Disaccharidase Activity in Rat Cecum and Colon with Hyperplasia Induced by Maltitol or Glucomannan

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Summary The existence of disaccharidases and an enzyme that hydrolyzes maltitol were investigated in the large intestine of rats. In addition, the properties of disaccharidases were studied in the cecum and colon with hyperplasia induced by the ingestion of nondigestible carbohydrates such as maltitol and glucomannan. Maltase activity was detected in the cecal and colonic mucosa of rats fed a regular diet, although it was a very low level as compared with that in the small intestinal mucosa. Maltitol hydrolysis was notably lower in the cecum and colon than in the small intestine. The Km of maltose was 5.56 mM in the small intestine and 5.59 mM in the cecum, while that in the colon was 2.56 mM. The Vmax of maltose was at very low levels in the cecum (0.38 μmol/mg protein/h) and colon (0.37 μmol/mg protein/h) in comparison with that in the small intestine (30.3 μmol/mg protein/h). With regard to the maltitol hydrolyzing enzyme, Km and Vmax were 2.00 mM and 2.51 μmol/mg protein/h in the small intestine, respectively. Km and Vmax in the cecum and colon could not be measured because the level was too low. The tissue weights of the cecum and colon increased significantly in both the maltitol (p<0.01, p<0.05) and glucomannan (p<0.01, p<0.05) groups in comparison with that of the control group. The specific activity of maltase decreased significantly in both the maltitol (p<0.01, p<0.05) and glucomannan (p<0.05, p<0.05) groups in comparison with that of the control group. The specific activity of maltase decreased significantly in the small intestine of the maltitol (p<0.05) and glucomannan (p<0.01) groups. However, maltase activity in the cecum and colon was not lowered by maltitol ingestion, although it decreased significantly in the cecum of the glucomannan group (p<0.01). Sucrase activity in the small intestine and cecum was decreased significantly by maltitol (p<0.05, p<0.01) or glucomannan (p<0.01, p<0.01) ingestion, whereas it was not decreased in the colon. Maltitol hydrolyzing activity did not decreased significantly in the small intestine of the maltitol group, although that in the cecum and colon was not measured exactly by the methods used here. These results demonstrate that disaccharidases exist in the cecal and colonic mucosa of rat, and that they are not induced even

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Sugar alcohols such as erythritol (1, 2), maltitol (3, 4), lactitol (5) and paratinit (6, 7) have been developed as reduced energy sweeteners that are useful in solving the problems of obesity and its complications such as diabetes mellitus and heart disease. Furthermore, sugar alcohols do not induce tooth decay (8) and do not stimulate the secretion of insulin (9). Erythritol is absorbed from the small intestine and excreted readily to the urine without any degradation, while other sugar alcohols are little hydrolyzed by digestive enzymes and instead are fermented by intestinal microbes (10). In addition, these sugar alcohols induce tissue enlargement in the cecum and colon of rats (1, 3).

It has also been found that dietary fiber causes morphological and physiological changes in the gastrointestinal tract, in particular in the large intestine (11–17). The effect of water-soluble fiber, glucomannan, is greater in the cecum than in the colon, whereas the effect of water-insoluble fiber, cellulose, is stronger in the colon than in the cecum (17–20). Nondigestible sugar alcohols and oligosaccharides as well as dietary fiber induce enlargement of the cecum and colon (3, 21). The tissue enlargement induced by dietary fiber ingestion is a hyperplasia that increases the number of mucosal cells, and can be recovered by the elimination of dietary fiber from the diet (14, 17). In addition to these morphological changes, the specific activity of (Na + K)ATPase increases proportionally to the tissue enlargement.

The existence and properties of disaccharidases and the enzyme that hydrolyzes maltitol have not been investigated previously in the large intestine. Furthermore, it is not clear whether disaccharidases in the cecal and colonic mucosa are affected by tissue enlargement induced by nondigestible carbohydrate ingestion. The purpose of this study is to clarify whether or not the properties of disaccharidases are different between the small and large intestines, and to investigate the influence of maltitol and glucomannan ingestion on disaccharidase activity in the cecal and colonic mucosa of rats.

METHODS

Animals and diets. Male Wistar rats (Nisseizai Co., Tokyo, Japan) weighing about 150 g were raised on a diet containing 10% maltitol or 10% glucomannan, as shown in Table 1, for 10 d. The only variable in the experimental diet was the nondigestible carbohydrate source. Four or five rats were randomly assigned one of three different diets and housed in stainless-steel, wire-bottomed cages in a room with a 12 h light-dark cycle at 25 ± 3°C. Food and water were provided ad libitum. Food was removed 18 h before the rats were studied.

Tissue weighing and preparation of mucosal homogenates of small intestine, cecum and colon. After decapitation and draining of the blood from the rat, the small
Table 1. Composition of diets. (%)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control</th>
<th>10% Maltitol</th>
<th>10% Glucomannan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch</td>
<td>70.0</td>
<td>60.0</td>
<td>60.0</td>
</tr>
<tr>
<td>Casein</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Salt mixture*</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mixture**</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Maltitol</td>
<td>0</td>
<td>10.0</td>
<td>0</td>
</tr>
<tr>
<td>Glucomannan</td>
<td>0</td>
<td>0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

*** AIN-76 composition.

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intestine, cecum and colon were removed immediately and the whole cecum including the contents was weighed. Then, intestine, cecum and colon were slit open, rinsed with ice-cold physiological saline and blotted with tissue paper. After measurement of the wet weight, the mucosa was scraped with a glass slide and homogenized with a Potter-Elvehjem homogenizer in nine volumes of the same saline solution. The homogenate was passed through two layers of cheese cloth to remove cell debris, and the aliquot of the filtered homogenate was suitably diluted and assayed for disaccharidase activity and the enzyme that hydrolyzes maltitol.

**Measurement of disaccharidase activity in intestinal mucosa.** The hydrolysis of maltose, sucrose and maltitol by the small intestinal, cecal and colonic mucosa homogenates was determined by the method of Dahlqvist with glucose oxidase (22). The substrate concentration was 56 mM in 100 mM sodium maleate buffer (pH 6.0). The reactions were performed at 37°C for a period adequate to determination. The activity was expressed as micromoles or nanomoles substrate hydrolyzed/mg protein/h. The protein concentration was measured by the method of Lowry et al using bovine serum albumin as the standard (23).

**Chemicals.** Maltitol (more than 99.9% pure) and maltose (more than 99% pure) were kindly provided by Hayashibara Co., Ltd. (Okayama, Japan), and glucomannan was purchased from Shimizu Chemical Co., Ltd. (Fukuyama, Japan). All other reagents employed were of analytical grade.

**Statistical analysis.** The results were expressed as mean values with standard deviation. Significance of differences was calculated by 2-way analysis of variance (ANOVA) followed by Tukey’s pairwise multiple-comparison procedure using a significance level of p < 0.05.

**RESULTS**

The hydrolysis of maltose and maltitol was measured using the mucosal homogenate of small intestine, cecum and colon of rats fed a regular chow. The
time course of maltose and maltitol hydrolysis is shown in Fig. 1. In the small intestine, maltitol hydrolysis was remarkably lower than maltose hydrolysis. Moreover, maltose hydrolysis in the cecum and colon was notably lower than that in the small intestine. However, maltitol hydrolysis in the cecum and colon was not detectable by the method used here.

According to these results, the Michaelis constant (Km), maximal velocity (Vmax) of maltose and maltitol hydrolysis were calculated by the graphical method of Lineweaver and Burk (Table 2). With regard to maltose hydrolysis, Km in the small intestine and cecum was 5.56 and 5.59 mM, respectively, while that in the colon was 2.56 mM. The Vmax of maltose was at very low levels in the cecum (0.38 µmol/mg of protein/h) and colon (0.37 µmol/mg of protein/h) in comparison with that in the small intestine (30.3 µmol/mg protein/h). With regard to the maltitol hydrolyzing enzyme in the small intestine, Km and Vmax were 2.00 mM and 2.51 µmol/mg protein/h, respectively. However, the Km and Vmax of maltitol hydrolysis were not obtained because the hydrolyzing activity was very low and undetectable in either the cecum or colon.

When rats were raised on a diet containing 10% maltitol or 10% glucomannan for 10 d, the average body weight of the maltitol group was not significantly different from that of the control group, while that of the glucomannan group was significantly higher than those of the control (p < 0.01) and maltitol groups (p < 0.05) (Table 3). The difference of body weight gain between the glucomannan and maltitol groups appears to be concerned with the difference in food intake. In fact, food intake was higher in the glucomannan group (22.7 g/d) than in the maltitol group (21.2 g/d). Further, the body weight gain in the maltitol group might have been restrained because maltitol ingestion caused slight diarrhea in the early period of the experiment. The weight of whole cecum including the contents in both maltitol and...
Table 2. Km and Vmax of maltose and maltitol in the homogenate of small intestine, cecum and colon of rats fed a regular diet.

<table>
<thead>
<tr>
<th></th>
<th>Maltose</th>
<th>Maltitol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Km (mm)</td>
<td>Vmax (μmol/mg protein/h)</td>
</tr>
<tr>
<td>Small intestine</td>
<td>5.56</td>
<td>30.3</td>
</tr>
<tr>
<td>Cecum</td>
<td>5.59</td>
<td>0.38</td>
</tr>
<tr>
<td>Colon</td>
<td>2.56</td>
<td>0.37</td>
</tr>
</tbody>
</table>

Km and Vmax values calculated by the graphical method of Lineweaver-Burk. Each value represents the mean of two rats with duplicate determinations. N.D., not determined.

Table 3. Effect of maltitol or glucomannan ingestion on cecum and colon weight in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Cecum</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tissue + contents (g)</td>
<td>Tissue (g)</td>
<td>Tissue (g)</td>
</tr>
<tr>
<td>Control (n = 5)</td>
<td>213 ± 14</td>
<td>2.42 ± 0.32 (1.14)</td>
<td>0.65 ± 0.04 (0.31)</td>
</tr>
<tr>
<td>Maltitol (n = 4)</td>
<td>230 ± 11</td>
<td>6.77 ± 0.93** (2.94)</td>
<td>1.76 ± 0.13** (0.77)</td>
</tr>
<tr>
<td>Glucomannan (n = 4)</td>
<td>275 ± 14**.5</td>
<td>11.5 ± 1.11*** (4.18)</td>
<td>1.66 ± 0.25** (0.60)</td>
</tr>
</tbody>
</table>

Rats were fed with a diet containing maltitol or glucomannan at a 10% concentration for 10 d.
*,** and *** Significantly different from the control group at p<0.05, p<0.01 and p<0.001, respectively.
* Significantly different from the maltitol group at p<0.05.
( ), tissue weight (g) per 100 g of body weight.

glucomannan groups increased greatly as compared with those of the control group (p<0.01 and p<0.001, respectively). The tissue weights of the cecum and colon in both maltitol and glucomannan groups also increased greatly as compared with those of the control group (p<0.01 and p<0.05 for maltitol and glucomannan groups, respectively). The tissue weight per 100 g of body weight also increased markedly for the cecum and colon in both maltitol and glucomannan groups. The
volume and weight of cecal content in the glucomannan group was greater than that in the maltitol group because maltitol is decomposed readily by intestinal microbes. In fact, glucomannan is excreted partly to the feces, while maltitol is not detected in the feces. It is believed that cecal enlargement is closely related to the volume of cecal content (14, 16).

Disaccharidase activity in the small intestine is increased by the ingestion of saccharides (24, 25). On the other hand, tissue enlargement of the cecum and colon is caused largely by water-soluble rather than water-insoluble dietary fiber and the change in physiological function, such as (Na + K)ATPase activity occurring in the mucosa (15, 16). A morphological change in the small intestine is also induced. Therefore, the hydrolysis of disaccharides and maltitol may be affected in the cecum and colon, with hyperplasia induced by maltitol ingestion.

The specific activity of maltose hydrolysis in the small intestine decreased significantly in both the maltitol (p < 0.05) and glucomannan (p < 0.01) groups as compared with that of the control group (Table 4). The effect of glucomannan ingestion was stronger than that of maltitol ingestion. Maltase activity in the cecum was reduced significantly, not increased, by glucomannan ingestion, and produced a marginally significant decrease in maltitol ingestion. However, maltase activity in the colon was not changed significantly by maltitol or glucomannan ingestion.

Sucrase activity in the small intestine also decreased significantly in both the maltitol (p < 0.05) and glucomannan (p < 0.01) groups as compared with the control

Table 4. Effect of maltitol or glucomannan ingestion on activity of maltase, sucrase and maltitol hydrolysis in the small intestine, cecum and colon of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Substrates</th>
<th>Small intestine</th>
<th>Cecum</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(µmol hydrolyzed substrate/mg protein/h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Maltose</td>
<td>22.6 ± 0.97</td>
<td>0.42 ± 0.056</td>
<td>0.39 ± 0.026</td>
</tr>
<tr>
<td>(n = 5)</td>
<td>Sucrose</td>
<td>5.18 ± 0.25</td>
<td>0.097 ± 0.016</td>
<td>0.045 ± 0.008</td>
</tr>
<tr>
<td></td>
<td>Maltitol*</td>
<td>1.43 ± 0.10</td>
<td>0.042 ± 0.008</td>
<td>0.039 ± 0.001</td>
</tr>
<tr>
<td>Maltitol</td>
<td>Maltose</td>
<td>17.8 ± 1.14*</td>
<td>0.34 ± 0.040</td>
<td>0.40 ± 0.048</td>
</tr>
<tr>
<td>(n = 4)</td>
<td>Sucrose</td>
<td>4.03 ± 0.32*</td>
<td>0.050 ± 0.011**</td>
<td>0.042 ± 0.012</td>
</tr>
<tr>
<td></td>
<td>Maltitol*</td>
<td>1.13 ± 0.21</td>
<td>0.037 ± 0.008</td>
<td>0.027 ± 0.005*</td>
</tr>
<tr>
<td>Glucomannan</td>
<td>Maltose</td>
<td>11.7 ± 0.74**</td>
<td>0.29 ± 0.013**</td>
<td>0.40 ± 0.019</td>
</tr>
<tr>
<td>(n = 4)</td>
<td>Sucrose</td>
<td>2.49 ± 0.11**</td>
<td>0.038 ± 0.017**</td>
<td>0.054 ± 0.008</td>
</tr>
</tbody>
</table>

Rats were the same as in Table 3.

* Maltitol hydrolyzing-activity of cecum and colon was not measured exactly by the method used here because the activity was very low.
* and ** Significantly different from the control group at p < 0.05 and p < 0.01, respectively.
group (Table 4). The effect of glucomannan ingestion was stronger than that of maltitol ingestion, as maltase, in the small intestine. Sucrase activity in the cecum was also decreased by maltitol \((p<0.01)\) or glucomannan ingestion \((p<0.01)\). However, a significant decrease in sucrase activity was not observed in the colon of either maltitol or glucomannan group. Namely, there were no significant differences in the specific activity of sucrase in the colon of the three groups.

The specific activity of maltitol hydrolysis in the small intestine and cecum was not changed significantly by maltitol ingestion but was at a very low level (Table 4). However, a significant effect on maltitol hydrolyzing activity in the colon was observed, although the activity was not measured exactly by the methods used here because the level was very low.

In conclusion, these results demonstrate that disaccharidases such as maltase and sucrase and the enzyme which hydrolyzes maltitol are not induced, even in the mucosa of the cecum and colon, with hyperplasia that is caused by maltitol or glucomannan ingestion.

**DISCUSSION**

The existence of disaccharidases and an enzyme that hydrolyzes maltitol were investigated in the large intestine of rats. In addition, the properties of disaccharidase were studied in the cecum and colon with hyperplasia caused by nondigestible carbohydrate ingestion.

Maltose hydrolysis was detected in the cecal and colonic mucosa of rats fed a regular chow, although this was at a very low level as compared with that in the small intestinal mucosa. The time-dependent increase of maltose hydrolysis strongly suggests the existence of maltase in the cecal and colonic mucosa (Fig. 1). Maltose which is taken directly from foods and produced from starch by hydrolysis is not transferred to the large intestine because it is digested by maltase and absorbed readily from the small intestine. Therefore, maltase in the large intestine does not seem to have any physiological significance. In contrast, maltitol is hydrolyzed very slowly by maltase in the small intestinal mucosa and competitively inhibits the hydrolysis of maltose \(4, 26\). However, it is believed that most of the maltitol which is ingested orally reaches the large intestine and is fermented readily by microbes \(27\). In fact, when sufficiently large amounts of maltitol are administered orally, it regularly produces a high osmotic diarrhea without significantly increasing blood glucose levels \(3, 9\). However, maltitol which reaches the large intestine might be hydrolyzed by the enzyme that is induced by maltitol ingestion because maltitol ingestion causes hyperplasia and the enhancement of \((\text{Na} + \text{K})\text{ATPase}\) activity in the cecum and colon of rats in the same manner as water-soluble dietary fiber \(16, 17\). But the possibility of maltitol being digested by digestive enzymes in the large intestine was denied by the results of this study. The hydrolyzing activity of maltitol was very low, less than \(1/8\) of maltose, in the cecum and colon, although it was not measured exactly by the method used here, and decreased, not increased,
after maltitol ingestion. It appears that maltitol is not actually digested in the large intestine.

The $K_m$ for maltose in the cecal mucosa homogenate was 5.56 mM and was very close to the $K_m$ (5.56 mM) in the small intestine. But it was clearly different from the $K_m$ (2.56 mM) for maltose in the colonic mucosa homogenate. These results suggest that maltase in the cecum is of the same origin as that in the small intestine, but maltase in the colon is of a different origin from that in the cecum and small intestine. The reason is unknown but may be related to the physical arrangement of the organs; the cecum is closely connected to the small intestine, while the colon is far away from the small intestine. The $V_{max}$ for maltose in the cecal mucosa was 0.38 $\mu$mol/mg protein/h and was very close to that in the colon, 0.37 $\mu$mol/mg protein/h. But these levels were only 1/100 of those in the small intestine.

The $K_m$ for maltitol in the small intestinal mucosa was 200 mM and was much higher than that for maltose, and the $V_{max}$ was 1/12 that of maltose. $K_m$ and $V_{max}$ for maltitol in the cecum and colon could not be determined because the hydrolyzing activity was at a very low level.

It has been reported that unavailable carbohydrates such as dietary fiber and sugar alcohols increase the length and weight of the large bowel in rats and cause hyperplasia, in which the cell number increases (14, 15). Both the maltitol and glucomannan used in this experiment caused tissue enlargement of the cecum and colon as shown in previous papers (3, 14). When rats were raised on a diet containing 10% maltitol or 10% glucomannan for 10 d, the effect on the weight of the whole cecum including contents was stronger with glucomannan ingestion than with maltitol ingestion, although the effect on the tissue weight of the cecum was not significantly different between the two groups. The effect on tissue enlargement was greater in the cecum than in the colon. This result was similar to the relation between Neosugar and glucomannan (21).

The hydrolysis of maltose increased linearly during the incubation periods in the cecum and colon of rats fed a diet containing 10% maltitol or 10% glucomannan for 10 d. Maltase activity in the cecal mucosa was higher in the control group than in the groups with maltitol or glucomannan ingestion, while it was not significantly different between the groups with maltitol ingestion and glucomannan ingestion. Similar results have been obtained from other experiments with dietary fiber (17). It seems that the epithelial surfaces of the cecum and colon are damaged nonspecifically by the large volume ingestion of nondigestible saccharides that have a bulking effect such as maltitol and glucomannan. These results demonstrate that the sufficient ingestion of nondigestible saccharides such as maltitol and glucomannan, which have a bulking effect, does not induce the enzyme which hydrolyzes maltitol, although it does cause tissue enlargement of the cecum and colon.
REFERENCES


