Digestibility Characteristics of Isomaltooligosaccharides in Comparison with Several Saccharides Using the Rat Jejunum Loop Method

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Received July 11, 1994

Isomaltooligosaccharides (IMO) are a mixture of isomaltose, isomaltotriose, panose, isomaltotetraose, etc. IMO and its hydrogenated derivative (IMH) were characterized for their luminal clearance from rat jejunum loops as the indication of their digestibility. They were compared with a disaccharide fraction (IM2) and a higher oligosaccharide fraction (IM3) prepared from IMO, typical digestible saccharides (maltose, maltotriose, and sucrose), and typical nondigestible saccharides (maltotriose, raffinose, and fructooligosaccharides (FO)). The clearance rate of IMO was significantly smaller than that of IM2, which was mainly composed of isomaltose (64.3%), and digestible saccharides, and significantly larger than that of nondigestible saccharides. That of IM2 was almost the same as that of sucrose or maltotriose but significantly smaller than that of maltose. That of IM3 tended to be smaller than that of IMO, and larger than that of nondigestible saccharides. That of IMH was significantly smaller than that of IMO and similar to that of maltitol. These results seem to indicate that IMO is slowly digested in the jejunum, that the components having higher degree of polymerization of IMO are less digestible, and that IMH is nondigestible.

Isomaltooligosaccharides (IMO) are enzymatically manufactured from starch as a mixture of α(1→6) linked glucosides, such as isomaltose, isomaltotriose, panose, and isomaltotetraose. IMO is a sugar substitute with 40% of the sweetness of sucrose and has been used widely in various foods and drinks. It has various physiological actions on human and animals such as promoting the growth of intestinal bifidobacteria.1-3)

The digestibility of IMO in digestive tract is proposed to be different from that of nondigestible saccharides, for example, fructooligosaccharides (FO), also a growth factor of bifidobacteria. IMO may be partly digested by the enzymes of the small intestine,4,6) while FO is hardly digested.7-10) Because of this difference in the gastrointestinal digestibility, the energy value evaluated from the human metabolic study was found to be 2.8–3.2 kcal/g for IMO6) and 1.5 kcal/g for FO.10) However, very few investigations have been undertaken to clarify the digestibility of IMO in the digestive tract.

In the digestive tract, saccharides are digested by secretory enzymes and membrane enzymes on the surface of the intestinal mucosa, followed by immediate absorption of the enzymatic digest, that is, monosaccharides.11) Therefore, an in vitro system is insufficient to estimate the digestion and absorption of saccharides, since there is no process by which the digest are absorbed. An in situ perfusion system is preferred to characterize the digestion and absorption of saccharides in the digestive tract.

The composition of IMO is very complicated, because its degree of glucose polymerization ranges from di- to hexasaccharides, and glucosidic linkage varieties exist, for example, maltotriose, isomaltotriose, and panose in trisaccharide components. Consequently, it is difficult to obtain the individual components of IMO in a definite amount.

In this study, two fractions of IMO were prepared with difference of polymerization degree and used.

The purpose of this study is to characterize the digestibility of IMO and its hydrogenated derivative (IMH) in comparison with the fractions prepared from IMO and several saccharides, by the in situ perfusion system using the rat jejunum loop, which was done to discover the residual amounts and compositional changes of the perfusate.

Materials and Methods

Test saccharides. Isomalto-900® (Showa Sangyo Co., Ltd., Tokyo, Japan) is a commercially available IMO, disaccharide fraction of IMO (IM2) and higher oligosaccharide fraction of IMO (IM3) were fractionated from

| Table | Composition of IMO, IM2, and IM3
<table>
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<tr>
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<tbody>
<tr>
<td>Components</td>
<td>IM0</td>
</tr>
<tr>
<td>DP1 Glucose</td>
<td>3.8</td>
</tr>
<tr>
<td>DP2 Maltose</td>
<td>4.5</td>
</tr>
<tr>
<td>Isomaltose</td>
<td>22.8</td>
</tr>
<tr>
<td>Others</td>
<td>13.1</td>
</tr>
<tr>
<td>DP3 Maltotriose</td>
<td>0.9</td>
</tr>
<tr>
<td>Panose</td>
<td>11.6</td>
</tr>
<tr>
<td>Isomaltotriose</td>
<td>16.7</td>
</tr>
<tr>
<td>DP4 Isomaltotetraose and others</td>
<td>17.7</td>
</tr>
<tr>
<td>DP5 Isomaltoolpentaose and others</td>
<td>7.2</td>
</tr>
<tr>
<td>DP6 Isomaltohexaose and others</td>
<td>1.7</td>
</tr>
<tr>
<td>Total of isomaltooligosaccharides</td>
<td>90.8</td>
</tr>
</tbody>
</table>

DP, degree of polymerization.

Abbreviations: IMO, isomaltooligosaccharides (Isomalto-900®); FO, fructooligosaccharides (MeioP-P); IM2, a disaccharide fraction prepared from IMO; IM3, a tri- and higher oligosaccharide fraction prepared from IMO; IMH, a hydrogenated derivative of IMO; PBS, phosphate-buffered saline; HPLC, high-performance liquid chromatography.
Digestibility Characteristics of Isomaltooligosaccharides

IMO by preparative high-performance liquid chromatography (HPLC). Their compositions are shown in Table. The hydroxyned derivative of IMO (IMH) was prepared using the Raney-nickel catalyzing method.\(^{11}\)

The condition of preparative HPLC is as follows. Column, Diaion UBK 530 (Mitsubishi Chemical Industries, Tokyo, Japan), which is an Na type of strongly acidic cation ion-exchanger, having a diameter of 5 cm and a length of 450 cm; eluant, deionized water at a rate of 59 ml/min; column temperature, 60 C; injection, 500 ml of a 50% (w/v) aqueous solution of IMO. The eluate from 4200 ml to 5000 ml after the injection was collected as the fraction of IM2 (79.0 g of dry matter), and the eluate from 1800 ml to 3400 ml as the fraction of IM3 (66.0 g).

Glucose, sucrose (Wako Pure Chemical Industries Ltd., Osaka, Japan), maltose, maltotriose, maltotetraose (Hayashibara Biochemical Laboratories Inc., Okayama, Japan), sorbitol (Towa Chemical Industry Co., Ltd., Tokyo, Japan), and raffinose (Fluka Chemie AG, Switzerland) were purchased and their purities were more than 98%. Besides these saccharides, Meioiogulo-\(^{6}\) (FO, Meiji Seika Kaisha Ltd., Tokyo, Japan), which was a commercially available fructooligosaccharide mixture, was also purchased. FO had the following composition: glucose (G) plus fructose (F), 1.0%; GF, 3.5%; GF2, 42.7%; GF3, 44.2%; GF4, 7.2%; GF5, 1.4%.

The oligosaccharide mixtures had the following average molecular weight: IMO, 486.5; IM2, 359.0; IM3, 624.0; IMH, 487.4; FO, 596.8.

In situ jejunum loop perfusion. Male Sprague-Dawley rats (Clea Japan Co., Tokyo, Japan), 7 weeks old, were raised in our animal facilities under a constant temperature (23 ± 1.5 C) and moisture (55 ± 7%), and maintained on a light-dark cycle (light on period, 07:00-19:00). Rats were given ad libitum water and commercial diet (type CE-2, Clea Japan Co., Tokyo, Japan) for 4-6 days to acclimatize them to their environment. They were starved for 18-21 h before the test and anesthetized with an intraperitoneal injection of urethane (25% solution) at 5 ml/kg body weight. The small intestine was exposed by laparotomy, and a section of jejunum approximately 25 cm long beginning at 15 cm from the pylorus was selected. Silicone tubes were cannulated at each end and connected to the perfusion system of the jejunum loop with a peristaltic pump (Atto Co., Tokyo, Japan). Animals were kept on a heating plate to maintain body temperature at 37 C. The perfusion test was done during a period of 09:00-15:00.

The perfusate was continuously gassed with 95%O\(_{2}\)-5%CO\(_{2}\) (v/v) and maintained at 37 C. The perfusion rate was 4.0 ml/min. After washing through with 40 ml of PBS (phosphate-buffered saline), the jejunum loop was perfused with 20 ml of the test saccharide solution (56 or 14 mm), which was made isotonic by adjusting the sodium chloride in PBS and stored in the volumetric reservoir (glass cylinder). The isotonicity of the perfusate was examined with osmotic pressure using an Osmometer (Fiske Inc., U.S.A.).

Each perfusate of test saccharide was recirculated for 60 min and 1.0 ml from the reservoir was collected into a sealed test tube every 15 min. The test tube was immediately heated for 10 min in a boiling water bath to deactivate the digestive enzymes desquamated from the intestinal surface, and then cooled down to room temperature. To deionize the perfusate, 1.0 g of amphoteric ion-exchange resin (MB-3, Organo Inc., Tokyo, Japan) was added to the test tube, which was then shaken vigorously and left for 10 min. The treated solution was collected and the remaining resin was washed with 0.5 ml distilled water. This washing was repeated three times, all of the solutions was combined, and the total volume was adjusted to 2.0 ml. Then, the solution was passed through a membrane filter with a pore size of 0.45 \(\mu\)m and the filtrate was analyzed by HPLC.

The volumetric change of the perfusate was measured by the residual volume of the reservoir, when 1.0 ml of perfusate was collected sequentially, and when the perfusate was completely removed from the jejunum loop immediately after the test period. After the test, the small intestine was resected and the length of the jejunum loop was measured.

Analysis of saccharide composition. Using HPLC under the two different conditions shown, the saccharide composition in the perfusate was analyzed. Condition A: column, TSKgel NH\(_{4}\)-60 (4.6 mm i.d. x 250 mm, Tosoh Co., Kanagawa, Japan); eluant, acetonitrile:H\(_{2}\)O (65:35, v/w); flow rate, 0.8 ml/min; column temperature, ambient; injection volume, 20 \(\mu\)l; detector, refractive index. Condition B: column, MCI GEL CK04S (8 mm i.d. x 200 mm, Mitsubishi Kasei Co., Tokyo, Japan); eluant, H\(_{2}\)O; flow rate, 0.35 ml/min; column temperature, 60 C; injection volume, 20 \(\mu\)l; detector, refractive index. The composition of IMO, IM2, IM3, IMH, M3, or FO was analyzed by the combination of conditions A and B, while the compositions of the other saccharides were analyzed by condition A.

Digestibility was estimated as luminal clearance of saccharides, which was represented by the difference between the beginning of perfusion and the time when the perfusate was sequentially collected, and expressed as mmol clearance/cm of jejunum segment perfused. The composition of the residual saccharide is represented by the absolute amount (mmol) in the perfusate.

Statistical analysis. Results are expressed as means ± SD of 6 rats. Difference of means among the all groups was evaluated by a Kruskal--Wallis’s test, and then, differences of means in all pairs of the groups were evaluated by a Turkey’s multiple range test.\(^{13}\) The differences were considered significant at \(p < 0.05\).

Fig. 1. Course of the Luminal Clearance of Saccharides (56 mm) in Rat Jejunum Loop. IMO, a mixture of isomaltooligosaccharides; IM2, dimer component concentrate of IMO; IM3, not less than trimer component concentrate of IMO; M3, maltose; M3, maltotriose; S, sucrose; G, glucose; SOR, sorbitol; R, raffinose; MII, maltitol; IMH, a hydrogenated IMO; FO, a mixture of fructooligosaccharides. Each bar represents micromoles of clearance per cm of jejunum segment perfused as mean ± S.D. of 6 rats. Means not sharing a common superscript letter are significantly different (\(p < 0.05\)).
Results

Luminal clearance of saccharide from the perfusate

The luminal clearance of each test saccharide per length of the jejunum loop used (μmol/cm) was represented at 15 min intervals up to 60 min (Figs. 1 and 2). The initial saccharide concentrations of the perfusate were 56 mM in Fig. 1 and 14 mM in Fig. 2, respectively. A gradual and time-dependent increase in luminal clearance was shown in cases of all saccharides and concentrations.

In the case of 56 mM (Fig. 1), the clearance of IMO was significantly smaller than that of IM2 after 45 min, that of maltotriose after 60 min, and also those of maltose, sucrose, and glucose after 30 min. Those of raffinose and FO were significantly smaller than that of IMO after 30 min and 45 min, respectively. Those of maltitol and IMH were also significantly smaller than that of IMO after 60 min. That of IM3 tended to be smaller than IMO but no significant difference was observed. That of maltotriose was significantly smaller than those of maltose and sucrose after 30 min, and also than that of glucose after 15 min. That of sucrose was significantly smaller than that of maltose after 30 min, and also than that of glucose after 15 min. Comparing raffinose, FO, and saccharide alcohols, the clearance of sorbitol was the largest, while that of raffinose was the smallest.

In the case of 14 mM (Fig. 2), the clearance of IMO was significantly smaller than those of maltose and glucose after 15 min, and also than that of maltotriose after 45 min. Comparing the saccharides except IMO, that of maltotriose tended to be smaller, while that of glucose tended to be larger, however, no significant difference was observed among them.

Composition of saccharides component in the perfusate

The composition of each saccharide component (mmol) except monosaccharide in the perfusate was represented every 15 min (Figs. 3–5). Figure 3 shows the compositional changes of IMO, IM2, and IM3 in the case of 56 mM. Figure 4 shows those of maltose, maltotriose, and IMH in the case of 56 mM. Figure 5 shows those of IMO, maltose, and maltotriose in the case of 14 mM.

In case of 56 mM (Figs. 3 and 4), IMO, IMH, and FO showed a gradual decrease of residual amount, while the ratio of their components remained constant. In the case of IM2, the decrease rate of the disaccharide component was faster than the other components. In the case of IM3, the decrease rate of the tri- and tetrasaccharides components were faster than the other components, while the disaccharide components gradually increased. Trisaccharide component appeared in the case of maltose, and di- and tetrasaccharides components appeared in the case of maltotriose.

In the case of 14 mM (Fig. 5), IMO also showed gradual decrease of the residual amount, while the ratio of components remained constant. In a similar manner as the case of 56 mM, a trisaccharide component appeared in the case of maltose, and di- and tetrasaccharides components ap-

![Fig. 2. Course of the Luminal Clearance of Saccharides (14 mM) in Rat Jejunum Loop. Abbreviations and conditions are described in Fig. 1.](image)

![Fig. 3. Course of Residual Amount of Saccharides Component (56 mM) in Rat Jejunum Loop. Abbreviations are described in Fig. 1. DP2, DP6 represent components having each degree of polymerization. Absolute amount (mmol) of each component were expressed as mean of 6 rats.](image)
peared in the case of maltotriose.

In both concentrations of 56 and 14 mm, the trisaccharide produced from maltose was identified as maltotriose, and the tetrasaccharide produced from maltotriose was identified as maltotetraose by HPLC. Although components which appeared to be different from the α(1→4) linkage of glucose were found on the chromatogram of HPLC, they could not be identified because there were only traces.

In the results of the analytical HPLC (data not shown), maltitol produced slight amounts of glucose and sorbitol, which were its saccharide moieties, in the perfusate. Raffinose did not produce glucose, fructose, or galactose, which were its saccharide moieties. Sucrose produced a slight amount of glucose and fructose, which are its saccharide moieties, but no trisaccharide. Glucose and sorbitol produced no other saccharide.

Discussion

In this study, the perfusate flowed at the rate of 4 ml/min, so the 20ml set in the reservoir at the beginning of the perfusion test circulated once in 5 min. The flow rate was assumed to be fast enough that the one milliliter collected from the reservoir to analyze its composition, could represent the state of the digestion and absorption in the jejunal loop.

The intestinal digestion and absorption of saccharides are generally considered to consist of sequential processes of the luminal digestion by secreted digestive enzymes (i.e., pancreatic α-amylase), the membrane digestion on the epithelial surface, and absorption from the intestine. The activity of disaccharidases, which are concerned in membrane digestion of disaccharides to monosaccharides, is considered to be higher in the jejunum than in both duodenum and ileum. In particular, the activity of disaccharidases at the upper section of jejunum is considered to be highest. In this study, the total length of a rat's small intestine was 90–110 cm, and the jejunum loop was the section of approximately 25 cm located below the duodenum. It is assumed that the jejunal loop in this experiment corresponds to the upper section of jejunum, where the disaccharidase activities must be highest. Furthermore, since the inside of the jejunal loop was washed out with PBS before recirculation and no pancreatic juice entered the loop, saccharides in this experiment may be degraded by the membrane digestion but not by the luminal digestion.

Raffinose and FO are considered to be hardly digested by the intestinal mucosa enzymes. However, their clearance from the perfusate was observed (Fig. 1). Since glucose, fructose, or galactose, which were all the saccharide moieties of raffinose, were never released in the perfusate, it was assumed that raffinose was not digested. In the common digestion process, disaccharides are digested to monosaccharides by membrane digestive enzymes before they are finally absorbed. It is also reported that a slight amount of sucrose, FO, and maltitol is absorbed without undergoing digestion. Therefore, the clearance of raf-
finose and FO from the perfusate is assumed to indicate that a part of such saccharides can be absorbed without undergoing digestion. It is also assumed that the clearance due to such kind of direct absorption occurs for all test saccharides, and that the amount of directly absorbed oligosaccharides is considered as on the same level as raffinose and FO in this study. The amount of direct absorption in the case of raffinose and FO is 5 μmol/cm² at 60 min. So half of the clearance of IMO at 60 min may be the result of direct absorption. This state may not represent the exact one in vivo, however the clearance distinction among test saccharides in this in situ system can give a suggestion of their relative digestibility in vivo.

Maltose, maltotriose, and sucrose are regarded as digestible saccharides, but maltitol, raffinose, and FO as nondigestible ones. In this study, the characteristics of the clearance of IMO were intermediate between those of such digestible and nondigestible saccharides mentioned above (Figs. 1 and 2). This result supports the conclusion that IMO is considered partly digestible, which has been reported in the studies of in vitro digestion by intestinal mucosa enzyme and also 14C-labeled IMO metabolism by humans. Since IM2 contains 64.3% isomalto, the digestibility of IM2 may be considered to represent that of isomalto. Therefore, IMO must be digested more slowly than isomalto. This agrees with the result of the in vitro digestion study.5,6

The clearance of IM2 was significantly smaller than that of maltose and almost the same as that of maltotriose or sucrose (Fig. 1). The mainly decreased component was disaccharides (Fig. 3). These results suggest that the digestibility of disaccharides in IMO components are similar to those of sucrose or maltotriose. On the other hand, with respect to the compositional change of IM3, the increase of disaccharides following the decrease of tri- and tetrascaccharides was observed, while little decrease of penta- and hexasaccharides was observed (Fig. 3). These results suggest that the components of tri- and tetrascaccharides are sequentially degraded into smaller components, and that the components having much higher degrees of polymerization were much harder to digest.

Isomalto, which has a (1→6) linkage of glucose, is digested by isomaltase of the intestinal mucosa membrane.11 It is reported that isomaltase and maltase are competitively inhibited by trehalose and maltitol, respectively.16,19 In this study, IMO had a complicated composition (Table), and its clearance was much more similar to that of IM3. Thus, it is assumed that the digestion of individual components is affected by each other, and that the components having higher degrees of polymerization in IMO may be much harder to digest.

In this study, the clearance of maltitol was larger than those of raffinose and FO (Fig. 1) which are regarded as nondigestible saccharides. A slight amount of production of glucose and sorbitol was observed in the perfusate. These results support the conclusion that a small part of maltitol is digested in vitro by intestinal disaccharidases.19 The clearance of IMH was significantly smaller than that of IMO, and its magnitude was quite similar to that of maltitol (Fig. 1). This seems to indicate that hydrogenation makes IMO hardly digestible.

In the cases of maltose and maltotriose, maltotriose and maltotetraose, respectively, were produced regardless of the concentration (Figs. 4 and 5). The degree of polymerization of each product is one more higher than each perfusate. This may be from the enzymatical transglucosidation in contact with the jejunum mucosa. Its magnitude seems to be affected by the concentration of perfusate, especially in the case of maltotriose.

In this in situ perfusion test using the rat jejunum loop, IMO is characterized as a slowly digestible saccharide, and the magnitude of digestion is intermediate between digestible and nondigestible saccharides. IMH seems to be characterized as a kind of nondigestible saccharide. The compositional changes of IMO and its fractions suggest that each component of IMO is sequentially degraded into smaller one, and that much larger components of IMO such as the penta- and hexasaccharides are much harder to digest. These results might suggest that remaining components of IMO, which could escape complete digestion, reached the lower intestine if a large amount was ingested. Further studies must be required to clarify the digestibility of individual IMO components, and the relationship between the ingestion amount and the amount to reach the lower intestine in vivo.

References