be a very good substrate for aminooligopeptidase (15). Diglycine was hydrolyzed at a faster rate when compared with these proline-containing peptides, however the rate was still much lower than that of Leu-Gly-Gly. With Leu-Pro-Gly-Gly, the rate of hydrolysis was 15–70 times higher than that for the other proline containing peptides tested and was approximately two thirds the rate of that for Leu-Gly-Gly.

DISCUSSION

As mentioned previously, peptides can be absorbed into the intestinal absorptive cells by peptide transport systems and/or by amino acid transport systems after hydrolysis by brush border membrane peptidases. In general, the intestinal absorption rates of amino acids from peptides are higher than from the corresponding

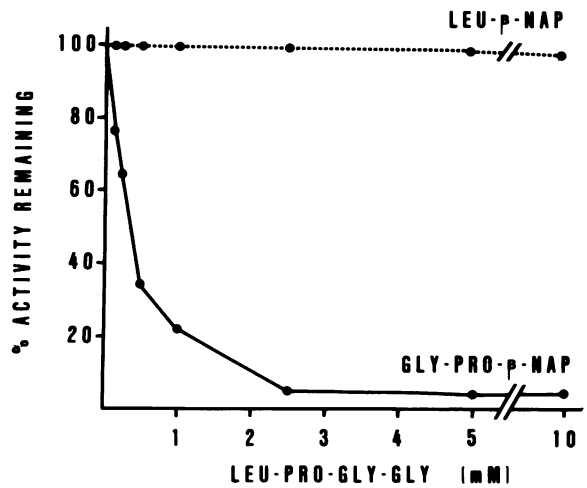

FIGURE 5 Inhibition of dipeptidyl aminopeptidase IV and aminopeptidase by Leu-Pro-Gly-Gly. Dipeptidyl aminopeptidase IV (solid line) and aminopeptidase N (dashed line) were assayed with 2 mM Gly-Pro-β-Nap and 2 mM Leu-β-Nap, respectively, in the presence of varying concentrations of Leu-Pro-Gly-Gly.

TABLE II
Hydrolysis of Peptides by Brush Border Membranes

Substrates	Products				
	Pro	Leu	Gly	Gly-Cly	Leu-Pro
	μmol/h/mg brush border membrane protein				
Leu-Pro	0.58±0.03	0.68±0.09	—*	—	—
Pro-Leu	1.28±0.24	1.39±0.23	—	—	—
Gly-Gly	—	—	18.86±2.18	—	—
Pro-Gly-Gly	3.20±0.37	—	0.53±0.05	2.85±0.45	—
Leu-Gly-Gly	—	71.90±1.22	1.23±0.09	67.80±1.22	—
Leu-Pro-Gly-Gly	1.95±0.21	2.67±0.26	8.37±0.45	40.66±2.84	46.70±1.71

Values are means±SD. Five different brush border membrane preparations were used.
* Spaces are left blank where values would represent the substrate under investigation or where the product could not result from hydrolysis of substrate.

amino acid mixture (5, 31) and this has been shown to be due to the greater absorption capacity of the peptide transport system (5, 22, 32). This study is in accordance with these studies and shows that the constituent amino acids were absorbed significantly faster from the tetrapeptide Leu-Pro-Gly-Gly than from an equivalent amino acid mixture. These results further indicate that the absorption of amino acids from Leu-Pro-Gly-Gly does not occur solely by the amino acid transport system. Analysis of the hydrolytic products after in vivo perfusion of Leu-Pro-Gly-Gly demonstrated that the major products detected in the perfusate were two dipeptides, Leu-Pro and Gly-Gly. Incubation of Leu-Pro-Gly-Gly with purified brush border membranes also yielded Leu-Pro and Gly-Gly as the major hydrolytic products. Only small amounts of free amino acids were detected in both experiments. This suggests that brush border membrane aminopeptidases do not play a major role in the terminal digestion of Leu-Pro-Gly-Gly and that there is another brush border membrane enzyme that hydrolyzes Leu-Pro-Gly-Gly into the two constituent dipeptides. Although brush border aminopeptidases have been shown to have broad substrate specificity and to hydrolyze amino acids sequentially from the amino terminus of a variety of peptides, proline-containing peptides are not hydrolyzed efficiently by these enzymes (10, 15). In contrast, dipeptidyl aminopeptidase IV is capable of releasing aminoacylproline dipeptides from the amino terminus of proline-containing oligopeptides (25, 26). This enzyme has been reported to be widely distributed among many tissues and to be associated with the intestinal brush border membranes of pig, rabbit, and man (20, 26, 27, 33, 34). Since the initial discovery of dipeptidyl aminopeptidase IV by Hopsu-Havu using synthetic substrates (25), this enzyme has been postulated to play an important role in the degradation of proline-containing peptides and proteins in various tissues. However, until this report, no direct evidence has been presented to substantiate this hypothesis. The data presented here show that dipeptidyl aminopeptidase IV co-purifies with other known brush border membrane hydrolases, and is localized to the rat intestinal brush border membrane, whereas post-proline endopeptidase activity is negligible in both the rat intestinal homogenate and brush border membranes. Inhibition studies with a Leu-Pro-Gly-Gly and Gly-Pro-β-naphthylamide indicate that these substrates are hydrolyzed by the same enzyme. The peptide Leu-Pro-Gly-Gly was hydrolyzed by the brush border membranes at a rate comparable with that of Leu-Gly-Gly, which is a very good substrate for intestinal aminooligopeptidase. Thus, data in this study indicate that intestinal brush border membrane dipeptidyl aminopeptidase IV plays a major role in the terminal digestion of Leu-Pro-Gly-Gly.

The major mechanisms involved in the intestinal assimilation of Leu-Pro-Gly-Gly most likely occur as follows: (*a*) Leu-Pro-Gly-Gly is initially hydrolyzed by brush border membrane dipeptidyl aminopeptidase IV into Leu-Pro and Gly-Gly; (*b*) Leu-Pro is then absorbed primarily intact by the peptide transport system, since it is not readily hydrolyzed by brush border membrane aminopeptidases; (*c*) Gly-Gly may be further hydrolyzed in part by brush border membrane aminopeptidases and absorbed by the amino acid transport system or absorbed intact by the peptide transport system; (*d*) absorbed Leu-Pro and Gly-Gly may be further hydrolyzed by prolidase (12, 35) and other dipeptidases (10, 11, 22) present in the cytosol fraction of the intestinal mucosal cells.

Since dietary proteins rich in proline are readily

digested and absorbed in the intestine, it is likely that a number of different mechanisms occur during the intestinal assimilation of proline-containing peptides depending on their structure and sequence. This study clearly shows that dipeptidyl aminopeptidase IV plays a significant physiological role in the hydrolysis and subsequent absorption of peptides containing proline at the penultimate position. However, further studies are necessary in order to fully understand the mechanisms involved in the digestion and absorption of other proline-containing peptides. These studies are currently underway in our laboratory.

ACKNOWLEDGMENTS

The authors wish to thank Miss Trish Harrington for invaluable assistance in the preparation of the manuscript.

This work was supported by grant AM 17938 from the National Institutes of Health and by the Veterans Administration Medical Research Service.

REFERENCES

1. Nixon, S. E., and G. E. Maurer. 1970. The digestion and absorption of protein in man. 2. The form in which digested protein is absorbed. *Br. J. Nutr.* 24:241–258.
2. Adibi, S. A., and D. W. Mercer. 1973. Protein digestion in human intestine as reflected in luminal, mucosal and plasma amino acid concentrations after meals. *J. Clin. Invest.* 52:1586–1594.
3. Chung, Y. C., Y. S. Kim, A. Shadchehr, A. Garrido, I. L. MacGregor, and M. H. Sleisenger. 1979. Protein digestion and absorption in human small intestine. *Gastroenterology.* 76:1415–1421.
4. Munck, B. G. 1981. Intestinal absorption of amino acids. *In* Physiology of the Gastrointestinal Tract. L. R. Johnson, editor. Raven Press, New York. 1097–1122.
5. Adibi, S. A. 1971. Intestinal transport of dipeptides in man: relative importance of hydrolysis and intact absorption. *J. Clin. Invest.* 50:2266–2275.
6. Addison, J. M., D. Burston, and D. M. Matthews. 1972. Evidence for active transport of the dipeptide glycylsarcosine by hamster jejunum in vitro. *Clin. Sci.* 43:907–911.
7. Adibi, S. A., and E. L. Morse. 1977. The number of glycine residues which limits intact absorption of glycine oligopeptides in human jejunum. *J. Clin. Invest.* 60:1008–1016.
8. Smithson, K. W., and G. M. Gray. 1977. Intestinal assimilation of a tetrapeptide in the rat. Obligate function of brush border aminopeptidase. *J. Clin. Invest.* 60:665–674.
9. Chung, Y. C., D. B. A. Silk, and Y. S. Kim. 1979. Intestinal transport of a tetrapeptide, L-leucylglycylglycylglycine, in rat intestine *in vivo*. *Clin. Sci.* 57:1–11.
10. Kim, Y. S., W. Bertwhistle, and Y. W. Kim. 1972. Peptide hydrolases in the brush border and soluble fractions of small intestinal mucosa of rat and man. *J. Clin. Invest.* 51:1419–1430.
11. Das, M., and A. N. Radhakrishnan. 1973. Glycyl-L-leucine hydrolase, a versatile master dipeptidase from monkey small intestine. *Biochem. J.* 135:609–615.
12. Sjostrom, H., and O. Noren. 1974. Structural properties of pig intestinal proline dipeptidase. *Biochim. Biophys. Acta.* 359:177–185.
13. Shoaf, C., R. M. Berko, and W. D. Heizer. 1976. Isolation and characterization of four peptide hydrolases from the brush border of rat intestinal mucosa. *Biochim. Biophys. Acta.* 445:694–719.
14. Kim, Y. S., and E. J. Brophy. 1976. Rat intestinal brush border membrane peptidases. 1. Solubilization, purification, and physicochemical properties of two different forms of the enzyme. *J. Biol. Chem.* 251:3199–3205.
15. Kim, Y. S., E. J. Brophy, and J. A. Nicholson. 1976. Rat intestinal brush border membrane peptidases. 2. Enzymatic properties, immunochemistry and interactions with lectins of two different forms of the enzyme. *J. Biol. Chem.* 251:3206–3212.
16. Schiller, C. M., T. Huang, and W. D. Heizer. 1977. Isolation and characterization of four peptide hydrolases from the cytosol of rat intestinal mucosa. *Gastroenterology.* 72:93–100.
17. Kania, R. K., N. A. Santiago, and G. M. Gray. 1977. Intestinal surface amino-oligopeptidases. II. Substrate kinetics and topography of the active site. *J. Biol. Chem.* 252:4929–4934.
18. Gray, G. M., and N. A. Santiago. 1977. Intestinal surface amino-oligopeptidases. II. Isolation of two weight isomers and their subunits from rat brush border. *J. Biol. Chem.* 252:4922–4928.
19. Sjostrom, H., O. Noren, L. Jeppeson, M. Stavin, B. Svenson, and L. Christiansen. 1978. Purification of different amphiphilic forms of a microvillus aminopeptidase from pig small intestine using immunoabsorbant chromatography. *Eur. J. Biochem.* 88:503–511.
20. Auricchio, S., L. Greco, B. DeVizia, and V. Buonocore. 1978. Dipeptidyl aminopeptidase and carboxypeptidase activities of the brush border of rabbit small intestine. *Gastroenterology.* 75:1073–1079.
21. Wojnarowska, F., and G. M. Gray. 1975. Intestinal surface peptide hydrolases: identification and characterization of three enzymes from rat brush border. *Biochim. Biophys. Acta.* 403:147–160.
22. Adibi, S. A., and Y. S. Kim. 1981. Peptide absorption and hydrolysis. *In* Physiology of the Gastrointestinal Tract. L. R. Johnson, editor. Raven Press, New York. 1097–1122.
23. Oya, H., I. Nagatsu, and T. Nagatsu. 1972. Purification and properties of glycylprolyl β-naphthylamidase in human submaxillary gland. *Biochim. Biophys. Acta.* 258:591–599.
24. Kessler, M., O. Acuto, C. Storelli, H. Murer, M. Muller, and G. Semenza. 1978. A modified procedure for the rapid preparation of efficiently transporting vesicles from small intestinal brush border membranes. Their use in investigating some properties of D-glucose and choline transport systems. *Biochim. Biophys. Acta.* 506:136–154.
25. Hopsu-Havu, V. K., and G. G. Glenner. 1966. A new dipeptide naphthylamidase hydrolyzing glycyl-prolyl-β-naphthylamide. *Histochemie.* 7:197–201.
26. Kenny, A. J., A. C. Booth, S. G. George, J. Ingram, D. Kershaw, D. J. Wood, and A. R. Young. 1976. Dipeptidyl peptidase IV, a kidney brush border serine peptidase. *Biochem. J.* 157:169–182.
27. Yoshimoto, T., M. Fischl, R. C. Orlowski, and R. Walter. 1978. Post-proline cleaving enzyme and post-proline dipeptidyl aminopeptidase: comparison of two pepti-

dases with high specificity for proline residues. *J. Biol. Chem.* 253:3708–3716.
28. Fujita, M., H. Ohta, K. Kawai, H. Matsui, and M. Nakao. 1972. Differential isolation of microvillus and basolateral membranes from intestinal mucosa: mutually exclusive distribution of digestive enzymes and ouabain-sensitive ATPase. *Biochim. Biophys. Acta.* 274:336–347.
29. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265–275.
30. Burston, D., E. Taylor, and D. M. Matthews. 1979. Intestinal handling of two tetrapeptides by rodent small intestine *in vitro*. *Biochim. Biophys. Acta.* 553:175–178.
31. Silk, D. B. A., P. D. Fairclough, N. J. Park, A. E. Lane, J. P. Webb, M. L. Clark, and A. M. Dawson. 1975. A study of relations between the absorption of amino acids, dipeptides, water and electrolytes in the normal human subject. *Clin. Sci. Mol. Med.* 49:401–408.
32. Matthews, D. M., and S. A. Adibi. 1976. Peptide absorption. *Gastroenterology.* 71:151–161.
33. Hopsu-Havu, V. K., and T. O. Ekfors. 1969. Distribution of a dipeptide naphthylamidase in rat tissues and its localisation by using diazo coupling and labeled antibody techniques. *Histochemie.* 17:30–38.
34. Svenson, B. M., M. Danielsen, M. Stann, L. Jeppeson, O. Noren, and H. Sjostrom. 1978. An amphiphilic form of dipeptidyl peptidase IV from pig small intestinal brush border membrane *Eur. J. Biochem.* 90:489–498.
35. Baksi, K., and A. N. Radhakrishnan. 1974. Purification and properties of prolidase (imidodipeptidase) from monkey small intestine. *Indian J. Biochem. Biophys.* 11:7–11.