THE EFFECTS OF MALTITOL ON RAT INTESTINAL DISACCHARIDASES

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Summary 1. The highest disaccharidase activity for sucrose, maltose and maltitol was found in the jejunum, followed by the ileum and duodenum. However, the disaccharidase activity for maltitol was extremely low compared with that for sucrose and maltose.

2. For maltitol, the $K_m$ value was very large and the $V_{\text{max}}$ value was very low compared with the values for sucrose and maltose.

3. The initial velocity ($v$) in the presence of sucrose and maltitol, was equal to the sum of the rates for individual substrates sucrose and maltitol ($v_1$ and $v_2$) respectively ($v = v_1 + v_2$). Thus, no competition between these substrates was observed. In the case of maltose and maltitol, the initial velocity ($v$) in the presence of both substrates was less than the sum of the individual rates for maltose and maltitol ($v_1 + v_2$) in the absence of the other substrate ($v < v_1 + v_2$). This finding demonstrates that there is competition between these two substrates for the same enzyme.

Furthermore, the apparent Michaelis constant ($K_m$) and the apparent maximal velocity ($V_{\text{max}}$) for pure and mixed substrates, i.e., maltose and maltitol, at various mole fractions of maltose showed dependence on the mole fraction of maltose. The obtained kinetic data provide strong evidence that both maltose and maltitol react at the single active center of maltase.

Maltitol is a sugar alcohol of the disaccharide maltose and is similar to it in structure, but is not found in nature.

Previous studies showed that maltitol is absorbed to a small extent by the small intestine and that the absorbed maltitol is relatively quickly excreted in the urine ($I$, $2$). A considerable amount of maltitol is found at the surface of the intestinal mucosa ($2$). This is very interesting from the point of view of

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Abbreviations used: Tris, tris (hydroxymethyl) amino methane; maltitol-U-14C, maltitol universally labeled with $^{14}$C.
current concepts of membrane digestion (3, 4), since the disaccharidases are also found on the intestinal surface membrane, i.e., the brush borders (3, 4).

It was therefore of interest to study the effect of maltitol on the kinetics of intestinal disaccharidases in the presence of a naturally occurring disaccharide, such as maltose or sucrose.

MATERIALS AND METHODS

Female rats of the Wistar strain, weighing 150-180 g, were given a standard laboratory chow (Oriental Yeast Co., Osaka). Food and water were given ad libitum. The animals were made to fast for 24 hr before being decapitated.

The entire small intestine was quickly removed and subdivided into the duodenum, jejunum and ileum. The subdivided intestines were slit open and washed with ice-cold saline. The mucosa was scraped off with a glass slide. Four parts of redistilled water were added, and the homogenization was performed in a Potter and Elvehjem homogenizer fitted with a teflon pestle. Then the homogenate was centrifuged in a refrigerated centrifuge at $1,000 \times g$ for 15 min to remove nuclei and larger cell debris. The supernatants were used for the experiment.

Disaccharidase activity was assayed by the method of DAHLQVIST (5). Sucrose, maltose and maltitol were used as substrates. The concentration of substrate solution was 0-56 mM in 0.1 M sodium maleate buffer, pH 6.0. An aliquot of the supernatant was diluted so that the solution contained a suitable amount of disaccharidase activity for the assay.

One-tenth milliliter of the diluted enzyme solution was mixed with 0.1 ml of substrate solution in a small test tube and incubated at 37°C. After an appropriate time, the amount of glucose produced was determined with Tris-glucose oxidase reagent. The Tris-glucose oxidase reagent was prepared according to the procedure of DAHLQVIST (5). Protein was determined by the LOWRY method (6), using bovine serum albumin as a standard.

Sucrose and glucose were purchased from the Kokusan Chemical Works, Ltd. Maltose and maltitol were kindly provided by the Nikken Chemical Co., Ltd. and Hayashibara Co., Ltd., while glucose oxidase was obtained from the Worthington Biochemical Company.

RESULTS

1. Distribution of disaccharidases in rat small intestine

The activity of disaccharidases was observed in various parts of the rat small intestine (Table 1). The activities of maltase and sucrase were the highest in the jejunum, followed by those in the ileum, while the duodenum exhibited very low activity. This pattern of enzymatic activity was also observed with maltitol, although the activity was much lower compared with that obtained with maltose.
Table 1. Specific activity of disaccharidases in the various parts of the small intestine of the rat.a

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>0.38±0.02b</td>
<td>2.23±0.17b</td>
<td>1.26±0.08b</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(10)</td>
<td>(5)</td>
</tr>
<tr>
<td>Maltose</td>
<td>2.86±0.19</td>
<td>6.68±0.28</td>
<td>5.42±0.24</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(9)</td>
<td>(5)</td>
</tr>
<tr>
<td>Maltitol</td>
<td>0.20±0.01</td>
<td>0.53±0.03</td>
<td>0.25±0.01</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(7)</td>
<td>(4)</td>
</tr>
</tbody>
</table>

( ): number of determinations.

The disaccharidase activity was assayed with 28 mM substrate in 0.1 M sodium maleate buffer, pH 6.0 and expressed as \( \mu \) moles substrate hydrolyzed/hour/mg protein. It was calculated from the amount of glucose produced. The hydrolysis of 1 mole of sucrose or maltitol produces 1 mole of glucose, but that of maltose produces 2 moles of glucose. Therefore, the value for maltose was divided by 2.

Mean±S.E.M.

and sucrose. The jejunum portion of the small intestine was used in all subsequent enzymatic studies because its disaccharidase activity was highest.

2. Properties of disaccharidases in rat intestine

The properties of disaccharidases were analyzed in the jejunum of rat small intestine. The disaccharidases activities were determined for maltose, sucrose and maltitol in the different substrate concentrations (Fig. 1). From these, the Michaelis constant \( (K_m) \) and the maximal velocity \( (V_{max}) \) for sucrose, maltose and maltitol were determined by the graphical method of the Lineweaver-Burk plots (Table 2). The \( K_m \) value for maltitol was extremely large compared with that for maltose and sucrose. This observation demonstrates that the disaccharidase has little affinity for maltitol. Furthermore, the lowest \( V_{max} \) was found when maltitol was used as substrate. These results indicate that maltitol is enzymatically not easily available to the rat.

3. The effect of maltitol on the hydrolysis of sucrose and maltose

Previous studies have shown that a considerable amount of orally administered maltitol-U-\(^{14}\)C is bound to the surface of the intestinal mucosa (2). However, in this study, maltitol was not significantly hydrolyzed by the preparation consisting of rat intestinal mucosa. It will be considered that maltitol combines with the disaccharidases and inhibits the hydrolysis of sucrose or maltose. Observations were carried out to determine how sucrose and maltose hydrolyze in the presence of maltitol.

If there were no interaction between two substrates, the initial velocity \( (v) \) in the presence of two substrates should be equal to the sum of those for the individual substrates, \( v_1 \) and \( v_2 \) \((v = v_1 + v_2)\). On the other hand, if there were an interaction between the two substrates, then \( v < v_1 + v_2 \) (7).
The effect of maltitol on sucrase activity was observed (Fig. 2). The initial velocity \( (v = \text{mm glucose produced/hr}) \) in the presence of sucrose and maltitol was 1.03, which was equal to the sum of the rate for the individual substrates, sucrose and maltitol, \( i.e., 0.78 \) and \( 0.28 \), respectively \( (v = v_1 + v_2) \). Thus, there was no competition between sucrose and maltitol.

Furthermore, the effect of maltitol on maltase activity was observed (Fig. 3). The initial velocity \( (v) \) for the mixture of maltose and maltitol was 7.35, which was about 13% less than the sum of the individual rates for maltose and maltitol, \( i.e., 8.08 \) and \( 0.39 \), respectively \( (v < v_1 + v_2) \) (Fig. 3). These results demonstrate the so-called “competition” between maltose and maltitol, and suggest that maltose and maltitol react at the same active site of the disaccharidase “maltase.”
Fig. 2. Effect of maltitol on sucrase activity. The initial velocity was determined with sucrase, maltitol, or the mixture of sucrase and maltitol. Substrate concentration was 28 mM. The ordinate shows mM glucose produced and the abscissa shows incubation time in minutes. From the figure, the initial velocity for sucrase, maltitol, or mixture of sucrase and maltitol is obtained as 0.78, 0.28, or 1.03, respectively. The initial velocity was expressed as mM glucose produced/hour instead of mM substrate hydrolyzed/hour.

Fig. 3. Effect of maltitol on maltase activity. The initial velocity for maltose, maltitol, or the mixture of maltose and maltitol was determined as described in Fig. 2. That for maltose, maltitol, or the mixture of maltose and maltitol is 8.08, 0.39, or 7.35, respectively.

In order to study the competitive mechanism between maltose and maltitol, the apparent Michaelis constant $K_m$ and the apparent maximal velocity ($V$) were determined for single and mixed substrates at various mole fractions of maltose. Lineweaver-Burk plots of $1/v$ against $1/s$ were obtained for mixed substrate solutions having various maltose mole fractions ($f$) (Fig. 4-a). These plots may reasonably be regarded as being straight lines, and from them the apparent Michaelis constant ($K_m$) and the apparent velocity ($V$) were calculated. The
dependence of $K_m$ and $V$ on the mole fraction ($f$) of maltose is shown in Fig. 4-b,c. Thus, the kinetic features above, obtained with the mixed substrate, maltose and maltitol, are consistent with those predicted for maltose and maltitol acting in competition for a single active center.

![Fig. 4. Lineweaver-Burk plots for mixed substrates: maltose and maltitol (a) and dependence of $K_m$ (b) and $V$ on $f$ (C). This kinetic study was carried out by changing maltose and maltitol concentrations, but without changing the ratio of each substrate concentration. The mole fractions ($f$) of maltose were 0, 0.17, 0.33, 0.50, and 1.00. In this case, a mole fraction of maltose ($f$) was calculated as follows: $f = a/a + b$, where $a$ and $b$ represent the substrate concentrations of maltose and maltitol, respectively. (The points corresponding to $f = 0$ and $f = 1$ represent the values for pure maltitol and maltose, respectively.) $K_m$ and $V_{max}$ was obtained from each fraction of maltose. $V$ is the initial rate for the total substrate concentration(s), which is the sum of the molar concentrations of maltose and maltitol.]

**DISCUSSION**

Previous studies in our laboratory have shown that a considerable amount
of orally administered maltitol-U-\(^14\)C is bound to the surface of rat intestinal mucosa, even though maltitol is not utilized by this animal \((1, 2)\). Recently, it has been demonstrated that the disaccharides are hydrolysed on the membranes of the small intestinal mucosa \((3, 4)\). This type of digestion is characterized by the proximity of enzymatic hydrolysis to the surface of cell membrane, which separates the intracellular and extracellular environments. The enzymes involved in these reactions are fixed onto the cell membrane.

In the present paper, we report the effect of maltitol on the activity of the intestinal disaccharidases, sucrase and maltase. Our studies demonstrate that maltitol and maltose compete for the same enzyme. The kinetic features obtained with the mixed substrates, maltose and maltitol, are consistent with the kinetics predicted for the competition for a single active center on the enzyme \((7)\). Therefore, it can be reasoned that maltitol is hydrolysed by maltase. However, for maltitol, the \(K_m\) value is very large and the \(V_{max}\) value is very small, as compared to these values for maltose. Therefore, only very little hydrolysis of this synthetic sugar alcohol would occur in the small intestine.

It has been reported that maltitol feeding causes growth retardation \((1)\). Our data suggest that this reduced growth would be due to the inhibition of the digestion of maltose by maltitol. Thus, in the presence of excess maltitol, the hydrolysis of maltose, a product of natural dietary carbohydrate, would be reduced; as a result, less monosaccharides will be available for absorption and consequently the calorie intake would be decreased.

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REFERENCES