Hairy Region Concentrate of Pectin Strongly Stimulates Mucin Secretion in HT29-MTX Cells, but to a Lessor Degree in Rat Small Intestine

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(Received February 26, 2020)

Summary Pectin enhances mucin secretion in the rat small intestine. However, what structural features of pectin to stimulate mucin secretion remain unclear. The study aimed to clarify active constituents of pectin using a human goblet cell line, HT29-MTX. Various pectins at 100 mg/L commonly stimulated MUC5AC secretion, irrespective of their differences in molecular size, plant origin and degree of methoxylation, whereas other dietary fiber materials at 100 mg/L did not show any effects, except fucoidan. Hairy region concentrate (HRC) and its further fractions (F1–F3) were prepared by polygalacturonase treatment of citrus pectin and successive anion exchange chromatography. Neutral sugars, such as galactose and arabinose were enriched in these fractions. HRC and F1–F3 at 30 mg/L significantly increased MUC5AC secretion, which were 3 times more potent compared with a starting material (citrus pectin). Further, a dose-dependent study showed that F1 significantly increased MUC5AC secretion from at 0.3 mg/L, much stronger than that of mucin-secretagogue lipopolysaccharides. Rats consumed 5% apple pectin diet showed significant increases of luminal mucin contents and Muc2 expression in the small intestine, while the luminal mucin contents in rats consumed 1.5% HRC diet were increased by 24% compared to those in rats consumed control diet, but the difference did not reach significant. Thus, HRC is supposed to be active constituents of mucin-secretory effect of pectin in vitro. At present, however, the effect of HRC has not been verified in vivo.

Key Words pectin, hairy region, mucin, goblet cell, rats

Epithelial surface of the small intestine is covered by a layer of mucus composed predominantly of mucin glycoproteins that are synthesized and secreted by goblet cells (1). Mucin is a key component of the gut barrier that prevents physical stimuli, potential pathogens and antigens from gaining access to the underlying epithelium (2, 3). Mucin also interacts with glycan side chains of secretory component and acts as a reservoir for secretory IgA (4). Consequently, dietary strategies to stimulate mucin secretion would be of benefit to protect a leaky gut associated with numerous disorders such as Crohn’s disease, celiac disease, and irritable bowel syndrome (5, 6).

We previously reported in rats that bulky (insoluble) and viscous (soluble) fibers generally upregulated baseline secretion of small intestine mucins by increasing the number of goblet cells in proportion to either the bulk-forming property or the viscosity of the respective fibers ingested (7–9). Among these, we found that low-methoxyl apple pectin (AP) was unique in that its mucin-secretory effect was not accompanied by goblet cell proliferation, but associated with an elevation of Muc2 mRNA gene expression in the small intestine (10). Further, the mucin-secretory effect of low-methoxyl AP was observed in human goblet cell line HT29-MTX, suggesting a direct interaction of low-methoxyl AP molecule and the small intestine epithelia (10). However, it is not yet known what structural features of low-methoxyl AP are involved in the mucin-secretory activity.

Pectins are heteropolysaccharides in the plant cell wall and contain two elements (11). Pectin molecule consists of smooth regions, which are partly methoxylated linear homogalacturan, and hairy regions with highly branched neutral sugar side chains, which are further classified into rhamnogalacturan (RG)-I and -II. RG-I is composed of disaccharide units (α-d-galacturonic acid-(1→2)-α-l-rhamnose-(1→4)), and the rhamnosyl residues is substituted with neutral sugar side chains (galactan, arabinan, and arabinogalactan). RG-II consists of a homogalacturan backbone with neutral sugar side chains consisting of 12 different types of sugars joined in more than 20 different linkages. Variations in the plant origin and extraction methods can yield various pectin fractions differing in molecular weight, degree of methoxylation, the structures and sugar compositions of RG-I and -II (12, 13), all of which
may affect diverse physiologic functions of pectins including mucin-secretory effect.

Recent studies on the anti-metastatic effects of modified citrus pectin (CP), a mixture of CP fragments, emphasize the potential importance of hairy regions (14–16). Pienta et al. (14) showed the reduction in the frequency of lung metastases by consumption of 0.1 and 1% modified CP in drinking water (w/v) in rats injected with MATLyLu cells into the hind limb. Similar anti-metastatic effects of modified CP were reported in mice inoculated with MDA-MB-435 and LSLiM6 cells (15). Judging from the results of fluorescence microscopy and flow cytometry studies, Gunning et al. (16) proposed that the hairy region of CP possesses a potential to interfere with galectin-3 mediated cell-cell interactions by a direct binding of modified CP to galectin-3 expressed on cancer cells. These findings implicate that hairy region of low-methoxyl AP would be also associated with its mucin-secretory effects because small intestine epithelia have been reported to express galectin receptors in rats (17, 18).

In the present study, we initially investigated that the mucin-secretory effect of pectins is unique when compared with various dietary fibers in HT29-MTX cells. Then, a wide variety of pectins with different origins, degree of methoxylation, and molecular weight were compared (Experiment 1). Further, we prepared hairy region concentrate (HRC) of pectin, and examined its mucin-secretory effects in HT29-MTX cells (Experiment 2) as well as in rat small intestine (Experiment 3).

MATERIALS AND METHODS

Materials. High-methoxyl apple pectin (AP) (Pectin classic AB901), low-methoxyl AP (Pectin classic AM201), AP with medium molecular weight of <45 kDa (AP45) (Pectin classic AU-L 074/09), low-methoxyl citrus pectin (CP) (Pectin classic CB901), high-methoxyl CP (Pectin classic CM201), CP with low molecular weight of <15 kDa (CP15) (Pectin classic CU901) were gifts from DSP Gokyo Food & Chemical Co., Ltd. (Osaka, Japan). Low-methoxyl orange pectin (OP) and oligogalacturonic acids with average molecular weight of 600 were gifts from POLA (Tokyo, Japan). Information for other dietary fibers used in the present study was provided in Table S1 (Supplemental Online Material).

Preparation and fractionation of HRC. HRC were prepared from CP15. Ten grams of CP15 and 750 μL of polygalacturonase (1,200 units/mL, Pectinase G Amano, Amano Enzyme Inc., Aichi, Japan) were incubated in 500 mL of 50 mMol/L acetate buffer with pH 4.5 under gentle stirring (150 rpm) at 30°C for 6 h, then heated in a boiling water for 10 min to stop the reaction. Preliminary experiment showed that amount of total sugars released from the reaction mixture reached maximum at 6 h. After cooling down to room temperature, the reaction mixture was centrifuged at 3,500 × g for 10 min to remove the insoluble matter. The supernatant was dialyzed against distilled water with Spectra/Per 7 tubular dialysis membranes (molecular weight cutoff values of 1,000, Repligen Corporation, Waltham, MA).

Fig. 1. The elution profile of HRC on anion exchange chromatography. Data represents sugar contents in each fraction as glucose equivalent. HRC, hairy region concentrate. HRC was eluted with distilled water, a stepwise gradient of sodium hydrogen carbonate solutions (0.1–0.4 mol/L) and 0.1 mol/L NaOH.

Twenty-six batches of the dialyzed supernatants were pooled, freeze-dried and used as HRC (yield, 22.6% by weight). A portion of HRC was further fractionated by anion exchange chromatography according to the method of Nishida et al. (19) with some modification. Briefly, HRC dissolved in distilled water was applied to diethylaminoethyl cellulose column (HCO3− form), washed with water and separated by stepwise elution with 0.1–0.4 mol/L of sodium hydrogen carbonate and finally 0.1 mol/L of NaOH solutions. Every 10 mL of eluant was monitored for total sugar contents, and three fractions (F1, F2 and F3) were obtained (Fig. 1). Each fraction was dialyzed against distilled water, freeze-dried and used for the present study.

Analyses of total sugar contents, sugar composition and molecular size distribution. Total sugar contents were measured by phenol-sulfuric acid method and calculated based on a standard curve obtained with glucose (20). Sugar compositions of CP15, HRC and F1–F3 of HRC were measured by the method of Yasuno et al. (21) with some modification. Briefly, neutral sugars and galacturonic acid were measured after hydrolysis with 4 mol/L of trifluoroacetic acid for 4 h and 2 mol/L of sulfuric acid for 3 h, respectively. The hydrolysis temperature was 100°C in both conditions. Released sugars were derivatized with 4-aminozobenzoic acid ethyl ester (ABEE) using a commercial labeling Kit (J-Oil Mills, Inc., Tokyo, Japan) and were applied to high performance liquid chromatography equipped with GlyScope HonenpakC18 column (4.6 mm × 75 mm) (J-Oil Mills, Inc.) according to the manufacturer’s instruction. The ABBE-labeled sugars were detected with a fluorescent detector (excitation 305 nm, emission 360 nm). A known concentration of each sugar (galacturonic acid, galactose, glucose, mannose, arabinose, rhamnose, xylose, fucose, and fructose) was used for generating standard curves. Molecular size distribution of F1 was measured by the method of Hung et al. (22) with some modification. 2,5-Dihydroxybenzoic acid was used as matrix (8 g/L) and approximately 100 pg of the F1 was deposited as a mixture together with the matrix on a stainless steel target, and subsequently dried under reduced pres-
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Table 1. Sugar compositions of CP15, HRC, F1, F2 and F3,

|          | CP15 | HRC | F1  | F2  | F3  |
|----------|------|-----|-----|-----|-----|
|          | mol% |     |     |     |     |
| Galacturonic acid | 92.6 | 69.3 | 72.2 | 57.0 | 76.5 |
| Rhamnose | 1.8  | 4.8 | 2.6 | 10.8 | 7.8 |
| Galactose | 3.7  | 12.6 | 11.0 | 17.8 | 6.1 |
| Arabinose | 1.2  | 8.6 | 9.2 | 8.6 | 6.3 |
| Xylose   | 0.2  | 1.3 | 1.4 | 1.4 | 1.6 |
| Fucose   | 0.5  | 3.4 | 3.6 | 4.4 | 1.6 |

1 Values are means from duplicate determinations. CP15, citrus pectin with low molecular weight of <15 kDa; HRC, hairy region concentrate.

Mucin-secretory effects of various pectins and other dietary fibers in HT29-MTX cells (Experiment 1). HT29-MTX cells were kindly provided by Dr. Thécla Lesuffleur (INSERM UMR S 938, Paris, France) (23). Cells were maintained in Dulbecco’s modified Eagle’s minimal essential medium (DMEM) (SIGMA-Aldrich, St Louis, MO) supplemented with 10% inactivated fetal bovine serum (Gibco, Grand Island, NY) and 1% antibiotic-antimycotic solution (Antibiotic-Antimycotic Solution; 1.0×10^7 units of penicillin, 10 g of streptomycin, and 25 mg of amphotericin B/L, Gibco) at 37°C in a 10% CO₂/90% air atmosphere. The cells were seeded at 1×10⁶ cells in 6-well plates (NUNC, Roskilde, Denmark) and pre-cultured for 7 d. The cells were washed with PBS 3 times and cultured with 1 mL of DMEM including 1% media supplement (Insulin-Transferrin-Sodium Selenite Supplement: 10 mg of human insulin, 5.5 mg of human transferrin, and 5.5 mg of sodium selenite/L, SIGMA-Aldrich) in the presence of 100 mg/L of pectins and other dietary fibers for 24 h. One milligram/L of citrus pectin with low molecular weight of <15 kDa; HRC, hairy region concentrate.

Mucin-secretory effects of HRC and F1–F3 in HT29-MTX cells (Experiment 2). HT29-MTX cells were prepared as in the Experiment 1. HRC and its fractions (F1, F2, and F3) dissolved in DMEM with 1% media supplement were passed through a 0.20 μm filter (Millipore, Bedford MA, USA) for sterilization. The cells were treated with 30 mg/L of HRC or F1–F3, or 30, 100 mg/L of CP15 for 24 h. In order to examine dose-dependent effects, the cells were treated with 0.1–30 mg/L of F1–F3 for 24 h. Secreted mucins into the media were measured as in the Experiment 1. Separately, time-dependent effects were also assessed in the cells treated with 1 or 10 mg/L of F1 for up to 24 h. The culture media and the cells were gathered and subjected to MUC5AC and quantitative RT-PCR analyses, respectively.

Mucin-secretory effects of low-methoxyl AP and HRC in rat small intestine (Experiment 3). Male Sprague-Daley rats (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were housed individually in screen-bottomed, stainless steel cages in a temperature- (23±2°C) and light- (lights on from 7:00–19:00) controlled room. The rats were acclimatized for 5 d and allowed free access to water and a control diet (25) formulated from 250 g/kg of casein, 652.5 g/kg of cornstarch and 50 g/kg of corn oil. The remainder of the diet consisted of AIN-76 vitamin mixture (10 g/kg), AIN-76 mineral mixture (35 g/kg), and choline bitartrate (2.5 g/kg). Animal experiment was performed according to protocol approved by the Animal Use Committee of Shizuoka University (approval no. 29-14), and the rats were maintained in accordance with the Shizuoka University guidelines for the care of laboratory animals. After acclimatization, 48 rats (90–112 g, 5 wk old) were divided into 4 groups of 12 rats and allowed free access to the control diet or a diet containing low-methoxyl AP (50 g/kg diet) or HRC (7.5 g and 15 g/kg diet) for 7 d. Low-methoxyl AP or HRC was added to the control diet at the expense of an equal amount of cornstarch. At the end of the experiment, rats were killed by decapitation under pentobarbital anesthesia, and the small intestine was excised. Luminal contents were collected as described previously (7) and stored at −30°C until luminal mucin analysis. The upper half of the small intestine except for the duodenum was defined as the jejunum and the lower half was defined as the ileum. Portions of the segments were used for histologic evaluation and mucosa preparation, which was used for the isolation of total RNA and quantitative RT-PCR analyses.

MUC5AC analysis. Released MUC5AC from HT29-MTX cells was measured by enzyme-linked immuno sorbent assay (ELISA) as described previously (10). Briefly, the supernatants were appropriately diluted with 0.1 mol/L bicarbonate–carbonate buffer (pH 9.6) and plated on 96-well plates (F96 MAXISORP NUNC IMMUNO PLATE, catalog number 430341, Thermo Fisher Scientific, Somerset, NJ). MUC5AC was detected by anti-human MUC5AC antibody (Clone 1-13M1 mAb, Abcam, Cambridge, UK) and peroxidase-conjugated anti-mouse antibody (SIGMA-Aldrich). A standard curve was generated by performing serial dilutions of mucin fraction obtained from the culture media of HT29-MTX cells.

RNA isolation and quantitative RT-PCR. Total RNA isolation and quantitative real-time PCR were performed as previously described (10). The primer pairs and protocols for PCR of MUC5AC (10), Muc2 and 18S rRNA
have been previously reported. 18S rRNA was used as an endogenous reference gene. Gene expression was quantified using the comparative CT method (26), and the data were expressed relative value to the control group.

Luminal mucin analysis. The luminal mucin fraction was isolated using the method of Lien et al. (27) with some modification (7), and was dissolved in 5.0 mL of distilled water for mucin analyses. After an appropriate dilution of the mucin fraction and plating on 96-well plates as in MUC5AC analysis, luminal mucin was measured by using rabbit anti serum against purified luminal mucin (1:12,000 dilution) (28), biotinylated goat anti-rabbit IgG (1:2,000 dilution) and peroxidase–avidin conjugate as descrived previ-ously (8, 28).

Histologic evaluation. The segments were placed in 10% buffered formalin for 24 h and then paraffin-embedded. Six 5-μm-thick cross-sections were prepared per rat from paraffin-embedded samples and stained with periodic acid Schiff, counter-stained with hematoxylin. Five complete villi (entire crypt/villus axis) per section were selected and villus length and the number of goblet cells per villus (left side) were determined at the light microscopic level (Olympus BH2).

Statistical analyses. Data were analyzed by one-way ANOVA. Differences between the groups were analyzed by Dunnett’s test or Tukey-Kramer test. Results from Experiment 1 and dose-dependent responses of F1–F3 of HRC in Experiment 2 were analyzed by Dunnett’s test to compare the control group with treatment groups. In other experiments, Tukey-Kramer test were employed to compare between all groups. The results were expressed as means±SE and a 5% level of probability was considered significant for all analyses. The statistical calculations were carried out using JMP8 software (SAS.
Institute, Cary, NC).

RESULTS

Mucin-secretory effects of pectins and other dietary fibers in HT29-MTX cells (Experiment 1)

HT29-MTX cells treated with various pectins for 24 h revealed that all types of pectin at the dose of 100 mg/L significantly increased MUC5AC secretion to a similar extent (50–80% increases) compared to the control. These effects were observed irrespective of the differences in the plant origin, degree of methoxylation and molecular weight (Table 2). Mucin-secretory effects of pectins were almost comparable to that of LPS (1 mg/L). In contrast, other dietary fibers at dose of 100 mg/L, except fucoidan, did not show any effects on MUC5AC secretion.

Mucin-secretory effects of HRC, F1–F3, and CP15 in HT29-MTX cells (Experiment 2)

HRC and F1–F3 at the dose of 30 mg/L significantly and equally increased MUC5AC secretion from HT29-MTX cells compared to the control, whereas CP15 as a starting material of HRC increased MUC5AC secretion only at the dose of 100 mg/L (Fig. 2A). The dose-dependent effects of F1–F3 showed that F1 significantly increased MUC5AC secretion from 0.3 mg/L, much stronger than that of LPS (1 mg/L), whereas the significant effects of F2 and F3 were obtained from 3 mg/L (Fig. 2B). The time-dependent effects of F1 revealed that 1 and 10 mg/L of F1 significantly increased MUC5AC secretion at 12 and 3 h after treatment, respectively (Fig. 3A). Further, transient increases in mRNA expression of MUC5AC were observed at 6 h after treatment of F1 at the both of 1 and 10 mg/L (Fig. 3B). Collectively, F1, F2 and F3 derived from HRC were supposed to be 30–100 times more potent in mucin-secretory effect than CP15.

Mucin-secretory effects of low-methoxyl AP and HRC in the rat small intestine (Experiment 3)

Food intake and body weight gain did not differ among the groups (Table 3). Villus length and the number of goblet cells in the jejunum and ileum did not differ among the groups. Muc2 expression in the jejunum was greater only in rats fed low-methoxyl AP diet than in

Table 3. Food intake, body weight gain, luminal mucin contents, and goblet cell numbers in the small intestine in rats fed the control diet or a diet containing 0.75 or 1.5% HRC or 5% low-methoxyl AP for 7 d.1

| Condition       | Control | 0.75% HRC | 1.5% HRC | 5% low-methoxyl AP | ANOVA |
|-----------------|---------|-----------|----------|-------------------|-------|
| Food intake, g/7 d | 102 ± 2 | 99 ± 2    | 107 ± 3  | 102 ± 3           | 0.22  |
| Body weight gain, g/7 d | 58 ± 1 | 60 ± 2    | 61 ± 2   | 60 ± 2            | 0.69  |
| Small intestine tissue |       |           |          |                   |       |
| Jejunum         |         |           |          |                   |       |
| Villus length, mm | 462 ± 10 | 438 ± 16  | 445 ± 13 | 433 ± 17          | 0.18  |
| Goblet cells, n/villus left side | 10.0 ± 0.1 | 9.9 ± 0.1 | 9.8 ± 0.1 | 10.1 ± 0.1        | 0.37  |
| Muc2, relative value | 1.0 ± 0.1b  | 1.1 ± 0.1b| 0.9 ± 0.1b| 1.4 ± 0.2a         | <0.01 |
| Ileum           |         |           |          |                   |       |
| Villus length, mm | 368 ± 10 | 380 ± 16  | 336 ± 14 | 329 ± 18          | 0.11  |
| Goblet cells, n/villus left side | 10.0 ± 0.0 | 10.0 ± 0.0| 9.9 ± 0.1 | 10.0 ± 0.1        | 0.63  |
| Muc2, relative value | 1.0 ± 0.1 | 1.2 ± 0.1| 1.0 ± 0.1 | 1.3 ± 0.2          | 0.14  |
| Small intestine contents |       |           |          |                   |       |
| Mucin, mg       | 1.7 ± 0.1b | 1.8 ± 0.2b| 2.1 ± 0.2ab| 2.7 ± 0.2a        | <0.01 |

1 Values are means ± SE, n = 12. Means in a row without a common letter are significantly different (p < 0.05) when analyzed by Tukey-Kramer test. AP, apple pectin; HRC, hairy region concentrate.
those fed the other diets, while Muc2 expression in the ileum was comparable among the groups. Consumption of low-methoxyl AP diet significantly increased luminal mucin contents in the small intestine. Luminal mucin contents in rats fed the 1.5% HRC diet increased by 24% compared to those in rats fed the control diet, but the difference did not reach significant.

**DISCUSSION**

In the present study, we first compared the mucin-secretory effects of variety of pectins with other dietary fiber materials in HT29-MTX cells, and then prepared the HRC of pectin, as possible active constituents for mucin secretagogue, and finally evaluated the biological effects of HRC in HT29-MTX cells and in rat small intestine.

All types of pectin significantly increased MUC5AC secretion in HT29-MTX cells, irrespective of molecular size, their plant origins and degree of methoxylated. In contrast, other dietary fiber materials including sodium alginate (uronic acid polymer) and oligogalacturonic acids did not show any effects, except fucoidan. Using an isolated vascularly perfused rat colon, Barcelo et al. reported that sulfated polysaccharides such as ulvan, carrageenan and fucoidan stimulated mucus discharge from the goblet cells depending on the abundance of sulfate moiety in the molecules (29). Thus, apart from action mechanisms, among dietary fiber materials, pectins and sulfated polysaccharides are likely to commonly stimulate mucin secretion.

Pectin molecule consists of smooth regions, which are partly methoxylated linear homogalacturonan, and hairy regions with highly branched neutral sugar side chains (11). Because both oligogalacturonic acid and sodium alginate, an uronic acid polymer and oligogalacturonic acids did not show any effects, except fucoidan. Using an isolated vascularly perfused rat colon, Barcelo et al. reported that sulfated polysaccharides such as ulvan, carrageenan and fucoidan stimulated mucus discharge from the goblet cells depending on the abundance of sulfate moiety in the molecules (29). Thus, apart from action mechanisms, among dietary fiber materials, pectins and sulfated polysaccharides are likely to commonly stimulate mucin secretion.

The casein-derived peptides (casomorphines) and LPS have been known to bind cell-surface-μ opioid receptor or toll-like receptor 4, respectively and stimulate mucin secretion in HT29-MTX cells through signal transductions (24, 30). Like these, one might consider that CP15, HRC, and F1 are recognized by a certain cell-surface receptor. Galactan side chain in RG-I of pectin molecule has been reported as an important structural feature for the direct binding of modified CP to galectin-3 on the cancer cells (16). In the present study, however, pretreatment of galactose, several galactooligosaccharides, or arabinogalactan (100 mg/L) did not compete and inhibit mucin-secretory effects of CP15, HRC, and F1 in HT29-MTX cells at all (data not shown).

In accordance with our previous studies (9, 10), ingestion of 5% low-methoxyl AP diet increased luminal mucin contents without an increase of the goblet cell numbers in the rat small intestine. Moreover, the increase in luminal mucin contents was accompanied by upregulation of Muc2 expression in the jejunum. Unexpectedly, the luminal mucin contents in rats consumed 1.5% HRC diet increased by 24% compared to those in rats consumed the control diet, but the difference did not reach significant. The present study was designed on the premise that the dietary levels of 0.75% and 1.5% HRC were corresponding to the dietary level of 5% pectin as a whole, because the recovery of HRC from pectin (CP15) was around 20%. The reason for the unexpected result was unclear, but might be related to differences in the intestinal behavior between HRC and intact pectin, in turn a luminal viscosity and its relation to the potential of adhesion to epithelial cells. In addition, there are limitations with the use of a cell culture model in this study. One limitation is that the responses of HT29-MTX cells may not precisely reflect the in vivo responses due to gene mutation and culture conditions, though the HT29-MTX cell model has proven to be a reliable tool for the study of gastrointestinal mucin secretion (30–34). Another limitation is that mucin secretory effects in HT29-MTX were examined by measurements of gastric mucin (MUC5AC) but not by intestinal mucin (MUC2). This is a technical limitation that there was no suitable antibody for detection of MUC2 by ELISA. However, previous studies investigating mucin secretory effects of probiotics showed that MUC2 and MUC5AC responses against probiotics were quite similar in HT29-MTX cells (33, 34). Nevertheless, in so far as the comparison in vitro the mucin-secretory effect of HRC was much stronger than the existing mucin secretagogues such as LPS and casomorphines. Therefore, future study would be worthwhile to test the mucin-secretory effects of HRC in rats with higher dietary level.

In conclusion, the mucin-secretory effect of pectin is unique among dietary fiber materials. The active constituent of pectin is supposed to be hairy region, i.e., HRC rather than the smooth region, which activity is much stronger than the existing mucin-secretagogues in HT29-MTX cells. At present, however, the effect of HRC has not been verified in the rat small intestine.
Authorship
Research conception and design: SH and TM; experiments: SH, NN; statistical analysis of the data: SH, NN; interpretation of the data: SH, NN, and TM; writing of the manuscript: SH and TM.

Disclosure of state of COI
S. Hino, N Nishimura and T. Morita, no conflicts of interest.

Acknowledgments
This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Supporting information
Supplemental online material is available on J-STAGE.

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