Effect of dietary protein level on intestinal aminopeptidase activity and mRNA level in rats

Kei Sonoyama, Shuhachi Kiriyama, and Ryoya Niki

Laboratory of Food Biochemistry, Laboratory of Nutritional Biochemistry, Department of Bioscience and Chemistry, Faculty of Agriculture, Hokkaido University, Sapporo, Japan

To investigate the mechanism of adaptive response of intestinal aminopeptidase on the dietary protein level, we determined the aminopeptidase activity and mRNA level in the intestinal mucosa of rats with different dietary protein levels. In the first experiment, the specific activity of aminopeptidase in the homogenate of intestinal mucosa from rats fed a 60% casein diet for 7 days was significantly higher than that from rats fed a 20% casein diet. A larger difference between the two groups was observed in the ileum than in the jejunum. In contrast to aminopeptidase, the specific activity of sucrase in the ileum of rats fed a 60% casein diet was significantly lower than that of rats fed a 20% casein diet. The activities of sucrase in the jejunum and alkaline phosphatase in the jejunum and ileum did not change between the two groups. Northern blot analysis of intestinal RNA using 1.1 kb aminopeptidase N cDNA as a probe did not show a significant difference in the level of aminopeptidase N mRNA between two dietary groups. In the next experiment, the aminopeptidase activity and mRNA level in the ileal mucosa were determined in rats fed 0, 10, 20, 40, and 60% casein diets for 7 days. While the specific activity of aminopeptidase increased in response to the dietary protein level, sucrase and alkaline phosphatase activities did not change. However, we did not observe any change in the level of ileal aminopeptidase N mRNA in all groups, and there was no significant correlation between the specific activity and the mRNA abundance. These data indicate that the catalytic activity of intestinal aminopeptidase responds to the dietary protein level, but the level of mRNA is preserved even under the protein malnourished condition. The data also suggest that translational and/or posttranslational regulations for the expression of intestinal aminopeptidase are necessary for the rapid response to changes in intraluminal nutrients. (J. Nutr. Biochem. 5:291–297, 1994.)

Keywords: aminopeptidase; mRNA; small intestine; dietary protein level; rat

Introduction

Aminopeptidase, which liberates amino acids from the N-terminus of oligopeptides,[1] is located in the brush border membrane of enterocytes and plays an important role in the final stages of protein digestion.[2,3] The catalytic activity of this enzyme increases with feeding a high-protein diet,[4–6] and the increase corresponds with the increase in the amount of aminopeptidase molecules.[6] Its synthesis is regulated by intraluminal peptide nutrients.[7] In this adaptive response of aminopeptidase, however, the regulatory mechanisms in the level of gene expression have not been well documented.

In this report, we measured the activity and mRNA abundance of intestinal aminopeptidase in rats fed diets containing different levels of protein. Thus, the purpose of our study was to determine whether the adaptive response of intestinal aminopeptidase to dietary protein level is controlled at the pretranslational level.

Address reprint requests to Dr. Kei Sonoyama at the Laboratory of Food Biochemistry, Faculty of Agriculture, Hokkaido University, Kita-9, Nishi-9, Kita-ku, Sapporo-shi, 060, Japan.
Received August 5, 1993; accepted January 10, 1994.

Methods and materials

Materials

Tris, mannitol, sucrose, *p*-nitrophenyl phosphate, Glucose-B-Test Wako, phenol, chloroform, and formaldehyde were purchased from Wako Pure Chemical Industries (Osaka, Japan); leucine-*p*-nitroanilide from Sigma Chemical Co. (St Louis, MO USA); *p*-hydroxymercuri benzoic acid (PHMB) from Aldrich Chemical Co. (Milwaukee, WI USA); guanidium thiocyanate from Fluka (Buchs, Switzerland); GeneAmp RNA PCR kit from Perkin Elmer Cetus (Norwalk, CT USA); agarose (SeaKem ME) from Takara (Kyoto, Japan); Silver Sequence DNA Sequencing Reagents and Staining

© 1994 Butterworth–Heinemann

Reagents from Promega (Madison, WI USA); DIG-DNA labeling kit and DIG-luminescent detection kit from Boehringer Mannheim (Mannheim, Germany); Hybond N+ from Amersham International plc. (Amersham, UK).

Animals and diets

Male Wistar rats (Japan SLC Inc., Hamamatsu, Japan), 5 weeks old at the start of the experiment, were housed in individual cages in a temperature-controlled (23 ± 2° C) room under a 12-hr light/dark cycle (light: 8:00–20:00). All rats were fed a 20% casein diet *(Table 1)* for 3 days prior to two experiments. In experiment 1, rats weighing 113 ± 2 g (n = 12) were divided into two groups, one of which was fed a 20% casein diet and the other a 60% casein diet *(Table 1)* ad libitum for 7 days. In experiment 2, rats weighing 114 ± 2 g (n = 30) were divided into five groups, and each group was fed a 0, 10, 20, 40, or 60% casein diet *(Table 1)* ad libitum for 7 days. On the last day of each experimental period, rats were decapitated without prior fasting, the small intestine was immediately removed, and the luminal contents were washed out with 20 mL of ice-cold saline. In experiment 1, the intestine was suspended vertically and two 10-cm portions of the intestine were excised, one at 10 cm distal from the ligament of Treitz (jejunal segment) and the other 30 cm proximal from the ileocecal valve (ileal segment), and stored at −45° C for enzymatic analyses. For RNA isolation, two other 10-cm portions were excised just distal from jejunal and ileal segments used for enzymatic analyses, and the mucosa was scraped off with slide glass and stored at −80° C until RNA extraction. In experiment 2, only the ileal segment used for enzymatic analyses and RNA extraction was excised.

The study was approved by the Hokkaido University Animal Use Committee, and animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Hokkaido University.

Enzymatic analyses

The mucosa was squeezed from the intestinal segments after thawing and gently homogenized in ice-cold 50 mM mannitol/2 mM Tris-HCl buffer pH 7.1 with a Potter-Elvehjem homogenizer. With these mucosal homogenates, activity of three enzymes and contents of protein and DNA were determined. Aminopeptidase activity was measured using leucine-*p*-nitroanilide as a substrate[12] in the presence of 0.5 mM PHMB, a known inhibitor of the cytosolic peptidases.[4] Sucrase activity was measured using sucrose as a substrate,[13] and the amount of glucose released was measured by Glucose-B-Test (Wako) (kit for glucose oxidase method). Alkaline phosphatase activity was measured using *p*-nitrophenyl phosphate as a substrate.[14] The contents of protein and DNA were determined by the methods of Lowry et al.[15] and Brunk et al.,[16] respectively.

Table 1 Composition of diets[a]

Dietary casein level (%) Dietary components	0	10	20	40	60
			g/100g		
Casein[b]	0.0	10.0	20.0	40.0	60.0
Sucrose	89.7	79.7	69.7	49.7	29.7
Corn oil[c]	5.0	5.0	5.0	5.0	5.0
Mineral mixture[d]	4.0	4.0	4.0	4.0	4.0
Vitamin mixture[e]	1.0	1.0	1.0	1.0	1.0
Vitamin E[f]	0.1	0.1	0.1	0.1	0.1
Choline chloride	0.2	0.2	0.2	0.2	0.2

[a]Rats were fed the 20 and 60% casein diets in experiment 1; and 0, 10, 20, 40, and 60% casein diets in experiment 2.
[b]Casein (ALACID; New Zealand Dairy Board, Wellington, New Zealand).
[c]Retinyl palmitate (7.66 μmol/kg diet) and ergocarciferol (0.0504 μmol/kg diet) were added to the corn oil.
[d]The mineral mixture is identical to MM2 described by Ebihara et al.[8]
[e]The vitamin mixture was prepared in accordance with the AIN-76 mixture,[9] except vitamin K as menadione and L-ascorbatic acid were added to give 5.81 μmol/kg[10] and 284 μmol/kg[11] of diet, respectively.
[f]Vitamin E (Granulated, Juvela, Eisai Co., Tokyo, Japan) supplied 423 μmol all-rac-α-tocopheryl acetate in kg diet.

PCR amplification of rat intestinal aminopeptidase N cDNA and sequencing

According to the method of Cohen et al.,[17] an upstream primer (5′-CTGAATGATGTGTACCGTGTG-3′) and a downstream primer (5′-GCAGTAGACAGTAGACCGAAG-3′) were synthesized with 380B DNA Synthesizer (Applied Biosystems Japan, Tokyo, Japan). Either ileal total RNA or kidney total RNA were used for reverse transcription-polymerase chain reaction (RT-PCR) as the template. Those RNA were prepared from ileal mucosa and kidney of adult male Sprague-Dawley rat (Japan SLC Inc., Hamamatsu, Japan) as described below. PCR amplification was carried out using GeneAmp RNA PCR kit as described by Cohen et al.[17] Ten percent of the individual PCR products were then electrophoresed on a 1% agarose gel[18] and visualized by ethidium bromide staining.

Partial sequencing of the PCR products from rat ileal RNA by dideoxy chain termination reactions was carried out using Silver Sequence DNA Sequencing Reagents. Incubation was carried out at 95° C for 2 min for denaturation followed by 60 cycles of PCR consisting of denaturation at 95° C for 30 sec, and annealing/extension at 70° C for 30 sec. Samples were then electrophoresed on an 8% polyacrylamide gel using a 20 × 45 cm Takara DNA sequencing electrophoresis unit, and detection was carried out using Silver Sequence DNA Staining Reagents according to the manufacturer's instructions.

RNA isolation and Northern blot analysis

Total RNA was isolated by the acid guanidium-phenol-chloroform method[19] from intestinal mucosa. Samples of total RNA (15 μg/lane in experiment 1 and 10 μg/lane in experiment 2) were electrophoresed on denaturing 2.2 M formaldehyde, 1% agarose gel[18] and transferred to nylon membrane (Hybond N+). Blots were hybridized with the 1.1 kb aminopeptidase N cDNA prepared by the PCR amplification as described above. Labeling of the probe was carried out using a nonradioisotopic system (DIG-DNA labeling kit), and prehybridization, hybridization, and detection were carried out with DIG-luminescent detection kit as recommended by the manufacturer. After the detection, each filter was then sequentially rehybridized with the rat β-actin cDNA[20] and the detection was carried out similarly. The relative abundance of mRNA was estimated by densitometry (Dual-Wavelength Flying-Spot Scanner CS-9000, Shimadzu) with the x-ray film.

Statistical analysis

Results were expressed as means ± SE and the statistical comparisons of the mean were done by Student's t test in experiment 1 and by Duncan's multiple range test in experiment 2.

Results

In experiment 1, the total food intake of rats fed 20% casein diet was similar to that of rats fed 60% casein diet, and there was also no significant difference in the final body weight between the two dietary groups *(Table 2)*. In experiment 2,

the total food intake of rats fed the 0% casein diet was significantly lower than that of rats fed the other four diets, and the intake of rats fed the 10% casein diet was also significantly lower than that of rats fed the 20, 40, and 60% casein diets. There was no significant difference in the total food intake between the 20, 40, and 60% casein diet-fed groups. The body weight of rats the fed 0% casein diet decreased for the experimental period, and that of rats fed the 10% casein diet maintained the initial level. Rats fed 20, 40, and 60% casein diets gained body weight well *(Table 2)*. Thus, rats fed 0% and 10% casein diets were protein malnourished.

As shown in *Table 3*, there was also no diet-dependent difference in the contents of protein and DNA and the protein to DNA ratio in jejunal and ileal mucosa in experiment 1. Reflecting the body weight gain in experiment 2, however, the mucosal protein content in ileal segment of rats fed 0% and 10% casein diets was significantly lower than that of rats fed 20, 40, and 60% casein diets, and total DNA content in the ileal mucosa of rats fed 0 and 10% casein diets was significantly lower than that of rats fed 40 and 60% casein diets *(Table 4)*. The ratio of protein content to DNA content tended to inversely decrease as the protein level in the diet was increased. There was no difference in those parameters in the ileal mucosa among the 20, 40, and 60% casein diet-fed groups.

Table 5 shows the specific activities of aminopeptidase, sucrase, and alkaline phosphatase in the homogenate of intestinal mucosa in experiment 1. We observed the adaptive increase in aminopeptidase activity both in jejunal and ileal segments of rats fed the 60% casein diet. However, the response in jejunal segment (19% higher than the 20% casein diet, $P < 0.05$) was smaller than that in ileal segment (41% higher than 20% casein, $P < 0.01$). In contrast, the sucrase activity in the ileal segment of rats fed the 60% casein diet was significantly lower than that of rats fed the 20% casein diet. The activities of sucrase in the jejunal segment and alkaline phosphatase in the jejunal and ileal segment were not significantly different between the two groups. In experiment 2, we observed the adaptive increase in aminopeptidase

Table 4 Effect of dietary protein level on protein content, DNA content, and protein/DNA ratio in homogenate of ileal mucosa of rats in experiment 2

Dietary casein level	Protein	DNA	Protein/DNA
(%)	(mg/10 cm segment)	(mg/10 cm segment)	
0	25.1 ± 1.9[a]	0.55 ± 0.07[a]	47.1 ± 3.1[a]
10	28.0 ± 2.2[a]	0.68 ± 0.10[ab]	43.8 ± 3.7[ab]
20	32.8 ± 1.1[b]	0.80 ± 0.05[bc]	41.7 ± 2.1[ab]
40	33.0 ± 0.8[b]	0.88 ± 0.04[c]	37.7 ± 1.6[b]
60	33.4 ± 1.2[b]	0.90 ± 0.05[c]	37.3 ± 0.9[b]

Within a column, values with different superscripts are significantly different among the dietary groups ($P < 0.05$). Values are means ± SE, $n = 6$ per group.

Table 2 Effect of dietary protein level on food intake and body weight of rats fed each test diet for the experimental period (7 days)

Dietary casein level	Experiment 1 Food intake	Experiment 1 Body weight Initial	Experiment 1 Body weight Final	Experiment 2 Food intake	Experiment 2 Body weight Initial	Experiment 2 Body weight Final
(%)	(g/7 days)	(g)		(g/7 days)	(g)	
0	—	—	—	44 ± 5[a]	114 ± 2	94 ± 1[a]
10	—	—	—	63 ± 3[b]	114 ± 2	117 ± 3[b]
20	100 ± 3	113 ± 2	161 ± 3	93 ± 3[c]	114 ± 3	159 ± 4[c]
40	—	—	—	96 ± 3[c]	114 ± 2	167 ± 3[d]
60	94 ± 3	113 ± 2	164 ± 3	89 ± 2[c]	114 ± 2	160 ± 3[c]

Within a column, values with different superscripts are significantly different among the dietary groups ($P < 0.05$). Values are means ± SE, $n = 6$ per group.

Table 3 Effect of dietary protein level on protein content, DNA content, and protein/DNA ratio in homogenate of intestinal mucosa of rats in experiment 1

Tissue	Dietary casein level	Protein	DNA	Protein/DNA
	(%)	(mg/10 cm segment)	(mg/10 cm segment)	
Jejunum	20	34.2 ± 1.5	0.93 ± 0.07	37.0 ± 1.3
	60	34.5 ± 1.1	0.93 ± 0.03	37.1 ± 1.0
Ileum	20	30.9 ± 0.7	0.95 ± 0.06	33.0 ± 1.6
	60	29.4 ± 1.0	0.95 ± 0.06	31.4 ± 1.6

Values are means ± SE, $n = 6$ per group.

Table 5 Effect of dietary protein level on specific activities of aminopeptidase, sucrase, and alkaline phosphatase in homogenate of intestinal mucosa of rats in experiment 1

Tissue	Dietary casein level	Aminopeptidase	Sucrase	Alkaline phosphatase
	(%)	(mU/mg protein)		
Jejunum	20	44.0 ± 2.3	68.5 ± 1.6	4642 ± 527
	60	52.4 ± 3.2*	62.4 ± 4.2	3829 ± 375
Ileum	20	51.2 ± 1.5	24.3 ± 1.6	517 ± 137
	60	75.1 ± 1.4**	15.7 ± 1.0**	367 ± 30

Values are means ± SE, $n = 6$ per group. *$P < 0.05$, **$P < 0.01$ as compared with 20% casein diet-fed group in each segment.

activity in response to the level of dietary protein (*Table 6*). Compared with the 0% casein diet-fed group, the specific activity of aminopeptidase in ileal mucosa of rats fed 10, 20, 40, and 60% casein diets was 33, 58, 100, and 138% higher, respectively, and there were statistically significant differences among all groups. In contrast, the activities of sucrase and alkaline phosphatase were not significantly different among all groups.

Figure 1 shows the results of amplification of rat ileal and kidney total RNA using oligonucleotide primers designed by Cohen et al.[17] The products were of the expected 1.1 kb lengths based on the distance between the primers. Sequencing of the first 113 bp at the 5′ end and 183 bp at the 3′ end of the PCR product from rat ileal total RNA revealed it to have 100% identity with kidney aminopeptidase N.[21] These results suggest that the PCR product from ileal total RNA represents the aminopeptidase N cDNA.

Figure 2 shows the representative Northern blot of intestinal RNA. A single transcript hybridized with 1.1 kb aminopeptidase N cDNA probe was detected in experiments 1 and 2. The relative abundance of aminopeptidase and β-actin

Table 6 Effect of dietary protein level on specific activities of aminopeptidase, sucrase, and alkaline phosphatase in homogenate of ileal mucosa of rats in experiment 2

Dietary casein level	Aminopeptidase	Sucrase	Alkaline phosphatase
(%)		(mU/mg protein)	
0	29.4 ± 2.5[a]	20.1 ± 2.0	213 ± 55
10	39.1 ± 2.9[b]	17.8 ± 1.4	345 ± 68
20	46.4 ± 1.0[c]	18.9 ± 1.5	394 ± 62
40	58.9 ± 1.6[d]	19.4 ± 2.5	384 ± 65
60	70.0 ± 3.1[e]	20.2 ± 3.3	382 ± 100

Within a column, values with different superscripts are significantly different among the dietary groups ($P < 0.05$). Values are means ± SE, $n = 6$ per group.

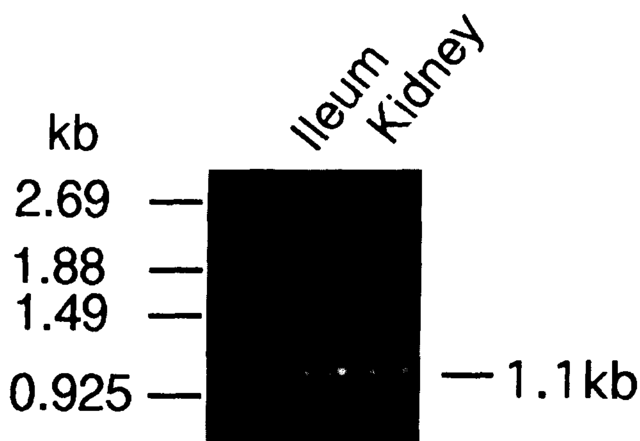
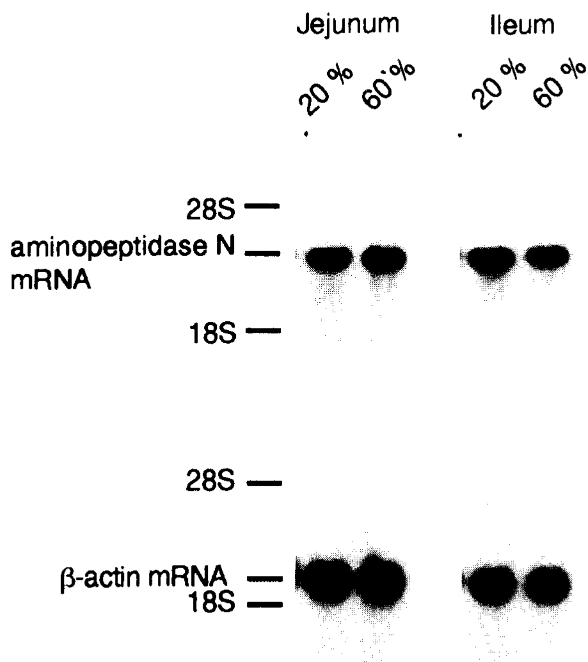


Figure 1 Agarose gel electophoresis of PCR products. Ileal and kidney total RNA from adult male Sprague-Dawley rats were used for PCR amplification as the template. Ten percent of each reaction product was then electrophoresed on a 1% agarose gel and visualized by ethidium bromide staining.



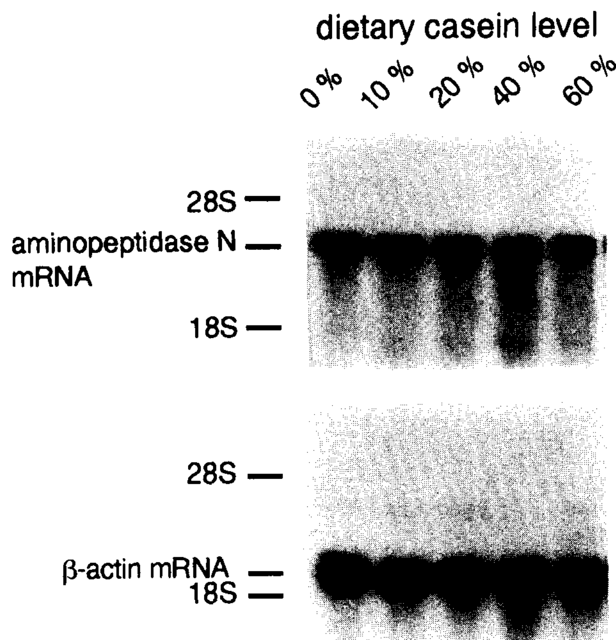


Figure 2 Representative Northern blots of intestinal RNA. Upper: experiment 1, lower: experiment 2. Total RNA (15 and 10 μg/lane in experiment 1 and experiment 2, respectively) isolated from intestinal mucosa of rats was electrophoresed on a denaturing 2.2 M formaldehyde, 1% agarose gel,[18] transferred to a nylon membrane (Hybond N+), and then probed with 1.1 kb aminopeptidase N cDNA[19] labeled with DIG-DNA labeling kit (Boerhinger Mannheim). Prehybridization, hybridization, and detection were performed with DIG-luminescent detection kit (Boerhiger Mannheim). The blot was sequentially rehybridized with the rat β-actin cDNA.[20] The positions of the 18S and 28S ribosomal RNA are shown.

mRNA estimated by densitometry with x-ray films and the ratio of aminopeptidase mRNA to β-actin mRNA in experiment 1 are shown in *Table 7.* We could not observe diet-dependent differences in the levels of aminopeptidase mRNA, β-actin mRNA, nor in the ratios of aminopeptidase mRNA to β-actin mRNA. Similarly, differences in the level of aminopeptidase mRNA and in the level of β-actin mRNA could not be detected among the groups in experiment 2 *(Table 8).* Relationship of mRNA abundance with specific activity of aminopeptidase in the ileal mucosa from all rats studied in experiment 2 is shown in *Figure 3.* We could not observe significant correlation between the two parameters ($r = 0.137$, $P > 0.1$). Thus, change in the specific activity of intestinal aminopeptidase was not accompanied by detectable change in the abundance of aminopeptidase mRNA.

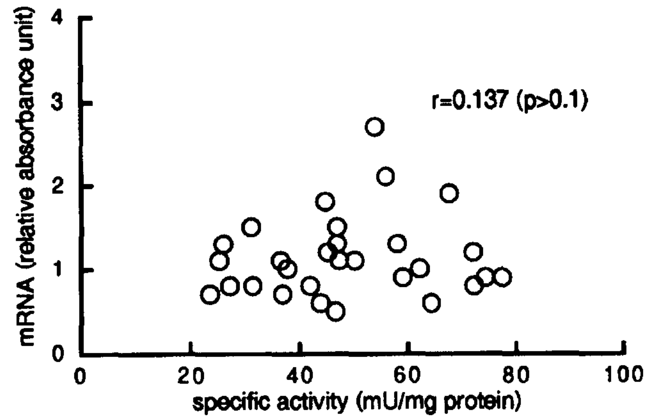


Figure 3 The relationship of the specific activity and the relative mRNA abundance of aminopeptidase in ileal mucosa of all rats studied in experiment 2. Northern blots were quantified by densitometry with x-ray film, and the relative abundance of aminopeptidase N mRNA was normalized by each value of β-actin mRNA and expressed relative to the mean value of 0% casein diet group.

Discussion

Although it has been documented that high-protein diets lead to increased activity of aminopeptidase in the intestinal mucosa compared with low-protein diets,[4–6] it is important to include adequate amounts of protein in the control diet to maintain growth when designing the studies of dietary adaptation.[5] In experiment 1, we chose a 20% casein diet as a normoprotein diet and a 60% casein diet as a high-protein diet. There were no differences in the food intake and the body weight gain between the two groups *(Table 2).* The contents of protein and DNA, and the protein to DNA ratio in the intestinal mucosa were also not influenced by the test diets *(Table 3).* Thus, similar nutritional effects on the intestine were obtained in the 20 and 60% casein diet groups. So in this condition, it seems reasonable to observe the effect of dietary protein levels on the expression of intestinal hydrolases.

Of the three hydrolases measured in experiment 1, only aminopeptidase increased in response to the 60% casein diet *(Table 5).* Raul et al.[6] reported that an increase in aminopeptidase activity due to feeding a high-protein diet for 15 hr paralleled the amount of corresponding aminopeptidase protein and suggested that the increase in the number of aminopeptidase protein resulted from either an increased rate of synthesis or a decreased rate of degradation of enzyme molecules. Although we did not determine the amount of enzyme protein, higher specific activity of aminopeptidase in the 60% casein diet group probably resulted from the increased amount of enzyme protein.

The relative abundance of aminopeptidase N mRNA and β-actin mRNA in the intestinal mucosa of rats was determined by Northern blot analysis. β-actin mRNA was used as the control, and the relative abundance was not changed between the two groups. It may be reasonable to use β-actin mRNA as the control for animals of the same age for short-term experiments. Results could not show the significant difference in the relative abundance of aminopeptidase mRNA between the two dietary groups *(Figure 2 and Table 7).* However, the mean level values of aminopeptidase mRNA tended to be higher in 60% casein diet groups in both the jejunum and ileum. Because the increase in the aminopeptidase activity was small (19% and 41% in the jejunum and ileum, respectively), we doubted whether such a small change could be detected in mRNA level. So we determined again the aminopeptidase activity and mRNA level in the intestine of rats fed diets containing different levels of protein in experiment 2. We also observed the

Table 7 Effect of dietary protein level on relative abundance of mRNA of aminopeptidase N and β-actin in intestinal mucosa of rats in experiment 1

Tissue	Dietary casein level	Aminopeptidase N	β-actin	Aminopeptidase N/β-actin
	(%)			
Jejunum	20	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
	60	1.1 ± 0.2	1.0 ± 0.2	1.1 ± 0.2
Ileum	20	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
	60	1.2 ± 0.3	1.1 ± 0.2	1.2 ± 0.3

Values are means ± SE, $n = 6$ per group. Northern blots were quantified by densitometry with x-ray film, and the relative abundance of each mRNA was expressed relative to the mean value of 20% casein diet-fed group, which was arbitrarily normalized to a mean value of 1.0.

Table 8 Effect of dietary protein level on relative abundance of mRNA of aminopeptidase N and β-actin in ileal mucosa of rats in experiment 2

Dietary casein level	Aminopeptidase N	β-actin	Aminopeptidase N/β-actin
(%)			
0	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
10	0.7 ± 0.1	0.8 ± 0.1	1.0 ± 0.2
20	0.8 ± 0.1	0.7 ± 0.1	1.2 ± 0.2
40	0.8 ± 0.1	0.7 ± 0.1	1.2 ± 0.3
60	1.0 ± 0.2	0.8 ± 0.2	1.1 ± 0.2

Values are means ± SE, $n = 6$ per group. Northern blots were quantified by densitometry with x-ray film, and the relative abundance of each mRNA was expressed relative to the mean value of 0% casein diet-fed group, which was arbitrarily normalized to a mean value of 1.0.

site-dependent difference in the response of aminopeptidase activity to the level of dietary protein in experiment 1 *(Table 5)*. The increase in aminopeptidase activity in rats fed the 60% casein diet was larger in the ileal segment than in the jejunal segment. McCarthy et al.[5] reported the same phenomenon and mentioned that the proximal intestine might normally function at a level close to the lower limit of its capacity to differentiate, and the prominent distal regulation of aminopeptidase suggested the ileum as an important site of protein digestion and nitrogen assimilation. So in experiment 2, we measured the activity and mRNA level of aminopeptidase in the ileal segment.

In experiment 2, we observed that aminopeptidase activity in the ileal mucosa increased depending on the dietary protein level *(Table 6)*. Two other hydrolases, sucrase and alkaline phosphatase, were not modified by the dietary protein level. Sucrase activity was constant among all dietary groups, even in the 0% casein diet-fed group in which animal's growth was repressed. Goda and Koldovsky[22] and Goda et al.[23] reported that the sucrase-active site of sucrase-isomaltase is degraded by the action of pancreatic proteases,[22] and that the intake of a high-protein diet leads to an acceleration of degradation of sucrase-isomaltase concomitantly with the increase of pancreatic proteases in the lumen.[23] In this experiment, preserved activity of sucrase in the ileum of rats fed 0% and 10% casein diets regardless of depressed growth might result from decreased secretion of pancreatic proteases in low-protein diets. A higher ratio of protein content to DNA content in the ileal mucosa of rats fed 0% and 10% casein diets *(Table 4)* suggests that the decrease in exocrine pancreatic secretion by feeding such low-protein diets leads to a decrease in turnover rate of mucosal protein. Northern blot analysis in experiment 2 could not show a significant difference in the abundance of aminopeptidase mRNA in the ileal mucosa among all dietary groups *(Figure 2 and Table 8)*. When we compared the data on the 60% casein diet-fed group with those on 0% casein diet-fed group, the relative abundance of aminopeptidase mRNA was not changed regardless of the 138% increase in the specific activity. Also, we observed no significant correlation between the aminopeptidase activity and mRNA level in the ileum of all rats studied in experiment 2 *(Figure 3)*. Thus, the level of aminopeptidase mRNA did not respond to the dietary protein level regardless of the increase of catalytic activity of this hydrolase.

Reisenauer and Gray[7] showed an abrupt induction of aminopeptidase synthesis in jejunum after a 30-min intraluminal perfusion of a tetrapeptide. Thus, it is possible that the adaptive increase in aminopeptidase activity in response to the dietary protein level may be the result of induction of aminopeptidase synthesis by the intraluminal peptide nutrient as the substrate of this hydrolase. In this phenomenon, however, the regulatory mechanism at the level of gene expression is not well known. Cohen et al.[17] reported in a study about the gene expression of intestinal aminopeptidase along the growing stages that the initial rise in the activity during the maturational period was regulated pretranslationally, whereas mechanisms operating at the translational and/or posttranslational levels appeared to be important after weaning. From this point of view, the adaptive response to the level of dietary protein in the expression of intestinal aminopeptidase of young adult rats we used may be regulated at the translational and/or posttranslational levels. Our observation that the abundance of aminopeptidase N mRNA in the intestinal mucosa of rats did not correspond to the change of aminopeptidase activity supports that speculation.

Because rats fed 0 and 10% casein diets seemed to be protein-malnourished *(Table 2 and Table 4)*, the lower activity of aminopeptidase in the ileal segment of rats fed the two diets might result from such a malnourished condition rather than the adaptive response to the dietary protein level. So in this condition, it may be unreasonable to discuss the adaptive response of intestinal aminopeptidase to the dietary protein level. However, preserved level of aminopeptidase mRNA in the ileum of rats even in the protein-malnourished condition suggests that the translational and/or posttranslational regulations for expression of ileal aminopeptidase are necessary for the rapid response to changes in the intraluminal peptide nutrient. Although we adopted 7-day feeding periods, the response of aminopeptidase activity to the dietary protein level was observed even under the more acute conditions in which rats were fed 60% casein diet only for 16 hr after a 32 hr-fast (unpublished data).

However, it remains unknown how the intracellular synthetic machinery of posttranscription is facilitated by the extracellular peptides. Therefore, further studies about the signal transduction caused by the intraluminal factors via plasma membrane of enterocytes are required.

Acknowledgments

Our special thanks to Dr. Milton M. Takeuchi for reading the manuscript and making a number of helpful discussions.

References

1. Kania, R.K., Santiago, N.A., and Gray, G.M. (1977). Intestinal surface amino-oligopeptidases. II. Substrate kinetics and topography of the active site. *J. Biol. Chem.* **252,** 4929–4934
2. Smithson, K.W. and Gray, G.M. (1977). Intestinal assimilation of a tetrapeptide in the rat. Obligate function of brush border aminopeptidase. *J. Clin. Invest.* **60,** 665–674
3. Ugolev, A.M. and Laey, P.D. (1973). Membrane Digestion. A concept of enzymic hydrolysis on cell membranes. *Biochim. Biophys. Acta* **300,** 105–128
4. Nicholson, J.A., McCarthy, D.M., and Kim, Y.S. (1974). The response of rat intestinal brush border and cytosol peptide hydrolase activities to variation in dietary protein content. Dietary regulation of intestinal peptide hydrolases. *J. Clin. Invest.* **54,** 890–898
5. McCarthy, D.M., Nicholson, J.A., and Kim, Y.S. (1980). Intestinal enzyme adaptation to normal diets of different composition. *Am. J. Physiol.* **239,** G445–G451
6. Raul, F., Goda, T., Gosse, F., and Koldovsky, O. (1987). Short-term effect of a high-protein/low-carbohydrate diet on aminopeptidase in adult rat jejunoileum. *Biochem. J.* **247,** 401–405
7. Reisenauer, A.M. and Gray, G.M. (1985). Abrupt induction of a membrane digestive enzyme by its intraintestinal substrate. *Science* **227,** 70–72
8. Ebihara, K., Imamura, Y., and Kiriyama, S. (1979). Effect of dietary mineral composition on nutritional equivalency of amino acid mixture and casein in rats. *J. Nutr.* **109,** 2106–2116
9. American Institute of Nutrition. (1977). Report of the American Institute of Nutrition ad hoc Committee on Standards for Nutritional Studies. *J. Nutr.* **107,** 1340–1348
10. American Institute of Nutrition. (1980). Second report of the ad hoc Committee on Standards for Nutritional Studies. *J. Nutr.* **110,** 1726

11. Harper, A.E. (1959). Amino acid balance and imbalance. 1. Dietary level of protein and amino acid imbalance. *J. Nutr.* **68,** 405–418
12. Appel, W. (1974). Aminopeptidase and Amino Acid Arylamidase. In *Methods of Enzymatic Analysis, Second English Edition,* (H.U. Bergmeyer, ed.), p. 950–978, Verlag Chemie, Weinheim/Academic Press, New York, NY USA
13. Dahlqvist, A. (1974). Disaccharidases. In *Methods of Enzymatic Analysis, Second English Edition,* (H.U. Bergmeyer, ed.), p. 916–922, Verlag Chemie, Weinheim/Academic Press, New York, NY USA
14. Walter, K. and Schutt, C. (1974). Alkaline Phosphatase in Serum (Continuous Assay). In *Methods of Enzymatic Analysis, Second English Edition,* (H.U. Bergmeyer, ed.), p. 860–864, Verlag Chemie, Weinheim/Academic Press, New York, NY USA
15. Lowry, O.H., Rosebrough, H.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with the Folin-phenol reagent. *J. Biol. Chem.* **193,** 265–275
16. Brunk, C.F., Jones, K.C., and James, T.W. (1979) Assay for nanogram quantities of DNA in cellular homogenates. *Anal. Biochem.* **92,** 497–500
17. Cohen, M.L., Santiago, N.A., Zhu, J.-S., and Gray, G.M. (1991). Differential regulation of intestinal amino-oligopeptidase gene expression in neonatal and adult rats. *Am. J. Physiol.* **261,** G866–G871
18. Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). *Molecular Cloning, A Laboratory Manual,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY USA
19. Chomczynski, P. and Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162,** 156–159
20. Nudel, U., Zakut, R., Shani, M., Neuman, S., Levi, Z., and Yaffe, D. (1983). The nucleotide sequence of the rat cytoplasmic β-actin gene. *Nucleic Acids Res.* **11,** 1759–1771
21. Watt, V.M. and Yip, C.C. (1989). Amino acid sequence deduced from a rat kidney cDNA suggests it encodes the Zn-peptidase aminopeptidase N. *J. Biol. Chem.* **264,** 5480–5487
22. Goda, T. and Koldovsky, O. (1985). Evidence of degradation process of sucrase-isomaltase in jejunum of adult rats. *Biochem. J.* **229,** 751–758
23. Goda, T., Raul, F., Gosse, F., and Koldovsky, O. (1988). Effects of a high-protein, low-carbohydrate diet on degradation of sucrase-isomaltase in rat jejunoileum. *Am. J. Physiol.* **254,** G907–G912