Cholestyramine and Bile Diversion Lower the Aminopeptidase Activity in the Intestinal Brush Border Membrane of Rats

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Summary To examine the effect of bile acids on the activity of intestinal aminopeptidase in vivo, we measured the activity of aminopeptidase in the intestinal mucosa from rats fed the diet containing cholestyramine which sequesters luminal bile acids (experiment 1) and from bile diverted rats (experiment 2). After 32 h fasting, rats were refed for 16 h either of a standard diet (25% casein diets), the same diet containing cholestyramine, or the fat-free diet in experiment 1. In the intestinal washing, the content of total bile acids was markedly decreased with feeding cholestyramine and activities of trypsin and chymotrypsin were also lowered with cholestyramine. Cholestyramine feeding decreased the specific activity of aminopeptidase in the homogenate of intestinal mucosa but increased the specific activities of sucrase and alkaline phosphatase. All these parameters were not modified by the fat-free diet. In experiment 2, bile diverted and sham operated rats were refed the standard diet for 16 h with prior 32 h fasting. Bile diversion, like cholestyramine feeding, lowered the content of total bile acids, the activities of pancreatic hydrolases in the intestinal washings, and the specific activity of aminopeptidase in the intestinal mucosa. The specific activity of sucrase in the intestinal mucosa was higher in bile diverted rats but the activity of alkaline phosphatase was not changed. These data indicate that the decreased abundance of intraluminal bile acid affects the activity of intestinal aminopeptidase not through the decreased absorption of dietary lipid. We propose that the intraluminal bile acids may be important for maintaining the activity of aminopeptidase while the degradation of sucrase by the pancreatic proteinases may be accelerated by the bile acids.

Key Words rat, cholestyramine, bile diversion, aminopeptidase, bile acids, enzyme regulation, sucrase, alkaline phosphatase

Aminopeptidase, which liberates amino acids from 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). Because the aminopeptidase is embedded within the brush border membrane and most of its domains including the active site are exterior to the cell surface, it is possible that the activity is influenced by some luminal factors, such as pancreatic proteases and bile acids. Other enzymes in the intestinal brush border membrane are known to be influenced by the luminal factors (4-11). For instance, sucrase-active site of sucrase-isomaltase is degraded by the action of pancreatic proteases (7,8), and the maintenance of enterokinase is modified by pancreatico-biliary secretion (9-11).

In this study, to evaluate the possible role of bile acids as an intraluminal effector on the activity of intestinal aminopeptidase in vivo, we measured the aminopeptidase activity of the intestine from rats fed the diet containing cholestyramine which sequesters intraluminal bile acids and from bile diverted rats.

**Materials and methods.**

**Materials:** Triton X-100, Tris, mannitol, sucrose, p-nitrophenyl phosphate, Total Bile Acid Test, Glucose-B-Test were purchased from Wako Pure Chemical Industries (Osaka, Japan); cholestyramine resin, N-α-p-toluuenesulfonfyl-L-arginine methyl ester (TAME), N-benzoyl-L-tyrosine ethyl ester (BTEE), leucine-p-nitroanilide from Sigma Chemical Co. (St Louis, MO); p-hydroxymercuri benzoic acid (PHMB) from Aldrich Chemical Company (Milwaukee, WI).

**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 12-h light/dark cycle (light: 8:00-20:00h). They were fed a standard diet (25% casein diet, Table 1) ad libitum and allowed free access to water prior to the experiment.

In experiment 1, after a 6-days-feeding of a standard diet, rats weighing 133±

| Table 1. Composition of standard diet. |
|----------------------------------------|
| Dietary component | g/kg diet |
|-------------------|-----------|
| Casein1           | 250       |
| Sucrose           | 647       |
| Corn oil2         | 50        |
| Mineral mixture3  | 40        |
| Vitamin mixture4  | 10        |
| Vitamin E5        | 1         |
| Choline chloride  | 2         |

1 Casein (ALACID; New Zealand Dairy Board, Wellington, New Zealand). 2 Retinyl palmitate (7.66 μmol/kg diet) and ergocalciferol (0.0504 μmol/kg diet) were added to corn oil. 3 The mineral mixture is identical with MM2 described by Ebihara et al. (23). 4 The vitamin mixture was prepared in accordance with the AIN-76 mixture (24), except that vitamin K as menadione and L-ascorbic acid were added to give 5.81 μmol/kg (25) and 284 μmol/kg (26) of diet, respectively. 5 Vitamin E (granulated, Juvela, Eisai Co., Tokyo, Japan) supplied 423 μmol all-rac-α-tocopheryl acetate in kig diet.

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2 g (n = 18) were divided into three groups, and starved for 32 h and then each group was fed a standard diet, the same diet containing cholestyramine (60 g/kg, Sigma Chemical Co.), or the diet which all fat was replaced with sucrose. After 16 h feeding, the animals were decapitated and the small intestine was excised and the luminal contents were washed out with 20 ml of ice-cold saline. The washings were collected quantitatively in plastic containers, freeze-dried, and stored at −45°C until analyses of bile acids content and pancreatic hydrolases. The intestine was suspended vertically and a 10 cm portion which was 30 cm proximal from the ileocecal valve was excised, and stored at −45°C for enzymatic analysis.

In experiment 2, after a 5-days-feeding of a standard diet, rats weighing 146 ± 2 g (n = 12) were starved for 24 h and then subjected to surgical operation under anesthesia by intraperitoneal injection of Nembutal (sodium pentobarbital 35 mg/kg body wt, Abbott Laboratories, North Chicago, IL). A tip of polyethylene catheter (SP 10; i.d. 0.28 mm, o.d. 0.61 mm; Natsume Seisakusyo, Tokyo, Japan) was inserted into the bile duct at a point just distal to the bifurcation of the common hepatic ducts, connected to silicone tubing (No. 00; i.d. 0.5 mm, o.d. 1.0 mm, Dow Corning Co., Kanagawa, Japan), and the distal end of cannula for returning the bile juice was placed through the fistula at a point 4-cm distal to the junction of cecum and colon. Sham surgery was performed as control group. Rats were not allowed food for the first 24 h postoperatively. Thereafter they were fed the standard diet for 5 days, and starved for 32 h and then each group was fed a standard diet. After 16 h feeding, the animals were decapitated and the tissue samples were obtained and stored as described in experiment 1.

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, Hokkaido University.

**Enzymatic analysis:** The freeze-dried intestinal washing was added to 25 ml of ice-cold saline containing 0.1% Triton X-100, suspended and filtered by aspiration. With this filtrate, the content of total bile acids was determined using Total Bile Acid Test, and amylase (12), trypsin (13), and chymotrypsin (14) activities were measured using procion yellow starch, TAME, and BTEE as substrates, respectively.

The mucosa was squeezed from the thawed intestinal segment and gently homogenized in ice-cold 50 mM mannitol/2 mM Tris-HCl buffer pH 7.1 with a Potter-Elvehjem homogenizer. With this mucosal homogenate, activities of three enzymes, contents of protein, and DNA were determined. Aminopeptidase activity was measured using leucine-p-nitroanilide as a substrate (15) in the presence of 0.5 mM PHMB, a known inhibitor of the cytosolic peptidases (16). Sucrase activity was measured using sucrose as a substrate (17) and the amount of glucose released was measured by Glucose-B-Test. Alkaline phosphatase activity was measured using p-nitrophenyl phosphate as a substrate (18). The contents of protein and DNA were determined by the methods of Lowry et al. (19) and Brunk et al. (20), respectively.

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Statistical analysis: Data were analyzed by one-way ANOVA and Fisher's least significant difference test was used to determine whether mean values were significantly different at \( p < 0.05 \) in experiment 1. And in experiment 2, Student's \( t \)-test was used to compare mean values at \( p < 0.05 \).

Results. As shown in Table 2, the body weights after 32 h fasting and after 16 h refeeding were not significantly different among the three diet-fed groups in experiment 1 but the body weights in bile diverted group were significantly lower than those in sham operated group in experiment 2. While the food intakes for 16 h refeeding period were also comparable among the groups in experiment 1, the intake in bile diverted group was lower than that in sham operated group in experiment 2.

The total bile acids content in the intestinal washing from rats fed the cholestyramine diet and from bile diverted rats were markedly lower than those from rats fed the standard and fat-free diets and from sham operated rats, respectively (Table 3). There was no significant difference in the bile acid contents between the standard and fat-free diet-fed groups in experiment 1. The activities of exocrine pancreatic hydrolases (amylase, trypsin, and chymotrypsin) in the intestinal washing are also shown in Table 3. In experiment 1, trypsin and chymotrypsin activities were significantly lower in cholestyramine-fed group than in the other two groups while amylase activity was not changed by the diets. The activities of all three hydrolases in bile diverted group were significantly lower than those in sham operated group in experiment 2.

Table 4 shows the wet weights of intestinal segment and mucosa, and the contents of protein and DNA in the homogenate of intestinal mucosa. There were no dietary-dependent differences in all parameters in experiment 1. In experiment

Table 2. Effects of diet (experiment 1) and bile diversion (experiment 2) on body weight and food intake.

| Treatments       | Body weight (g) | Food intake (g/16h) |
|------------------|-----------------|---------------------|
|                  | Fasting        | Refeeding          |                    |
| Experiment 1     |                 |                     |                    |
| Standard         | 117±2           | 132±2               | 13.3±0.7           |
| Cholestyramine   | 118±3           | 135±3               | 14.9±0.6           |
| Fat-free         | 117±2           | 133±2               | 14.4±0.5           |
| Experiment 2     |                 |                     |                    |
| Sham             | 156±2           | 175±2               | 16.0±0.7           |
| Bile diversion   | 143±3*          | 156±4*              | 12.4±0.7*          |

Values are M±SEM, \( n = 6 \) per group. All rats were refed test diets for 16 h with prior fasting for 32 h. In experiment 1, there was no significant difference in all parameters among three groups when tested by one-way ANOVA and Fisher's least significant difference test at \( p < 0.05 \). In experiment 2, asterisk shows the significant difference between sham and bile diversion when tested by Student's \( t \)-test at \( p < 0.05 \).
Table 3. Effects of diet (experiment 1) and bile diversion (experiment 2) on total bile acids content and activities of exocrine pancreatic hydrolases in intestinal washings.

| Treatments       | Total bile acids (μmol/total washing) | Amylase (U/total washing) | Trypsin (U/total washing) | Chymotrypsin (U/total washing) |
|------------------|---------------------------------------|---------------------------|---------------------------|-------------------------------|
| **Experiment 1** |                                       |                           |                           |                               |
| Standard         | 18.7 ± 1.6a                           | 619 ± 82                  | 218 ± 8a                  | 139 ± 10a                     |
| Cholestyramine   | 1.4 ± 0.3b                            | 583 ± 53                  | 141 ± 15b                 | 51 ± 4b                       |
| Fat-free         | 21.5 ± 1.7a                           | 694 ± 70                  | 206 ± 9a                  | 140 ± 7a                      |
| **Experiment 2** |                                       |                           |                           |                               |
| Sham             | 30.8 ± 1.5                            | 1,167 ± 88                | 216 ± 16                  | 121 ± 10                      |
| Bile diversion   | 1.0 ± 0.9*                            | 541 ± 58*                 | 139 ± 14*                 | 52 ± 12*                      |

Values are M±SEM, n=6 per group. In experiment 1, values in a column not sharing a common superscript letter are significantly different when tested by one-way ANOVA and Fisher's least significant difference test at p<0.05. In experiment 2, asterisk shows the significant difference between sham and bile diversion when tested by Student's t-test at p<0.05.

Table 4. Effects of diet (experiment 1) and bile diversion (experiment 2) on segmental weight, mucosal weight, and protein and DNA content in homogenate of intestinal mucosa.

| Treatments        | Segmental (mg/10 cm) | Mucosal (mg/10 cm) | Protein (mg/10 cm) | DNA (mg/10 cm) |
|-------------------|----------------------|-------------------|--------------------|---------------|
| **Experiment 1**  |                      |                   |                    |               |
| Standard          | 449 ± 19             | 229 ± 9           | 35.9 ± 0.8         | 0.81 ± 0.04   |
| Cholestyramine    | 434 ± 17             | 210 ± 16          | 35.8 ± 1.4         | 0.80 ± 0.04   |
| Fat-free          | 438 ± 19             | 231 ± 12          | 38.4 ± 1.2         | 0.87 ± 0.06   |
| **Experiment 2**  |                      |                   |                    |               |
| Sham              | 460 ± 11             | 233 ± 12          | 33.8 ± 1.2         | —             |
| Bile diversion    | 421 ± 9*             | 214 ± 18          | 31.4 ± 0.8         | —             |

Values are M±SEM, n=6 per group. In experiment 1, there was no significant difference in all parameters among three groups when tested by one-way ANOVA and Fisher's least significant difference test at p<0.05. In experiment 2, asterisk shows the significant difference between sham and bile diversion when tested by Student's t-test at p<0.05. DNA content in the mucosal homogenate was not measured in experiment 2.

2, however, the wet weight of intestinal segment of bile diverted rats was significantly lower than that of sham operated rats.

The specific activities of three hydrolases in the homogenate of intestinal mucosa are shown in Table 5. The aminopeptidase activity was significantly decreased by feeding cholestyramine but not by feeding fat-free diet. By contrast, the activities of sucrase and alkaline phosphatase were significantly increased by

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Table 5. Effects of diet (experiment 1) and bile diversion (experiment 2) on specific activities of aminopeptidase, sucrase, and alkaline phosphatase in homogenate of intestinal mucosa.

| Diet               | Aminopeptidase (mU/mg protein) | Sucrese (mU/mg protein) | Alkaline phosphatase (mU/mg protein) |
|--------------------|--------------------------------|-------------------------|-------------------------------------|
| **Experiment 1**   |                                |                         |                                     |
| Standard           | 36.7±1.9<sup>a</sup>           | 21.4±0.7<sup>b</sup>    | 295±40<sup>b</sup>                 |
| Cholestyramine     | 23.5±1.3<sup>b</sup>           | 34.9±2.7<sup>a</sup>    | 588±53<sup>a</sup>                 |
| Fat-free           | 34.5±2.1<sup>a</sup>           | 26.7±2.0<sup>b</sup>    | 333±63<sup>b</sup>                 |
| **Experiment 2**   |                                |                         |                                     |
| Sham               | 44.7±2.4                       | 25.7±2.1                | 296±84                              |
| Bile diversion     | 26.3±2.4<sup>*</sup>           | 38.9±5.5<sup>*</sup>    | 274±113                             |

Values are M±SEM, n=6 per group. In experiment 1, values in a column not sharing a common superscript letter are significantly different when tested by one-way ANOVA and Fisher's least significant difference test at p<0.05. In experiment 2, asterisk shows the significant difference between sham and bile diversion when tested by Student's t-test at p<0.05.

feeding cholestyramine and were not influenced by feeding fat-free diet. The aminopeptidase activity in bile diverted rats was significantly lower than that in sham operated rats while the sucrase activity was significantly higher in bile diverted rats and the alkaline phosphatase activity was not changed.

Discussion. Evidence from a number of studies showed that some enzymes presented in the intestinal brush border membrane were influenced by the luminal factors (4–11). Goda and Koldovsky (7) reported that the sucrase-active site of sucrase-isomaltase in the small intestine of rat is degraded by the action of pancreatic proteinases. Goda et al. (8) showed that the intake of a high-protein, low-carbohydrate diet accelerated the degradation of sucrase-isomaltase by means of the increase of luminal pancreatic proteinases. Enterokinase which is another brush border enzyme cleaves the pancreatic trypsinogen, resulting in trypsinogen activation (21). Newman et al. (10,11) reported that trypsinogen and bile acids were the main agents responsible for the maintenance of enterokinase in the proximal intestine of rats.

In this study, rats were fed cholestyramine to obtain the information about the effect of luminal bile acids on the intestinal aminopeptidase. Cholestyramine is known to increase the excretion of bile acids into the feces because this anion-exchange resin adsorbs the bile acids in the intestinal lumen. The content of total bile acids measured in the intestinal washing was markedly decreased with feeding cholestyramine, thus the adsorption of the bile acids with orally administered cholestyramine led to the deficiency of the free bile acids in the lumen. It is not clear why the activities of trypsin and chymotrypsin in the intestinal washing were also lowered with cholestyramine. We observed the similar decrease in the activities of trypsin and chymotrypsin in the intestinal washing of bile diverted rats.
in experiment 2, and this observation suggests that the deficiency of luminal bile acids may be responsible for the decrease in the activities of trypsin and chymotrypsin with cholestyramine. Hadorn et al. (9) and Newman et al. (10, 11) reported the necessity of bile acids for the activation of trypsinogen by the enterokinase in the proximal intestine, and Green and Nasset (22) showed that the bile acids protected trypsin and chymotrypsin from autodigestion in the small intestine. Thus, the decrease in the activities of trypsin and chymotrypsin with cholestyramine or bile diversion may have resulted from the decreased activation and/or the decreased stability through the deficiency of luminal bile acids.

We observed the decrease in the aminopeptidase activity in the intestinal mucosa of rats fed cholestyramine or bile diverted rats. One of possible reasons for such a decrease is that the exocrine pancreatic proteinases with the lower activities in the intestinal washing from rats fed cholestyramine or bile diverted rats may supply the smaller quantity of oligopeptides as substrate for aminopeptidase. However, such a possibility may be neglected because our preliminary experiment showed that the aminopeptidase activity in rats fasted for 48 h was not changed when compared with that in rats fed the standard diet (unpublished data). And the fat-free diet did not influence the luminal bile acids, exocrine pancreatic proteinases, and the intestinal aminopeptidase. Although the decrease in luminal bile acids with cholestyramine or bile diversion may lead to the suppression of lipids digestion and absorption, the data on the fat-free diet suggest that the decrease in lipids absorption was not responsible for the decrease in aminopeptidase activity with cholestyramine or bile diversion. So the luminal bile acids may modulate directly the activity of intestinal aminopeptidase. But it is unclear how the luminal bile acids act as the effector on the intestinal aminopeptidase.

By contrast, the sucrase activity in the intestinal mucosa was increased with cholestyramine or bile diversion. As mentioned above, cholestyramine and bile diversion lowered the activities of luminal trypsin and chymotrypsin probably by means of decreased abundance of luminal bile acids. Because the sucrase-active site is degraded by the action of pancreatic proteinases (7, 8), it is possible that the increase in sucrase activity with cholestyramine or bile diversion might result from the decrease in degradation by the luminal proteinases. The activity of alkaline phosphatase was increased with cholestyramine but not with bile diversion, so this enzyme may be affected by other factors than the luminal bile acids.

In conclusion, we propose that the intraluminal bile acids may be important for maintaining the activity of aminopeptidase while the degradation of sucrase by the pancreatic proteinases may be accelerated by the bile acids.

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