Small Intestinal Goblet Cell Proliferation Induced by Ingestion of Soluble and Insoluble Dietary Fiber Is Characterized by An Increase in Sialylated Mucins in Rats

Shingo Hino, Naoki Takemura, Kei Sonoyama, Akio Morita, Hirokazu Kawagishi, Seiichiro Aoe, and Tatsuya Morita

Department of Applied Biological Chemistry, Faculty of Agriculture, and Graduate School of Science and Technology, Shizuoka University, Shizuoka, Japan; Division of Applied Bioscience Graduate School of Agriculture, Hokkaido University, Sapporo, Japan; and Laboratory of Nutritional Biochemistry, Otsu Women’s University, Chiyoda-ku, Tokyo, Japan

Abstract

The study aimed to examine the effects of insoluble and soluble fibers on mucin sialylation and sulfation in the small intestine. First, diets containing soluble (konjac mannan (KM), psyllium, or guar gum; 50 g/kg) or insoluble (polystyrene foam, wheat bran, or cornhusk; 80 g/kg) fiber were fed to rats for 13 d. The fiber-fed groups had more goblet cells in the ileum than the fiber-free control. High-iron diamine/alcan blue staining showed more sialylated mucin-producing cells in the fiber-fed groups than in the control, whereas sulfated mucin-producing cells were fewer (insoluble fibers) or unchanged (soluble fibers). Second, feeding KM (50 g/kg) and beet fiber (BF) (80 g/kg) diets for 7 d yielded a higher ileum Siat4C expression than the control, but Gal3ST2 and Gal3ST4 expression was comparable. Luminal mucin content correlated with sialic acid (r = 0.96; P < 0.001) or sulfate (r = 0.62; P < 0.01), but the slope of the sialic acid-derived equation was greater than that of the sulfate-derived equation, indicating a preferred increase in sialylated mucins. Third, rats were fed the control diet for 10 d while receiving antibiotic treatment. Analysis of the luminal mucin showed that sialylated mucins were more vulnerable to bacterial degradation than sulfated mucins. Finally, a study of bromo-deoxyuridine incorporation in rats fed a BF diet indicated that goblet cell proliferation accompanied by increased sialylated mucin appeared to be related to accelerated ileal epithelial cell migration. We conclude that intestinal goblet cell responses to insoluble and soluble fibers are characterized by increases in sialylated mucin production.

Introduction

The absorptive surface of the intestine is covered by a layer of mucins that are synthesized and secreted by specialized goblet cells. Mucins are heavily glycosylated molecules that consist of threonine-/serine-rich-polypeptide backbones and oligosaccharide side chains. These mucus gels present a barrier that prevents potential pathogens and antigens from gaining access to the underlying epithelium and also serve as binding sites for Ig, particularly for secretory IgA. Mucin oligosaccharide chains are often terminated with sialic acid or sulfated sugar, which accounts for their polyanionic nature and visco-elastic properties. Because the presence of high levels of sulfate in a mucin decreases its susceptibility to bacterial glycosidases and limits the rate and extent of degradation, it has been proposed that reduced mucin sulfation might be closely correlated with the increase in bacterial translocation in murine models of gut disease and the exacerbation of colitis in humans.

Consumption of dietary fiber appears to enhance the total capacity for mucin secretion in the small intestinal lumen, although the stimulatory effect on mucin secretion depends on the quantity as well as the quality of dietary fiber ingested. Our previous studies showed that small intestinal mucins were secreted in proportion to the settling volume in water (a numerical representation of bulk-forming properties) of water-insoluble dietary fibers or the viscosity of water-insoluble dietary fibers. The stimulatory effects of both soluble and insoluble fibers on mucin secretion appear to be linked to epithelial cell turnover and the subsequent increase in goblet cell number. However, there appears to have been little investigation into the changes in the patterns of small intestinal mucin sulfation and sialylation in response to different types of dietary fiber.
Cassidy et al. (11) conducted histochemical analyses using high-iron diamine/alcian blue (HID/AB) staining to differentiate HID* sulfomucin and AB* sialomucin-containing goblet cells in rat intestine. They reported that, compared with a fiber-free control, feeding of both gel-forming and bulking insoluble fibers at a dietary level of 5–10% of the diet tended to produce an increase in the percentage of HID* goblet cells in the terminal ileum. Piel et al. (12) also suggested that a diet containing 10% carboxymethylcellulose predominantly increased sulfomucin-containing goblet cells in the ileum of pigs. However, in the course of our studies of the mucin secretory effects of dietary fiber, we noticed that, compared with rats fed a fiber-free, semipurified diet, those fed a nonpurified diet had a higher number of total goblet cells in the ileum as well as in the jejunum. This increase could be accounted for by an increased number of AB* goblet cells, accompanied by a corresponding decrease in the number of HID* goblet cells. This nonpurified diet contains a number of fiber types and has a total dietary fiber value >16%, which is generally regarded as a high-bulk diet (13,14). These observations are just the opposite of the previous findings that indicated that bulky fiber ingestion increases the number of HID* goblet cells (11). This finding prompted us to examine whether insoluble (bulky) and soluble (viscous) fibers with a capacity for induction of goblet cell proliferation share common characteristics in terms of their influence on the pattern of sulfation and sialylation of the oligosaccharide chains of small intestinal mucins.

For this purpose, we fed several fibers with a bulky or viscous nature to rats, and then conducted histochemical analyses by HID/AB staining, measurements of the sulfate and sialic acid content of in the small intestinal mucins, and gene expression analyses of the sialyltransferases Sia4C4 and the sulfotransferases Gal3ST2 and Gal3ST4 in the ileum tissue. We also examined differences in the susceptibility of sialylated and sulfated mucins to bacterial mucinase by measurements of the sialic acid and the sulfate content of small intestinal mucins in rats with or without antibiotic treatment. Finally, the effects of a nonpurified diet on the epithelial cells in the ileum that were observed in our preliminary study were reevaluated and compared with those of a semipurified diet including a bulky fiber.

**Methods**

**Materials.** Konjac mannan [KM, a copolymer of glucose and mannose (1:1.6) joined through β-1,4-glucosidic linkages] was provided by Shimizuy Chemical (PROPOL A, 1000–2000 kDa). Guar gum (GG; Sunfiber) and psyllium (PS) were provided by Taiyo Kagaku and Bizen Chemicals, respectively. Polystyrene foam (PSF), with an experimentally determined expansion ratio (7) of 54.9, was provided by JSP. PSF was powdered using a Wiley mill with an adjusted mesh size of 30–100. Wheat bran (WB; 30–70 mesh), beet fiber (BF; 30–70 mesh), and corn husk (CH; 30–70 mesh) were gifts from Nisshin Seifun Group, Nippon Beet Sugar Manufacturing, and Nihon Shokuhin Kako, respectively. WB preparation was washed in boiling water to remove starch and was then repeatedly washed with 99% ethanol and dried. Dietary fiber contents (dry matter basis), as determined by the Prosky method (15), were: KM (95%), GG (81.5%), PS (90%), WB (77%), BF (78%), and CH (89%). The viscosity of a 1.0% solution of each soluble dietary fiber, defined as the area under the viscosity curve described by Dikeman et al. (16), was 599 Pa (KM), 165 Pa (GG), and 67.6 Pa (PS), respectively (9). The settling volume in water (7) of each insoluble dietary fiber was 10 mL/g (PSF), 9.0 mL/g (BF), 9.0 mL/g (WB), and 5.0 mL/g (CH), respectively.

**Care of animals.** The study (no. 22–18) was approved by the Animal Use Committee of Shizuoka University and animals were maintained in accordance with the guidelines of Shizuoka University for the care and use of laboratory animals. Male rats of the Wistar strain (purchased from Shizuoka Laboratory Animal Center) were housed in individual, wire screen-bottomed, stainless steel cages in a temperature (23 ± 2°C) and lighting (lights on from 0800–2000 h) controlled room. For adaptation, rats were fed a control diet for at least 5 d. This diet (7) was formulated from 250 g/kg casein, 652.5 g/kg cornstarch, and 50 g/kg corn oil. The remainder of the diet consisted of vitamins including choline (12.5 g/kg) and minerals (35 g/kg). Subsequently, rats were allocated to groups on the basis of body weight to give a similar mean body weight and allowed free access to experimental diets and water. Each dietary fiber was added at the expense of an equal amount of cornstarch in the diet. Accordingly, dietary starch levels differed in diets and were 572.5 g/kg (insoluble fiber-added diet) or 602.5 g/kg (soluble fiber-added diet). Body weight and food intake were recorded every morning before replenishing the diet. In the present series of experiments, dietary inclusions of soluble and insoluble dietary fibers were set at 50 and 80 g/kg diet, based on the settling volume and the viscosity of the respective dietary fibers, to ensure a sufficient quantity of fiber for induction of goblet cell proliferation.

**Expt. 1.** Forty-two rats weighing 120–140 g (age 6 wk) were allocated to 7 groups of 6 rats each and were allowed free access to the control diet or to a diet containing 50 g/kg KM, PS, or GG, or 80 g/kg PSF, WB, or CH for 13 d. The diets were withdrawn overnight and rats were then killed by decapitation and the small intestine was excised. Luminal contents were collected by flushing with 15 mL of ice-cold PBS (pH 7.4) containing 0.02 mol sodium azide/L and the same volume of air. The contents were freeze-dried and stored for luminal mucin analysis. For histological examination, the upper one-half of the small intestine except the duodenum was defined as the jejunum, and the lower one-half was defined as the ileum. The mid-portions of ileum segments (~5 cm) and the terminal ileum (~5 cm in length, cut at a distance of 2 cm from the ileo-cecal valve) were removed, opened longitudinally, placed in 10% buffered formalin, and used for tissue examination.

**Expt. 2.** Thirty-six rats weighing 127–147 g (age, 6 wk) were allocated to 3 groups of 12 rats each and were fed the control diet or a diet containing 50 g/kg KM or 80 g/kg PSF for 7 d. Then, each dietary group was further divided into 2 equal groups. One group was killed by decapitation without prior food deprivation. A part of the ileal segment (~5 cm) was opened longitudinally and the mucosa was scraped with a glass slide and used for total RNA isolation. The other group of rats was feed-deprived overnight and killed by decapitation. Luminal mucin sampling procedures and intestinal tissue collection were as described for Expt. 1.

**Expt. 3.** Twelve rats weighing 130–156 g (age, 6 wk) were allocated to 2 groups of 6 rats each and were allowed free access to the control diet with or without antibiotics (benzyl penicillin potassium, 50 kIU/L; neomycin sulfate, 2000 mg/L; cefoperazone sodium, 500 mg/L; WAKO Chemicals) in the drinking water for 10 d. The rats were then killed by decapitation and the small intestinal contents and the cecal contents were gathered. The small intestinal mucin sampling procedure was as described for Expt. 1. The cecal contents were used for the measurement of organic acids (17) and for bacterial culture.

**Expt. 4.** To reevaluate our preliminary study, 12 rats weighing 133–158 g (age 6 wk) were allocated to 3 groups of 4 rats each and were allowed free access to the control diet to a diet containing 80 g of BF/kg diet or to a nonpurified diet (MF-2, Oriental Yeast) for 10 d. For examination of epithelial cell migration, 5-bromo-deoxyuridine (BrdU) (50 mg/kg body weight) was i.p. injected into the rats on d 9 (1000–1100 h). At 24 h after administration, without feed deprivation, the rats were killed by decapitation and the mid-portions (~5 cm) of the ileum were removed and treated as described for Expt. 1.

---

8 Abbreviations used: BF, beet fiber; BrdU, 5-bromo-deoxyuridine; CH, corn husk; GG, guar gum; HID/AB, high-iron diamine/alcian blue; KM, konjac mannan; PAS, periodic acid Schiff; PS, psyllium; PSF, polystyrene foam; WB, wheat bran.
Mucin analysis. The mucin fraction was isolated by the method of Lien et al. (18), with some modification, as previously described (7), and was dissolved in 5.0 mL of distilled water for analyses. After an appropriate dilution of the mucin fraction, O-linked oligosaccharide chains were measured as previously described (7). Standard solutions of N-acetylglucosamine (Sigma-Aldrich) were used to calculate the amount of oligosaccharide chains liberated from mucins during the procedure.

Sialic acid determination. Part of the mucin fraction (0.1 mL) was hydrolyzed with 50 mmol sulfuric acid/L for 60 min at 100°C and sialic acid was determined by a previously described method (19). N-acetylneuraminic acid was used as a standard.

Sulfate determination. An appropriate volume of the mucin fraction (~20 µg protein) was completely dried, reconstituted in 200 µL of 4 mol/L HCl, and hydrolyzed at 100°C in a heating block for precisely 4 h. Determination of sulfate in the mucin fraction was basically performed using the method of Harrison and Packer (20). Solutions of 0.79, 1.59, 3.18, 6.38, and 12.8 mmol sulfatel. (Multi-anion standard solution-1, Wako Pure Chemicals) were used as standards.

Histochemical analyses. Six 5-µm-thick cross-sections were prepared from paraffin-embedded samples of each tissue for each staining. Five complete villi (entire crypt/villus axis) per section were selected and villus length and the number of epithelial cells and goblet cells per villus (left side) were determined. Two observers (unaware of treatments) independently analyzed each section by light microscopy using an Olympus BH2 instrument fitted with a micrometer eyepiece. Goblet cells were stained with periodic acid Schiff (PAS) and counter-stained with hematoxylin. HID/AB staining was performed using the method of Spencer (21) with a slight modification. Briefly, de-paraffinized and rehydrated sections were immersed in HID solution (240 mg of N, N-dimethyl-m-phenylenediamine dihydrochloride, Sigma-Aldrich), 40 mg of N, N-dimethyl-p-phenylenediamine hydrochloride (Wako Pure Chemicals), and 4.2 mL of 40% ferric chloride (Wako Pure Chemicals) in 100 mL of distilled water for 21 h. After washing with running tap water for 5 min, the sections were immersed in 1% alcian blue in 3% acetic acid (pH 2.5) for 1 h. The sections were then rinsed with distilled water, dehydrated with increasing concentration of ethanol, cleared with xylene, and mounted with mounting media (MP500, Matsunami Glass). This HID/AB technique stains sulfomucin black/brown and stains sialomucin blue. Both types of reaction, i.e., HID+ and AB+ (2–4 counts/villus, left side) were observed in a small population of goblet cells. We ascribed these cells to one type of reaction, depending on the predominant tone.

RNA isolation and real-time qPCR. Total RNA was isolated using the Takara RNAiso reagent (Takara Bio) according to the manufacturer’s instructions. One microgram of total RNA was reverse transcribed using the Takara Prime Script RT reagent (Takara Bio) at 37°C for 15 min. The synthesized cDNA was amplified by PCR using a LightCycler System (Roche Applied Science). The primer pairs and protocols for PCR of Muc2, Muc3 (22), Slat4c, Gal3ST4 (23), and 18S rRNA (24) have been reported. 18S rRNA was used as an endogenous reference gene. PCR reactions were carried out in a total volume of 20 µL containing 400 nmol/L each of gene-specific primers, cDNA, and SYBRPremix Extaq II (Takara Bio). To confirm amplification specificity, the PCR products from each primer pair were subjected to a melting curve analysis and subsequent agarose gel electrophoresis. Gene expression was quantified using the comparative Ct method (25) and the data were expressed relative to the control group. In the present study, Ct values were 18S rRNA values among the dietary treatments were 9.1 ± 0.1 (control), 9.2 ± 0.1 (BF), and 9.2 ± 0.1 (KM) and there were no differences between the groups.

Bacterial culture. After the rats were killed, the cecal contents were immediately removed, weighed, and then placed in grinding tubes containing anaerobic phosphate buffer. The cecal contents were homogenized under oxygen-free carbon dioxide gas (26). Bacteriological procedures and media were essentially the same as the method previously described (26,27).

BrdU staining. Six 5-µm-thick cross-sections of intestinal tissue per animal were collected on aminopropyltriethoxysilane-coated slides. After de-paraffinization and hydration, the sections were immersed in preheated 10 mmol/L citrate buffer (pH 6.0) and heated at 100°C for 20 min for antigen retrieval. BrdU staining was then performed as previously described (9). BrdU-positive cells were subsequently counted in the same manner as for goblet cell staining.

Statistical analyses. Data were analyzed by 1-way ANOVA and significant differences among means were identified by the Tukey-Kramer test. The results were expressed as means ± SEM and a 5% level of probability was considered a significant difference for all analyses. When variances were not homogenous by the Bartlett test, data were logarithmically transformed. When variances were not homogenous even after logarithmic transformation, the data were presented as medians with ranges and were then analyzed by Kruskal-Wallis ANOVA followed by Kolmogorov-Smirnov 2-sample tests. For Expt. 3 and 4, differences were analyzed by Student’s t test. Regression analysis was used to examine the relationship between O-linked oligosaccharide chains (as mucin) and sialic acid or sulfated sugar in the mucin fractions. If a significant correlation was observed, the sialic acid or sulfate content as a response variable was predicted from the contents of O-linked oligosaccharide chains as a function of regressor variables by each regression line. When the intercept was not zero, the mean slopes were compared by ANCOVA with O-linked oligosaccharide chains as a covariate. All calculations were done using the JMP8 software (SAS Institute).

Results

Expt. 1. Rats fed the KM and GG diets had lower food intakes than those fed the control, PSF, WB, and CH diets (Table 1). Food intakes in the viscous fiber-fed groups were also lower than in the bulky fiber-fed groups. Body weight gain in rats fed the GG diet was lower than in those fed the control and the other fiber-added diets, except the KM diet. The total amount of O-linked oligosaccharide chains (measured as mucin) in the small intestinal contents was significantly greater in the KM, PS, PSF, and WB groups than in the control. The difference between the KM and GG groups was also significant. However, neither the sialic acid nor sulfate content of the mucin fraction differed significantly among the dietary groups. Linear regression analysis showed a significant correlation between the mucin content and sialic acid or sulfate content, but the slope of the sialic acid-derived equation was significantly greater than that of the sulfate-derived equation, indicating that fiber ingestion predominantly increased sialeylated mucins (Fig. 1 A,B). In the mid ileum, villus heights in rats fed viscous fiber diets were significantly greater than in those fed bulky fiber diets. The difference between the control and PS groups was also significant. All of the fiber-fed groups had a higher number of PAS+ goblet cells than the control group, with the PS and PSF groups having significantly more cells than in the GG and CH groups. These increases were accounted for by an increase in the number of AB+ goblet cells in the epithelial cells. On the other hand, only the PSF, WB, CH, and GG groups had fewer HID+ goblet cells than the control group (Table 1; Supplemental Fig. 1). In the terminal ileum, villus heights in rats fed the PS diet were significantly greater than in those fed the control, PSF, WB, and CH diets. The fiber-fed groups, except the CH group, had a higher number of PAS+ goblet cells than the control group. The PAS+ goblet cells in the PS and PSF groups were also greater than in the CH group. The AB+ and HID+ goblet cells in the GG, PSF, and WB groups were higher and lower than in the control group (Table 1; Supplemental Fig. 2).
Expt. 2. In the 7-d feeding study of KM and BF, the KM group had less food intake and body weight gain than the control and BF groups (Table 2). The amount of O-linked oligosaccharide chains (mucins) in the small intestinal contents was greater in the KM and BF groups than in the control group. In the mucin fraction, the contents of sulfate and sialic acid were significantly greater in the BF group than in the control group. There was a significant correlation between the mucin content and the number of AB+ goblet cells, while a lower number of HID+ cells, the nonpurified diet gave similar results to the BF diet in the ileum (Fig. 1C,D). In the ileum tissue, the number of PAS+ goblet cells was significantly higher in the KM and BF groups than in the control group. These increases were accounted for by an increase in the number of AB+ goblet cells, while a lower number of HID+ goblet cells was observed in the BF group compared with the control group (Table 2; Supplemental Fig. 3). Mac2 gene expression was slightly but significantly greater in the BF group compared with the other groups. Stat4C expression was 6–10 times higher in the KM and BF groups than in the control, whereas the gene expression of Gal3ST2 and Gal3ST4 did not differ among the groups (Table 2).

Expt. 3. Food intake for 10 d was significantly lower in rats with antibiotic treatment (117 ± 3 g) than in those without antibiotic treatment (144 ± 4 g), but the body weight gain did not differ between rats with (55 ± 2 g) or without (52 ± 2 g) antibiotic treatment due to a large increase in the weight of cecal contents in rats treated with antibiotics (15.9 ± 0.9 g vs. control 1.9 ± 0.1 g). The concentration of cecal organic acids (the sum of acetate, propionate, butyrate, lactate, and succinate) was 58.7 ± 2.3 μmol/g content in rats without antibiotic treatment, but only negligible amounts of total organic acids were detected in rats with antibiotic treatment (<0.3 μmol/g). The total number of anaerobes, lactobacillus, and clostridia in the cecal contents was significantly reduced in rats with antibiotic treatment (4.0 ± 0.5, 0.4 ± 0.3, and 2.7 ± 0.9 log of cfu/cecum, respectively) compared with the number in rats without antibiotic treatment (13.5 ± 0.4, 12.7 ± 1.6, and 11.2 ± 0.2 log of cfu/cecum, respectively). The amount of O-linked oligosaccharide chains and sialic acid in the small intestinal contents increased in rats with antibiotic treatment by 500% compared with the amount in rats without antibiotic treatment and the amount of sulfate increased by 200% with antibiotic treatment (Fig. 2).

Expt. 4. Food intake and body weight gain did not differ between rats fed the control and BF diets. The villus height and number of epithelial cells in the ileum tissue also did not differ between the 2 groups (Table 3). The number of PAS+ goblet cells in the ileum was higher in rats fed the BF diet than in those fed the control diet (Table 3; Supplemental Fig. 4). The number of AB+ and HID+ goblet cells was higher and lower, respectively, in rats fed the BF diet than in rats fed the control diet. In terms of goblet cell variability and BrdU-labeled cells, the nonpurified diet gave similar results to the BF diet (Table 3; Supplemental Fig. 4).

Discussion

In accordance with our previous studies (7–9), an increase in small intestinal mucin content was consistently observed after bulky or viscous fiber ingestion (Expt. 1). The amount of small intestinal mucins in these fiber-fed groups increased in propor-

---

**TABLE 1** Food intake, body weight gain, amount of O-linked oligosaccharide chains, sulfate, and sialic acid in the small intestinal mucin fraction, and histological variables in the small intestinal tissue in rats fed a control diet, a diet containing 50 g/kg KM, PS, or GG, or a diet containing 80 g/kg PSF, WB, or CH for 13 d (Expt. 1).1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>5% KM2</th>
<th>5% PS3</th>
<th>5% GG4</th>
<th>8% PSF5</th>
<th>8% WB6</th>
<th>8% CH7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake, g/13 d</td>
<td>179 ± 5b</td>
<td>150 ± 4e</td>
<td>171 ± 5c</td>
<td>140 ± 3d</td>
<td>210 ± 7a</td>
<td>208 ± 6a</td>
<td>197 ± 5a</td>
</tr>
<tr>
<td>Body weight gain, g/13 d</td>
<td>66 ± 3e</td>
<td>59 ± 2abc</td>
<td>68 ± 4abc</td>
<td>49 ± 3d</td>
<td>69 ± 2ab</td>
<td>79 ± 4a</td>
<td>70 ± 1b</td>
</tr>
</tbody>
</table>

1 Data are mean ± SE, n = 6. Means in a row with superscripts without a common letter differ, P < 0.05. CH, corn husk; GG, guar gum; KM, konjac mannan; PS, psyllium; PSF, polystyrene foam; WB, wheat bran.
2 Diet containing 50 g/kg KM.
3 Diet containing 50 g/kg PS.
4 Diet containing 50 g/kg GG.
5 Diet containing 80 g/kg PSF.
6 Diet containing 80 g/kg WB.
7 Diet containing 80 g/kg CH.
or a diet containing 80 g/kg PSF, WB, or CH for 13 d (Expt. 1, rats were fed the control diet, or a diet containing 50 g/kg KM, PS, or GG, or a diet containing 80 g/kg PSF, WB, or CH for 13 d (A, B). In Expt. 2, rats were fed the control diet, or a diet containing 50 g/kg KM or 80 g/kg BF for 7 d (C, D).

FIGURE 1 Correlations between the amount of O-linked oligosaccharide chains and the amount of sialic acid or sulfate. In Expt. 1, rats were fed the control diet, or a diet containing 50 g/kg KM, PS, or GG, or a diet containing 80 g/kg PSF, WB, or CH for 13 d (A, B). In Expt. 2, rats were fed the control diet, or a diet containing 50 g/kg KM or 80 g/kg BF for 7 d (C, D).

ation to the number of PAS+ goblet cells in the mid-ileum (r = 0.80; P < 0.05) as well as in the terminal ileum (r = 0.72; P = 0.07). Under the conditions of our HID/AB staining assay of the mid-ileum, it appeared that bulky fiber ingestion stimulated an increase in the number of AB+ goblet cells with a concomitant decrease in the number of HID+ goblet cells, and viscous fiber ingestion resulted in a greater number of AB+ goblet cells with a constant number of HID+ goblet cells. These results remained essentially similar in the terminal ileum, except that there were no differences in the total (PAS+) goblet cell numbers in rats fed the CH diet compared with the control. These findings are very different from those of Cassidy et al. (11), who reported that chronic ingestion (4 wk) of viscous and bulky fibers by rats led to an alteration in the intestinal goblet cell population from predominantly AB+ to predominantly HID+ goblet cells. The reason for this discrepancy is unclear but could be explained by the differences in the duration of the feeding studies. Thus, there may be a chronic effect of dietary fiber on changes in the pattern of sulfation and sialylation of intestinal mucins. Another possible explanation may be that HID/AB-stained goblet cells were categorized differently in the 2 studies. When this technique is used, a small population of goblet cells demonstrates both types of reaction. We ascribed these cells to one type of reaction, depending on which tone was predominant, whereas Cassidy et al. (11) designated these cells as “mixed”.

Mucins have been categorized as neutral mucins, sialomucins, or sulfomucins on the basis of the density and types of acidic groups present in their oligosaccharide side chains. However, as indicated by Robertson and Wright (28), the intensity of HID/AB staining does not necessarily correlate with actual biochemical measurements of mucin sulfate levels and, furthermore, the quantity of sulfate needed to qualify a mucin for categorization as a sulfomucin is unclear. In the present study, therefore, we biochemically measured both sialic acid and sulfate content in the small intestinal mucin fractions in rats fed a bulky or viscous fiber diet. There was a significant correlation between the mucin content and the sialic acid or sulfate content. However, the slope of the sialic acid-derived equation was significantly greater than that of the sulfate-derived equation (Expt. 1 and 2). These results indicate that fiber ingestion increases mucin sialylated oligosaccharides rather than sulfated oligosaccharides.

Studies on the transcription levels of the sialyltransferase Siat4C and the sulfotransferases Gal3ST2 and Gal3ST4 genes provided further support for the predominant increase in sialylated mucins following fiber ingestion. Both bulky WB and viscous KM ingestion for 7 d strongly upregulated the gene expression of Siat4C (by 6- to 10-fold) compared with the fiber-free control, whereas the expression levels of Gal3ST2 and Gal3ST4, which are the major mucin sulfotransferases in the intestine (23,29), were comparable among the dietary groups (Expt. 2). Thus, the results of gene expression analyses are in accordance with those of the histochemical analyses of the ileum. On the other hand, the relative amounts of sialic acid and sulfate in the small intestinal mucin fractions do not necessarily reflect expression of the related genes or the ileum histochemistry (Expt. 1 and 2). This result may be partly due to the differences in susceptibility of sialomucin and sulfomucin in the small intestinal fluid to bacterial degradation.

Enteric bacteria possess both sialidases and glycosulfatases that are essential for mucin degradation (30), but the optimum pH of the glycosulfatases (pH 5.0) is much lower than that of the sialidases (pH 7.8) (31). Therefore, in small intestinal fluid of neutral pH, bacterial degradation of sulfomucin might be considerably less than that of sialomucin. Indeed, in the presence of antibiotics, not only the amount of O-linked oligosaccharide chains but also the amount of both sialic acid and sulfate in the small intestinal contents was significantly greater than that in the absence of antibiotics. However, the increase in sialic acid (500%) was much greater than that in sulfate (200%) in the antibiotic-treated rats (Fig. 2). These findings suggest that there might have been a greater underestimation of the sialic acid contents measured in Expt. 1 and 2.
than of the sulfate contents due to differences in their susceptibility to bacterial degradation. This possibility may partly explain the disparity between the sialic acid and sulfate content of luminal mucins and the results of gene expression or ileum histochemical analyses. Accordingly, at least as far as the findings of histochemical analyses, biochemical measurements, and gene expression are concerned, it is plausible to conclude that the ingestion of bulky and viscous fibers predominantly increases sialylated mucins rather than sulfated mucins in the rat small intestine.

Decreased mucin sulfation has been proposed to lead to enhanced mucin degradation and penetration of the secreted mucus barrier by microbes, thereby giving increased access to the epithelial cell surface (32). Dawson et al. (33) reported that NaS1 sulfate transporter null (NaS1−/−) mice, which display increased urinary sulfate excretion and hyposulfatemia, had reduced intestinal sulfomucin content, enhanced susceptibility to toxin-induced colitis, and an impaired intestinal barrier to bacterial translocation (4). In this regard, the predominant increase in sialylated mucin by dietary fiber ingestion might be considered less beneficial for mucusal physiology. However, it has been repeatedly observed in animal experiments that the incidence of bacterial translocation is low in rats fed a high-fiber diet or in rats fed a nonpurified diet compared with those fed a highly defined elemental diet or those treated with total parenteral nutrition (34–36). Because we also observed in rats fed a fiber-added diet or a nonpurified diet (Table 3; Supplemental Fig. 4), a marked increase in the total number of goblet cells is linked to the accelerated epithelial cell migration (23,37,38). Possibly, an increased capacity for mucin secretion accompanied by accelerated epithelial cell turnover may effectively function as an intestinal barrier, irrespective of sialylation/sulfation of mucins, at least under normal conditions. However, further studies are needed to clarify the physiological relevance of such an alteration in the pattern of sulfation and sialylation of small intestinal mucins.

At present, the mechanism by which bulky and viscous fiber ingestion increases sialylation of mucins in the small intestine remains unclear. However, Specian and Oliver (39) showed that immature goblet cells in the small intestine produce neutral mucins that contain less sialic acid, but, as they mature and migrate to the villus tip, the mucins become increasingly sialylated. This observation may suggest that accelerated epithelial turnover may be linked with an increase in sialylated mucin production. Besides dietary fiber, administration of medium-chain TG (38) or silver nanoparticles (40) also increases the level of sialylated mucin and is accompanied by goblet cell proliferation and accelerated epithelial turnover.

In conclusion, goblet cell responses to the ingestion of insoluble (bulky) and soluble (viscous) fibers are characterized by a predominant increase in sialylated mucin of the rat small intestine.

### TABLE 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>5% KM</th>
<th>8% BF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake, g/7 d</td>
<td>102 ± 2a</td>
<td>83 ± 1b</td>
<td>95 ± 2b</td>
</tr>
<tr>
<td>Body weight gain, g/7 d</td>
<td>39 ± 1a</td>
<td>32 ± 1b</td>
<td>36 ± 1b</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intestinal contents</th>
<th>O-linked oligosaccharide chains, μmol/intestine</th>
<th>Sulfate, μmol/intestine</th>
<th>Sialic acid, μmol/intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villus height, μm</td>
<td>335 ± 4a</td>
<td>365 ± 4a</td>
<td>325 ± 4a</td>
</tr>
<tr>
<td>PAS* cells, n/villus, left side</td>
<td>11.4 ± 2b</td>
<td>15.8 ± 0.3b</td>
<td>15.1 ± 0.3b</td>
</tr>
<tr>
<td>HID* cells, n/villus, left side</td>
<td>6.2 (5.8–7.0)</td>
<td>6.6 (3.7–8.6)</td>
<td>1.1 (0.5–1.8)*</td>
</tr>
<tr>
<td>AB* cells, n/villus, left side</td>
<td>3.3 (2.6–3.5)</td>
<td>7.1 (4.8–9.8)*</td>
<td>11.4 (10.7–12.7)*</td>
</tr>
</tbody>
</table>

Gene expression

| Muc2     | 1.0 ± 0.1a | 1.7 ± 0.1b | 1.3 ± 0.1bd |
| Siat4c   | 1.0 ± 0.1a | 10.3 ± 2.2b| 7.5 ± 3.7n  |
| Gal3ST2  | 1.0 ± 0.6  | 0.7 ± 0.5  | 0.2 ± 0.0   |
| Gal3ST4  | 1.0 ± 0.1  | 0.8 ± 0.0  | 0.8 ± 0.1   |

1 Data are mean ± SE or median (range), n = 6 or 12 (food intake, body weight gain). Means in a row with superscripts without a common letter differ, P < 0.05. BF, beet fiber; HID/AB, high-iron diamine/alcian blue; KM, konjac mannan; PAS, periodic acid Schiff.
2 Diet containing 50 g/kg KM.
3 Diet containing 80 g/kg BF.
4 The effects of dietary treatment were examined by Kruskal-Wallis 1-way ANOVA, followed by Kolmogorov-Smirnov 2-sample tests.
*Different from control, P < 0.05; †different from 5% KM, P < 0.05.
TABLE 3

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>8% BP</th>
<th>Nonpurified diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake, g/10 d</td>
<td>150 ± 12</td>
<td>134 ± 3</td>
<td>168 ± 8</td>
</tr>
<tr>
<td>Body weight gain, g/10 d</td>
<td>47 ± 6</td>
<td>44 ± 3</td>
<td>44 ± 5</td>
</tr>
<tr>
<td>Villus height, μm</td>
<td>364 (303–302)</td>
<td>337 (324–339)</td>
<td>335 (330–340)</td>
</tr>
<tr>
<td>Total epithelial cells, n/villus, left side</td>
<td>79 ± 3</td>
<td>71 ± 5</td>
<td>68 ± 1</td>
</tr>
<tr>
<td>PAS+ cells, n/villus, left side</td>
<td>10.2 ± 0.2</td>
<td>13.6 ± 0.3*</td>
<td>15.3 ± 0.4</td>
</tr>
<tr>
<td>HID+ cells, n/villus, left side</td>
<td>5.3 ± 0.3</td>
<td>1.3 ± 0.2*</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>AB+ cells, n/villus, left side</td>
<td>2.8 ± 0.3</td>
<td>10.2 ± 0.2*</td>
<td>12.9 ± 0.8</td>
</tr>
<tr>
<td>Position of upper-most BrdU-labeled cell</td>
<td>23.2 ± 1.3</td>
<td>32.9 ± 3.0*</td>
<td>42.7 ± 2.5</td>
</tr>
</tbody>
</table>

1 Data are mean ± SE or median (range), n = 4. *Different from control, P < 0.05 (Student’s t test). BF, beet fiber; BrdU, 5-bromo-deoxyuridine; HID/AB, high-iron diamine/alcian blue; PAS, periodic acid Schiff.
2 Diet containing 80 g/kg BF.
3 Values indicate the highest position of BrdU-labeled cells from the bottom of the villus at 24 h after BrdU injection.

Acknowledgments
T.M. designed the research and wrote the manuscript; S.H., N.T., A.M., and H.K. conducted the research; and K.S. and S.A. analyzed the data. All authors read and approved the final manuscript.

Literature Cited


