The Responses of Rat Intestinal Brush Border and Cytosol Peptide Hydrolase Activities to Variation in Dietary Protein Content

DIETARY REGULATION OF INTESTINAL PEPTIDE HYDROLASES

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ABSTRACT The effects of variation in dietary protein content on small intestinal brush border and cytosol peptide hydrolase activities have been investigated. One group of rats was fed a high protein diet (55% casein) and another group was fed a low protein diet (10% casein). After 1 wk, brush border peptide hydrolase activity (L-leucyl-β-naphthylamide as substrate) and cytosol peptide hydrolase activity (L-prolyl-L-leucine as substrate) were determined in mucosae taken from the proximal, middle, and distal small intestine. As judged by several parameters, brush border peptide hydrolase activity was significantly greater in rats fed the high protein diet when data for corresponding segments were compared. In contrast, no significant difference was seen in cytosol peptide hydrolase activity.

In a second study, brush border and cytosol peptide hydrolase activities were determined in the proximal intestine by utilizing an additional three peptide substrates: L-leucyl-L-alanine, L-phenylalanylglycine, and glycyl-L-phenylalanine. Sucrase, maltase, and alkaline phosphatase activities were also determined. As before, brush border peptide hydrolase activities were significantly greater in rats fed the high protein diet. However, activities of the nonproteolytic brush border enzymes did not vary significantly with diet. In contrast to the results obtained with L-prolyl-L-leucine as substrate for the cytosol enzymes, cytosol activity against the three additional peptide substrates was greater in rats fed the high protein diet.

It is suggested that the brush border peptide hydrolase response to variation in dietary protein content represents a functional adaptation analogous to the regulation of intestinal disaccharidases by dietary carbohydrates.

The implication of the differential responses of the cytosol peptide hydrolases is uncertain, since little is known of the functional role of these nonorgan-specific enzymes.

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INTRODUCTION

The dietary regulation of intestinal mucosal enzymes which subserve carbohydrate and fat digestion has been well established (1–4). In contrast, little is known about the dietary factors which influence intestinal peptide hydrolases. In earlier studies of the effects of protein malnutrition and starvation, no differentiation was made between the brush border and the cytosol peptide hydrolases (5–8). However, evidence obtained from studies of electrophoretic mobility, chain length specificity, heat stability, and the effects of inhibitors, suggests that the peptide hydrolases bound to the intestinal brush border are distinct from those in the cell cytosol (9–11). More recently, starvation was shown to have different effects on brush border and cytosol peptide hydrolase activities; brush border activity fell while cytosol activity increased (12). It is therefore important to distinguish between the peptide

hydrolases present in these two cellular compartments.

The present studies were undertaken to investigate the responses of both brush border and cytosol peptide hydrolase activities in the rat small intestine to variation in the protein content of the diet.

In the first experiment, brush border was distinguished from cytosol peptide hydrolase activity by the use of two substrates: L-leucyl-β-naphthylamide which is hydrolyzed principally by brush border enzymes (13, 14), and L-prolyl-L-leucine which is hydrolyzed solely by cytosol enzymes (9, 15, 16). Enzyme activities were determined in mucosae of standard segments taken from the proximal, middle, and distal small intestine and were related to mucosal protein content, DNA content, and wet weight.

In a second experiment, enzyme activities were determined in mucosae from the proximal intestine by utilizing an additional three peptide substrates: L-phenylalanylglycine, glycyl-L-phenylalanine, and L-leucyl-L-alanine. Sucrase, maltase, and alkaline phosphatase activities were also determined in the proximal intestine. This experiment allowed further characterization of the effects of dietary variation on brush border and cytosol peptide hydrolase activities.

METHODS

Male Wistar rats were used in all experiments. Laboratory chow pellets (Ralston Purina, St. Louis, Mo.) were fed to the rats before the experimental periods. The experimental "high," "normal," and "low" protein diets were isocaloric and were obtained in powdered form (General Biochemicals Div., Mogul Corp., Chagrin Falls, Ohio).

Substrates and standards used in the assays of peptide hydrolase activity were purchased from commercial sources. L-phenylalanylglycine, glycyl-L-phenyl-alanine, L-leucyl-L-alanine, and L-prolyl-L-leucine were obtained from Cyclo Chemical Corp., Los Angeles, Calif., L-leucyl-β-naphthylamide hydrochloride and β-naphthylamine were obtained from Sigma Chemical Co., St. Louis, Mo., and L-leucine, L-phenylalanine, and L-proline from Mann Research Labs, Inc., New York. L-Amino acid oxidase (*Crotalus adamanteus*, Sigma Chemical Co., Type I), horseradish peroxidase, *o*-dianisidine, and *p*-hydroxymercuribenzoate were all obtained from Sigma Chemical Co. and were used in one method of assay for peptide hydrolase activity. 3',5'-Diamino benzoic acid dihydrochloride, 99% pure, used in the assay of DNA, was obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis., and was not recrystalized before use. Highly polymerised calf thymus DNA (Sigma Chemical Co.) was used as the DNA standard.

Experiment I

Diet and feeding protocol. Rats were maintained on Purina Laboratory Chow (Ralston Purina, St. Louis, Mo.) for 5 days after purchase. 12 rats were then placed in individual metabolic cages, so constructed as to prevent contamination of food with feces. The rats were then fed the normal protein diet ad lib for five days. Thereafter, the rats were weighed and divided into two groups of six on the basis of their weights, so that the means and ranges of the weights of the two groups were similar. One group was then fed the high protein diet, and these are subsequently designated HP rats;[1] the other group was fed the low protein diet (LP rats). The compositions and caloric value of these diets are shown in Table I. In preliminary free feeding experiments, it had been established that rats consumed greater quantities of the low protein diet than of the high protein diet. To ensure that HP and LP rats consumed similar quantities of calories, rats were paired, and all were provided daily with an amount equal to the previously determined average daily high protein intake. Little manipulation of daily provision was necessary to maintain pairing, since the rats usually consumed the food provided. Water was provided ad lib.

TABLE I

Composition of the Experimental Diets

Dietary constituent	Dietary content		
	HP	NP	LP
	% *weight*		
Protein (casein)	55	26	10
Carbohydrate (corn starch)	30	59	75
Fat (cotton-seed oil)	8	8	8
Salt mix U. S. P. 14	4	4	4
Vitamin mix*	3	3	3

The caloric value of all diets was 4.11 Cal/g.

* Composition of the vitamin mix is given in Hegsted and Chang (38).

Preparation of tissues. After 7 days on the experimental diets, rats were sacrificed in the morning without prior fasting. The animals were taken to a cold room maintained at 4°C, stunned by a blow on the head, and then decapitated. Immediately after decapitation, the small intestine from the pylorus to the ileocecal valve was removed in one piece and stripped of mesentery and fat. The intestine was then suspended vertically in front of a meter rule with a 10-g weight attached to its ileal extremity. With the bowel at constant tension, three 10-cm segments were marked out and excised as follows: segment I, distal to a point 12 cm from the pylorus; segment II, between two points 5 cm on either side of the midpoint of the small intestine; and segment III, immediately proximal to the ileal extremity. Further preparations of mucosal homogenates and of cytosol and particulate fractions for assay of peptide hydrolase activity is described in detail in a previous paper (17).

Assays of protein and of peptide hydrolase activity by using L-leucyl-β-naphthylamide and L-prolyl-L-leucine as substrates were performed on homogenates and on cytosol and particulate fractions. In addition, homogenates were assayed for DNA.

Protein was determined by the method of Lowry, Rosebrough, Farr, and Randall (18). Bovine serum albumin was used as the protein standard.

DNA was determined by the method of Kissane and Robins (19).

[1] *Abbreviations used in this paper:* HP rats, high protein-fed rats; LP rats, low protein-fed rats; LAOR, L-amino acid oxidase reagent; NP rats, normal protein-fed rats.

TABLE II
Rat Weight and Dietary Intake

Expt no.	Diet	Initial weight	Weight at sacrifice	Dietary intake
		g	*g*	*g/day*
I	HP	217±6	254±6	15.6±0.6
	LP	222±6	237±3	16.5±0.4
II	HP	197±9	258±6	16.6±0.5
	LP	200±8	228±21	15.9±2.3

Each value represents the mean±1 SD of data from six rats.

Assay of peptide hydrolase activity by using L-leucyl-β-naphthylamide as substrate was performed by a modification of the method of Panvelliwalla and Moss (20). 0.1 ml of a 10 mM solution of L-leucyl-β-naphthylamide hydrochloride in methoxy-ethanol was diluted with 4.9 ml 0.1 M potassium phosphate buffer, pH 7.3, containing 0.2% Triton X-100. 0.1 ml of this substrate was incubated with 0.1 ml suitably diluted mucosal enzyme preparation for 10 min at 37°C. The reaction was stopped by addition of 2.0 ml ice-cold 50 mM glycine-5mM EDTA buffer, pH 10.4. The assay tubes were then kept on an ice bath until fluorescence was measured. The amount of β-naphthylamine released was calculated by comparison with a standard fluorescence curve determined with each batch of analyticals. Standards contained from 0 to 1.0 nmol of β-naphthylamine. Fluorescence was measured at 460 nm with excitation at 340 nm.

Assay of peptide hydrolase activity by using L-prolyl-L-leucine as substrate was performed by the Matheson and Tattrie modification of the Yemm and Cocking procedure (9, 21).

Experiment II

The diet and feeding protocol described above was repeated with a further two groups of six rats. After sacrifice, the intestine was removed and suspended as before, but only segment I as previously defined was excised. In addition, a 5-cm segment (I*a*) immediately distal to segment I was also excised.

Mucosal homogenates and cytosol and particulate fractions were assayed for DNA and protein as before, but in this experiment, peptide hydrolase activities were assayed by using L-phenylalanylglycine, glycyl-L-phenylalanine, and L-leucyl-L-alanine as substrates. Since the Matheson and Tattrie method gave high blank values when these dipeptides were used as substrates, a one-step modification[2] of previously reported L-amino acid oxidase methods was used for assays of peptide hydrolase activities (16, 22, 23). Each dipeptide was dissolved in 0.5 ml 50 mM Tris-HCl buffer, pH 8.0. 1 ml of L-amino acid oxidase reagent (LAOR) was then added to these solutions. LAOR was prepared by dissolving 20 mg L-amino acid oxidase, 2 mg horseradish peroxidase, and 10 mg *o*-dianisidine in 100 ml of the Tris-HCl buffer. 25 μl of suitably diluted enzyme preparation was then added to the tubes containing substrates and LAOR and the reactions incubated for 20 min at 37°C. The reactions were stopped by the addition of 0.74 ml of 50% sulfuric acid. Absorbance of the purple color produced was measured spectrophotometrically at 530 nm. The amount of L-leucine or L-phenylalanine released was estimated by comparison with standard absorbance curves prepared by incubation of 0–100 nmol of L-leucine or L-phenylalanine dissolved in 0.5 ml of Tris-HCl buffer with 1 ml of LAOR and 25 μl of the enzyme diluent (14% glycerol) for 20 min at 37°C followed by the addition of 0.74 ml of 50% sulfuric acid. The absorbances of reagent blanks and enzyme blanks were determined with each batch of analyticals. This method was used for the assay of hydrolase activities in the homogenates and in the cytosol and particulate fractions from segment I. In addition, particulate fractions were reassayed under the same conditions but with the addition of *p*-hydroxymercuribenzoate at a concentration of 0.5 mM in the final assay volume of 1.525 ml.

Mucosae from segment I*a* were homogenized in ice-cold 0.9% saline and assayed for protein and DNA contents and for sucrase, maltase, and alkaline phosphatase activities. Sucrase and maltase were determined by the method of Dahlqvist (24), and alkaline phosphatase was determined by the method of Garen and Levinthal using *p*-nitrophenyl phosphate as substrate (25).

Normal protein-fed rats (NP rats). In an independent study, a group of six male rats was submitted to the feeding protocol described above, except that in this instance, a restricted normal protein diet was fed for 7 days after the initial period of ad lib feeding. Assays of enzyme activity using L-leucyl-β-naphthylamide as substrate were performed on mucosae from segments I, II, and III.

Enzyme units. All units of enzyme activity represent micromoles of substrate hydrolyzed per minute at 37°C, except for alkaline phosphatase which was assayed at 25°C.

[2] Nicholson, J. A., and Y. S. Kim. Submitted for publication.

TABLE III
Tissue Parameters (Expt I)

			Segmental protein content							
	Mucosal wet weight		Homogenate		Cytosol		Particulate		Segmental DNA content	
Segment	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP
	g				*mg*				*mg*	
I	0.223±0.043	0.194±0.024	24.0±2.9	20.3±3.7	15.2±1.63	13.3±0.8	8.9±0.3	6.1±1.0	1.03±0.23	0.96±0.38
P					<0.05		<0.001			
II	0.278±0.066	0.239±0.052	30.5±7.4	19.4±4.3	18.4±2.1	13.2±2.7	12.0±2.9	7.3±1.4	1.16±0.24	1.06±0.28
P			<0.01		<0.005		<0.01			
III	0.202±0.069	0.172±0.023	20.4±5.2	18.7±3.2	12.9±3.5	11.1±2.7	6.0±1.8	5.7±1.6	1.04±0.31	1.04±0.20

Each value represents the mean±1 SD of data from six rats. Only statistically significant differences between data from the two groups are indicated.

Statistics. Comparisons between data from HP and LP rats were performed by means of Student's t test. Values for P of less than 0.05 were regarded as statistically significant.

RESULTS

Table II details the initial weights, dietary intakes, and weights at sacrifice of the groups of rats used in the two experiments. In both experiments, HP rats showed greater weight gains than LP rats.

Experiment I

Effects of differences in protein intake on tissue parameters. (*Table III*) In segment I, cytosol and particulate fraction protein were significantly greater in HP rats than in the corresponding segment of LP rats; there was no significant difference in DNA content. Similar differences in segmental protein were seen for segment II when data from the two groups were compared; again there was no significant difference in DNA content. In segment III, no significant differences were seen between data from the two groups of rats for any of the tissue parameters studied.

Peptide hydrolase studies. The results of assays of particulate activities using L-leucyl-β-naphthylamide as substrate and of cytosol activities using L-prolyl-L-leucine as substrate are shown in Fig. 1 for segment I from the two groups of rats. Particulate peptide hydrolase activity was significantly higher in HP rats as judged by any of the four parameters used to express enzyme activity. In contrast, there was no significant difference in cytosol activity between HP and LP rats.

When the recovery of enzyme activity using L-prolyl-L-leucine as substrate was studied in segment I, activity was wholly accounted for by the cytosol fractions in both HP and LP rats (Table IV).

Results obtained for activities in homogenates by using L-prolyl-L-leucine as substrate and in particulate fractions by using L-leucyl-β-naphthylamide as substrate are shown for segments II and III in Table V. As for segment I, there was no statistically significant difference in the cytosol enzyme activity (L-prolyl-L-leucine substrate) in the corresponding segments of the HP and LP rats. In contrast, particulate activity (L-leucyl-β-naphthylamide substrate) was again significantly greater in HP rats in the three parameters of activity for segment II and in two of the three parameters for segment III.

Assays of particulate enzyme activity using L-leucyl-β-naphthylamide as substrate in mucosae from the group of six NP rats (mean initial weight, 204±14 g; mean weight at sacrifice, 242±12 g; daily intake, 16.4 ±0.9 g) gave values intermediate between those obtained for LP and HP rats. For segment I, specific activity was 0.047±0.023 U/mg particulate protein (corresponding values for LP and HP rats were 0.034 ±0.008 and 0.053±0.011, respectively); for segment II, activity for NP rats was 0.053±0.012 U/mg particulate protein (LP, 0.045±0.010; HP, 0.069±0.021); and for segment III, activity for NP rats was 0.046±0.024 U/mg particulate protein (LP, 0.033±0.014; HP, 0.051±0.023).

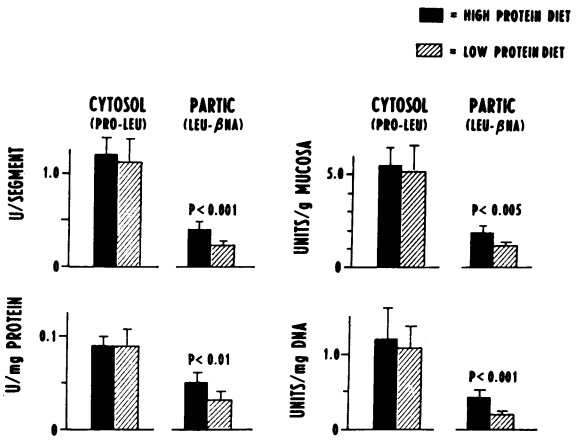

FIGURE 1 (Expt. I) Peptide hydrolase activities in particulate and cytosol fractions from the proximal small intestine (segment I) of rats fed high and low protein diets. Substrates are L-prolyl-L-leucine (PRO-LEU) for cytosol enzyme activity and L-leucyl-β-naphthylamide (LEU-βNA) for particulate (PARTIC) activity. Each histogram represents the mean+1 SD of data from six rats. Only statistically significant differences between data from the two groups are indicated. Units (U) are micromoles of substrate hydrolyzed per minute at 37°C. Units per milligram protein refer to units per milligram particulate protein when LEU-βNA is substrate and units per milligram cytosol protein when PRO-LEU is substrate.

Recovery data shown for segment I demonstrate that similar recoveries of enzyme activities using L-

TABLE IV

Similarity of Homogenate and Cytosol Activities when Using L-Prolyl-L-leucine as Substrate (Expt I)

Activity	HP group		LP group	
	Homogenate	Cytosol	Homogenate	Cytosol
U/segment	1.2±0.3	1.2±0.1	1.0±0.2	1.1±0.2
U/mg DNA*	1.1±0.4	1.2±0.4	1.0±0.1	1.1±0.2
U/g mucosa	5.4±1.9	5.4±1.0	5.3±1.0	5.2±1.4
U/mg protein‡	0.05±0.02	0.09±0.01	0.05±0.01	0.08±0.02

Units (U) represent micromoles of substrate hydrolyzed per minute at 37°C. Each value represents the mean±1SD of data from six rats (segment I).

* DNA was measured in homogenates only. Hence the denominator has the same value for both homogenate and cytosol activities when activity is expressed as U/mg DNA.

‡ Protein was measured both in homogenates and in cytosol fractions.

TABLE V

Enzyme Activity in Middle and Distal Small Intestine (Expt I). Activities in Homogenates when Using L-Prolyl-L-leucine (PRO-LEU) and in Particulate Fractions when Using L-Leucyl-β-naphthylamide (LEUβNA) as Substrates

Segment	Parameter of enzyme activity*	Enzyme activity			
		PRO-LEU		LEUβNA	
		HP group	LP group	HP group	LP group
II	U/segment	1.5±0.7	1.2±0.4	0.54±0.14	0.36±0.11
	P			<0.05	
	U/mg protein	0.06±0.02	0.05±0.01	0.07±0.02	0.04±0.01
	P			<0.01	
	U/mg DNA	1.25±0.50	1.15±0.31	0.52±0.06	0.38±0.07
	P			<0.005	
III	U/segment	1.1±0.6	0.8±0.4	0.29±0.09	0.17±0.05
	P			<0.02	
	U/mg protein	0.05±0.01	0.05±0.01	0.05±0.02	0.03±0.01
	U/mg DNA	0.09±0.55	0.08±0.30	0.28±0.04	0.16±0.03
	P			<0.001	

* Units (U) are μmol of substrate hydrolyzed per minute at 37°C. Each value represents the mean±1 SD of data from six rats. Only statistically significant differences between data from the two groups are indicated.

leucyl-β-naphthylamide as substrate were obtained from the cytosol and particulate fractions for both HP and LP rats (Table VI, see Percentage recovery). The majority of the recovered activity using this substrate was particulate bound (Table VI, see Subcellular distribution).

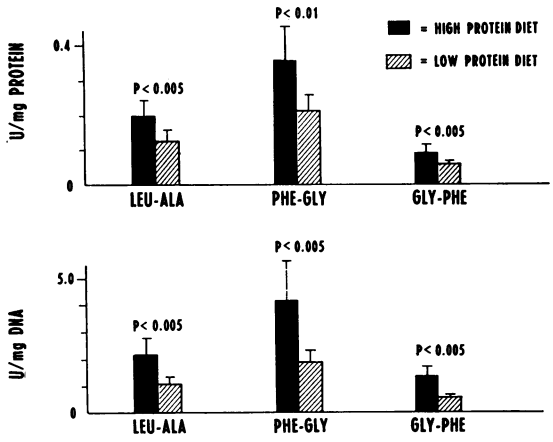

FIGURE 2 (Expt. II) Peptide hydrolase activities in particulate fractions from the proximal small intestine of HP and LP rats. Substrates are L-leucyl-L-alanine (LEU-ALA), L-phenylalanylglycine (PHE-GLY) and glycyl-L-phenylalanine (GLY-PHE). Each histogram represents the mean+1 SD of data from six rats. Only statistically significant differences between data from the two groups are indicated. Units (U) are micromoles of substrate hydrolyzed per minute at 37°C.

Experiment II

Peptide hydrolase studies in the proximal intestine (segment I). The recoveries of peptide hydrolase activities in the cytosol and particulate fractions for expt II are also shown in Table VI. For each substrate there was no significant difference in the percentage of activity recovered in these fractions between data from HP and LP rats. The subcellular distributions of recovered activities are shown in Table VI. The majority of the activities for both glycyl-L-phenylalanine and L-leucyl-L-alanine were associated with the cytosol fractions in both HP and LP rats. In contrast, only one-third of activity using L-phenylalanylglycine was associated with the cytosol fraction. The small differences in subcellular distribution of enzyme activities observed between data from the two groups is consistent with the relatively greater responses of the particulate-bound peptide hydrolases to variation in protein content of the diet (*vide infra* Fig. 2–4).

The activities of particulate fractions when using L-phenylalanylglycine, glycyl-L-phenylalanine, and L-leucyl-L-alanine as substrates are shown for the two groups of rats in Fig. 2. Activities were significantly greater in the HP rats for all three substrates.

When particulate fractions were assayed in the presence of *p*-hydroxymercuribenzoate, activities were again significantly greater in the HP rats (Fig. 3).

The activities of cytosol fractions when using L-phenylalanylglycine, glycyl-L-phenylalanine, and L-leucyl-

TABLE VI

Percentage Recovery and Subcellular Distribution of Enzyme Activity against Various Substrates

Substrate*	% Recovery‡		Subcellular distribution§			
			% Of recovered activity in cytosol fraction		% Of recovered activity in particulate fraction	
	HP	LP	HP	LP	HP	LP
LEUβNA	102±17	102±25	12±2	14±1	88±2	86±1
LEU-ALA	94±10	95±10	87±2	88±3	10±2	8±1
PHE-GLY	85±4	82±5	31±5	36±6	69±5	63±4
GLY-PHE	80±11	80±15	90±2	93±2	14±3	12±3

Each value represents the mean±1 SD of data from six rats.

* Substrates are L-leucyl-β-naphthylamide (LEUβNA), L-leucyl-L-alanine (LEU-ALA), L-phenylalanylglycine (PHE-GLY), and glycyl-L-phenylalanine (GLY-PHE).

‡ Percentage recovery of enzyme activity is calculated as follows: % recovery = (units (U) in particulate fraction per segment) + (U in cytosol fraction per segment)/(U in homogenate per segment) × 100%.

§ Subcellular distribution is calculated as follows: percentage of recovered activity in cytosol fraction = (U in cytosol fraction/segment)/(U in particulate fraction/segment) + (U in cytosol fraction/segment) × 100%. Percent of recovered activity in particulate fraction is similarly calculated with U in particulate fraction/segment as numerator.

L-alanine as substrates are shown in Fig. 4. Results when using L-prolyl-L-leucine (expt I) are included for comparison. In contrast to the previous results where no difference in activity using L-prolyl-L-leucine was seen, activities using the three additional substrates were greater in HP rats. In the case of L-phenylalanylglycine, though the difference was not statistically significant when results were expressed per milligram protein, segmental activity was significantly greater in the HP rats; (2.02±0.60 U/segment vs. 1.19±0.33 U/segment; $P < 0.02$). As in expt I, segment I cytosol protein was again greater in the HP rats, while segmental DNA did not vary.

Sucrase, maltase, and alkaline phosphatase. The results of assays of sucrase, maltase, and alkaline phosphatase activities in mucosal homogenates taken from segments I*a* are shown in Fig. 5 for three HP and three LP rats. For each enzyme, no significant difference was seen between data from the two groups of rats.

DISCUSSION

Evidence from in vitro (26) and in vivo (27) studies has been interpreted as suggesting two modes of peptide transport, (*a*) surface hydrolysis by mechanisms closely linked to the amino acid entry mechanisms, and (*b*) peptide entry into mucosal cells by a special mechanism, followed by intracellular hydrolysis. It was of interest, therefore, to determine whether or not the activities of intestinal peptide hydrolases are regulated by dietary protein in a manner analagous to the regulation of intestinal disaccharidases by dietary carbohydrate.

In the first experiment, brush border peptide hydrolase activity was characterized by the activity in particulate fractions when using L-leucyl-β-naphthylamide as substrate. In addition, since peptide hydrolase activity is present throughout the small intestine (28, 29), studies were performed on the proximal, middle,

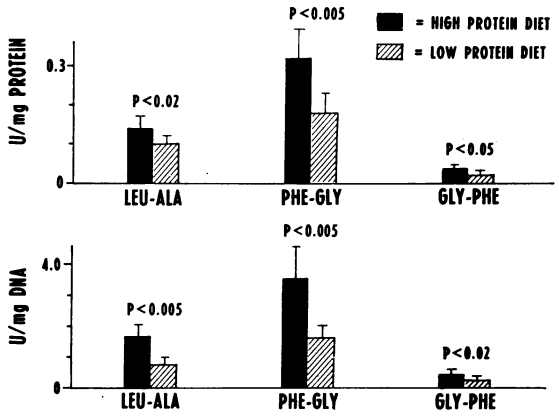

FIGURE 3 (Expt. II) *p*-Hydroxymercuribenzoate (PHMB) resistant particulate activities. Particulate fractions from the proximal small intestine were reassayed in the presence of 0.5 mM PHMB. Substrates and units (U) of enzyme activity are as described in Fig. 2. Histograms represent the mean + 1 SD of data from six rats. Only statistically significant differences between data from HP and LP rats are indicated.

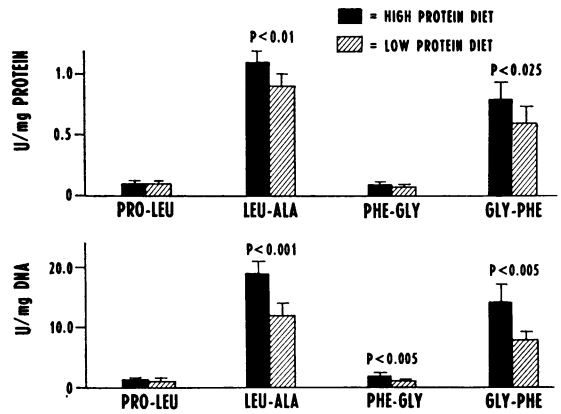

FIGURE 4 (Expt. II) Peptide hydrolase activities in cytosol fractions from the proximal intestine of rats fed high and low protein diets. Results from expt. I using L-prolyl-L-leucine (PRO-LEU) as substrate for cytosol activity are included for comparison. Other substrates and all units (U) of activity are as described in Fig. 2. Histograms represent the mean+1 SD of data from six rats. Only statistically significant differences between data from the two groups are indicated.

and distal small intestine. The demonstration in expt I that rats fed a high protein diet had greater activity for this substrate in all three regions of the small intestine than rats fed a low protein diet suggested that brush border peptide hydrolase activity was indeed affected by the protein content of the diet. Recently, however, Heizer and Shoaf (30, 31) purified three enzymes from the intestinal brush border of the rat. Although several peptides are hydrolyzed by more

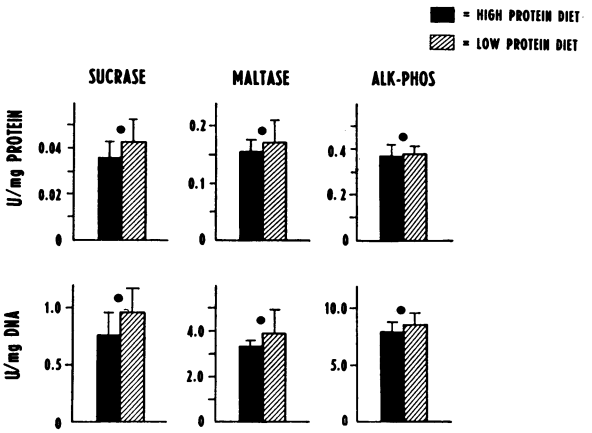

FIGURE 5 (Expt. II) Sucrase, maltase, and alkaline phosphatase (ALK-PHOS) activities in homogenates from the proximal small intestine of rats fed high and low protein diets. Histograms represent the mean+1 SD of data from three rats. The statistical significance of differences between data from the two groups is indicated: ● = $P > 0.05$. Units (U) are micromoles of substrate hydrolyzed per minute at 37°C, except for *alk-phos* which was assayed at 25°C.

than one of the enzymes, there are differences in the rapidity with which a particular substrate is split by each of the three enzymes. Hence, studies of brush border peptide hydrolase activity which employ only one substrate give an incomplete characterization of the total brush border peptide hydrolase activity. Consequently, a second experiment utilizing three additional substrates, L-phenylalanylglycine, glycyl-L-phenylalanine, and L-leucyl-L-alanine was performed on particulate fractions from the proximal intestinal segment. As a means of defining the brush border peptide hydrolase activity more clearly, particulate fractions were also assayed in the presence of *p*-hydroxymercuribenzoate. This substance has been shown to abolish cytosol peptide hydrolase activity, whilst brush border activity is not inhibited by its presence (11). Particulate enzyme activities were greater in the HP rats for all three substrates both in the absence and presence of *p*-hydroxymercuribenzoate, which suggests a true increase in brush border peptide hydrolases.

The differences in brush border peptide hydrolase activities observed between the HP and LP rats were not paralleled by differences in activities of the three nonproteolytic membrane-bound enzymes. Indeed, as might be expected, since the low protein diet contained more carbohydrate than the high protein diet, the mean sucrase and maltase activities were somewhat greater in LP rats, although the differences did not achieve statistical significance. This suggests that in this region of the intestine, the observed response of brush border peptide hydrolases to variation in the protein content of the diet is an adaptive phenomenon and not merely a reflection of a nonspecific response common to all membrane-bound enzymes.

Differences in the responses of the various cytosol peptide hydrolases to the two diets were observed. Whilst cytosol activity for L-prolyl-L-leucine did not vary with diet, cytosol activities for the other three substrates studied were greater in the HP rats. That cytosol activities against different peptide substrates may show differences in their responses to a common stimulus is not surprising, since data indicate the presence of multiple enzymes in the cytoplasm (9, 32).

The higher activities of the cytosol enzymes hydrolyzing L-phenylalanylglycine, glycyl-L-phenylalanine, and L-leucyl-L-alanine observed in the rats fed the greater quantity of protein is of interest. Data presented by several authors have indicated that some dipeptides may be absorbed into the enterocyte without prior hydrolysis (33–36). Hence, one possible function of the cytosol peptide hydrolases may be the hydrolysis of absorbed dipeptides. However, many other organs contain cytoplasmic peptide hydrolases which show the same electrophoretic patterns as those of the intestine

(9). This lack of organ specificity suggests that these enzymes may have some more general function in cellular protein metabolism rather than a specific role in the digestion of absorbed peptides. Hence, the effects of differences in protein intake on the cytosol activities against these three dipeptide substrates may not necessarily indicate a functional adaptation for the optimal digestion of absorbed peptides but may be analagous to the regulation by dietary carbohydrate of nondigestive intestinal cytosol enzymes which subserve intracellular carbohydrate metabolism (37).

Predictably, the rats fed the high protein diet gained more weight than the low protein-fed groups (38). However, after a perinatal period of development, peptide hydrolase specific activity does not vary between age 3 wk and adulthood (39); moreover our experience[3] with groups of rats with weight differences between 150 and 350 g showed no differences in particulate or cytosol peptide hydrolase activities over this weight range. Hence, the relatively small weight differences between the two groups observed in these experiments were an unlikely cause of the differences in peptide hydrolase activity.

It is unlikely that the differences in enzyme activities observed were due to the fractionation procedures employed, since no significant difference in enzyme recoveries are seen when data from the two groups of rats are compared (Table VI). Little cross contamination of cytosol and particulate fractions was observed; activity in particulate fractions using L-prolyl-L-leucine as substrate was 0–3% of the homogenate activity and *p*-hydroxymercuribenzoate resistant activities in cytosol fractions were below the limits of detectable activity.

In the absence of any data on enzyme turnover or activation (40), we can offer no explanation of the precise mechanisms underlying the observed differences in intestinal peptide hydrolase activities between the HP and LP rats. Nevertheless, the demonstration that brush border peptide hydrolase activities were greater in rats fed the high protein diet is analagous to the dietary regulation of disaccharidases by dietary carbohydrate, and may represent a similar functional adaptation. The implication of the observed differential responses of cytosol peptide hydrolases, however, are less obvious, and interpretation of these responses awaits definition of the functions of these widely distributed enzymes.

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[3] Kim, Y. S., and W. Fong. Unpublished observations.

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