

**Changes in the small intestinal mucin contents in rats were evaluated by two methods, viz., a newly established ELISA and a method based on the measurement of O-linked oligosaccharide chains (OSC) as a mucin marker. Significant correlation was observed between the values of ELISA-derived mucins and OSC. The results confirm the usefulness of measurement of OSC as an alternative method for mucin determination.**

**Key words:** mucins; ELISA; O-linked oligosaccharide chains; rat

The main functional properties of the mucin secreted by goblet cells are lubrication of the gut and action as a diffusion barrier against microorganisms. There is now growing evidence in experimental animals that luminal mucins are influenced by diet. Recently, Satchithanandam et al. evaluated luminal mucin secretion using ELISA in rats, and found that diets supplemented with 5% citrus fiber significantly increased the small intestinal mucin content, but not those with 20% cellulose or rice bran. In contrast, we found that small intestinal mucins, as assessed by O-linked oligosaccharide chains (OSC), were secreted in a way related to the bulk-forming property of dietary indigestible components in diets. Luminal mucin secretion by dietary fiber, then, is somewhat complicated. Although previous studies using ELISA for mucin determination did not define the bulk-forming property of the dietary fibers, it is also possible that different methods of mucin determination might lead to different results. In the present study, therefore, an attempt was made to evaluate small intestinal mucins by the two methods above. The results were compared and are discussed here.

Small intestinal mucins were purified according to the method of Satchithanandam et al. Briefly, mucus in the intestinal surface was sucked into a bottle with a phosphate buffered saline (PBS, pH 7.4) containing 1% sodium azide. The mucus was homogenized and centrifuged at 6,000 × g for 30 min at 4 °C to solubilize the mucins. The supernatant was dialyzed against 0.02% sodium azide solution, and then the sample was applied to a Superdex 200 pg column (26 × 60 cm) followed by a Sepharose 4B column (26 × 60 cm) to isolate the mucin fraction. The columns were eluted with 0.1 M phosphate buffer (pH 7.1) containing 0.02% sodium azide, and 5-ml fractions of each were collected. Proteins and glycoproteins in all fractions were measured by the methods of Lowry et al. and Mantle and Allen. Identification of purified mucins was analyzed by SDS/PAGE and Western-blotting to test the reactivity of peanut agglutinin toward the mucins. Anti-serum against rat small intestinal mucins was prepared by the method of Vaitukaitis et al., with modifications. Purified mucins (100 μg) were mixed with Freund’s complete adjuvant and then injected intradermally. The same process was repeated weekly for 2 weeks, except that incomplete adjuvant was substituted for complete adjuvant. Then three injections of purified mucins (50 μg) were made intravenously into rabbits at weekly intervals. One week after the final injection, blood was drawn and the serum was collected and stored at −80 °C. Recognition of intestinal mucins by anti-mucin antiserum was confirmed immunohistologically using rat ileal tissue. Sections (4 μm) of paraffin-embedded rat ileal tissue were incubated with blocking serum, and then with anti-mucin antiserum (1:500 dilution) at 4 °C overnight. The sections were sequentially exposed to biotinylated goat anti-rabbit IgG (1:2,000 dilution), peroxidase–avidin conjugate (1:2,000 dilution), and 3,3′-diaminobenzidine solution. All the reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan).

An animal experiment was conducted to produce...
different mucin contents in the rat small intestine. Male Wistar rats (purchased from Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were maintained at 23 ± 2°C under a 12-h light/12-h dark cycle under the approval of the Animal Use Committee of Shizuoka University, Faculty of Agriculture. Rats had free access to food and water during the experiment. A basal diet10) was 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 was essential vitamins and minerals. After adaptation to the basal diet, rats weighing 131–159 g were divided into 2 groups (15 rats per group) and fed the basal diet or one containing 7% polystyrene foam (PSF, 30–50 mesh) for 7 d. PSF, a model substance for high-bulk forming dietary fiber,4,10) was added to the diet at the expense of an equal amount of cornstarch. At autopsy, small intestinal contents were gathered and the mucin fraction was recovered as a 60% ethanol precipitate and the contents were finally dissolved in 5.0 ml of distilled water, as described previously.4) The obtained mucin fraction was used for preparation of hyperimmune sera. Immunohistochemical analyses of rabbit anti-mucin antiserum clearly showed that antiserum specifically reacted with goblet cells in the ileum (Fig. 1B). Hence, we established an ELISA method for mucin determination using the above anti-mucin antiserum. The equation of standard curve produced by ELISA was as follows: Y = 401.6X + 1.44 (r = 0.998), where Y is mucin-derived protein (ng protein/ml) from purified small intestinal mucins, and X is absorbance at 450 nm. ELISA is highly reproducible and had a working range of 15 to 500 ng of mucin protein/ml. The anti-mucin antiserum did not react at all with rat-stomach mucins, which are composed mainly of MUC-5,11) but showed a cross-reactivity of 35% against rat-colonic mucins (mainly MUC-3, but also 2 and 411)) as compared with rat-small intestinal mucins (mainly MUC-2 11)) as 100%. These findings suggest that the anti-mucin antiserum prepared in the present study recognizes the peptides near the linker of mucin molecules. These features of our antiserum are very similar to those of Satchithanandam et al.2)

Food intake and body weight gain did not differ between the groups. Mucins as assessed by ELISA (ELISA-mucins), OSC, and sialic acid in the small intestinal contents were significantly greater in the PSF

![Fig. 1. SDS/PAGE and Western Blot Analyses of Rat Purified Mucins (A) and Immunohistochemical Analyses of Rabbit Anti-Mucin Antiserum on Rat Ileal Mucosa (B).](image)
group than those in the control group (Table 1). Figure 2 shows linear regression analyses among ELISA-mucins and other variables. ELISA-mucins were significantly correlated with OSC and sialic acid, but not with protein.

Accordingly, it is concluded that changes in small intestinal mucin contents are predictable by both methods, viz., measurements of OSC and ELISA mucins. Also, the present results endorse the previous finding\(^4\) that dietary indigestible components with a high-bulk forming property stimulate luminal mucin secretion. Therefore, it is plausible to assume that the discrepancy between the results of Satchithanandam et al.\(^3\) and ours\(^4\) are due primarily to differences in the bulk-forming properties of the dietary fiber preparations used in the experiments. Further, the present results suggest that measurement of OSC should be of value in mucin determination as an alternative method of ELISA, which is relatively sensitive but is laborious in the preparation of mucin anti-sera.

**References**


9) Vaitukaitis, J., Robbins, J. B., Nieschlag, E., and Ross,
