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Inhibitory Action of Palatinose and Its Hydrogenated Derivatives on the Hydrolysis of α-Glucosylsaccharides in the Small Intestine

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This study was conducted to investigate the inhibitory effects of palatinose and Palatinit, which are disaccharides (or disaccharide alcohol) connected through an α-1,6-glucosyl linkage, on the hydrolysis of other carbohydrates, using an enzyme extract from the rat small intestine and a purified sucrase−isomaltase complex. Palatinose and its hydrogenated product, Palatinit, an equimolar mixture of α-*O*-D-glucopyranosyl-1,6-D-sorbitol (GPS) and α-*O*-D-glucopyranosyl-1,6-D-mannitol (GPM), inhibited the hydrolysis of sucrose and maltose. Palatinose and Palatinit also inhibited the hydrolysis of dextrin and soluble starch. Kinetic analysis of the enzymatic inhibition by GPS and GPM on sucrose hydrolysis revealed that both GPS and GPM competitively inhibit sucrase catalytic activity. These results suggest that disaccharides with an α-1,6-glucosyl linkage competitively inhibit intestinal α-glucosidases and may reduce the rate of hydrolysis of sucrose and other α-glucosylsaccharides.

KEYWORDS: Palatinose; α-*O*-D-glucopyranosyl-l,6-D-sorbitol; α-*O*-D-glucopyranosyl-1,6-D-mannitol; sucrase−isomaltase complex

INTRODUCTION

Considerable attention has been paid in recent years to the quality and quantity of dietary carbohydrates (*1, 2*). Psomaltulose (6-*O*-α-D-glucopyranosyl-D-fructose) is a noncariogenic disaccharide (*3*), a compound of glucose and fructose connected through an α-1,6-glucosidic linkage. Isomaltulose is digested and absorbed more slowly than sucrose (*4–6*). Palatinit, a hydrogenated product of palatinose, is an equimolar mixture of α-D-glucopyranosyl-l,6-D-sorbitol (GPS) and α-D-glucopyranosyl-1,6-D-mannitol (IUPAC: α-D-glucopyranosyl-1,1-D-mannitol) (GPM). Palatinit is minimally hydrolyzed by human digestive enzymes (*7*). We have previously demonstrated in intestinal studies that when a mixture of palatinose and sucrose is ingested, the increase in blood glucose concentration is reduced as compared with the situation when sucrose is digested alone (*8*). However, it is unclear whether the inhibition of sucrose digestion in the presence of palatinose is due to a specific action of palatinose at the active site of sucrase. It is also unclear whether the property of palatinose, that is, a disaccharide with an α-1,6-glucosidic linkage, plays an important role in this inhibition. In this study, we investigated the inhibitory effects of palatinose, GPS, and GPM on the intestinal hydrolytic activities of various α-glucosylsaccharides using a rat intestinal enzyme preparation and a purified sucrase−isomaltase complex.

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Table 1. Inhibitory Effects of Palatinose and Its Hydrogenated Product, Palatinit, on the Hydrolysis of Sucrose and Maltose[a]

	rate of glucose production (μmol glucose produced/tube/min)
28 mM sucrose	0.262 ± 0.002
28 mM maltose	2.198 ± 0.042
28 mM palatinose	0.049 ± 0.002
28 mM Palatinit	0.017 ± 0.002
28 mM sucrose + 28 mM palatinose	0.230 ± 0.005**
28 mM sucrose + 28 mM Palatinit	0.235 ± 0.002**
28 mM maltose + 28 mM palatinose	1.962 ± 0.020##
28 mM maltose + 28 mM Palatinit	1.892 ± 0.108#

[a] Data were expressed as means ± SEM for three determinations. Palatinit is an equimolar mixture of GPS and GPM. ** denotes a significant difference as compared with the value obtained for 28 mM sucrose at $p < 0.01$. #,## denotes significant differences as compared with the value obtained for 28 mM maltose at $p < 0.05$ and $p < 0.01$, respectively.

MATERIALS AND METHODS

Enzyme Preparation. Intestinal acetone powder from rats (Sigma-Aldrich Inc., Montana) was used as a source of α-glucosidase. The intestinal acetone powder was dissolved in 10 volumes of 0.1 M phosphate buffer (pH 6.8) and centrifuged at 12000*g* for 15 min, and the supernatant was filtrated through a 0.8 μm membrane filter before use as a crude enzyme preparation. The crude intestinal enzyme preparation was further used for purification of the sucrase−isomaltase complex by the method of Kolinska et al. (*9*), which involves fractionation with ammonium sulfate, and column chromatographies using DEAE-cellulose and DEAE-Sephadex A25. The specific activity of the purified enzyme was 1300 U/mg for sucrase activity, which is

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Table 2. Inhibitory Effects of Palatinose and Its Hydrogenated Product, Palatinit, on the Hydrolysis of Soluble Starch and Dextrin[a]

	rate of glucose production (μmol glucose produced/tube/min)
28 mM soluble starch[b]	1.504 ± 0.097
28 mM dextrin[b]	1.535 ± 0101
28 mM palatinose	0.049 ± 0.001
28 mM Palatinit	0.017 ± 0.002
28 mM soluble starch[b] + 28 mM palatinose	1.360 ± 0.038
28 mM soluble starch[b] + 28 mM Palatinit	1.345 ± 0.079
28 mM dextrin[b] + 28 mM palatinose	1.278 ± 0.079*
28 mM dextrin[b] + 28 mM Palatinit	1.268 ± 0.061*

[a] Data were expressed as means ± SEM for three determinations. Palatinit is an equimolar mixture of GPS and GPM. * denotes a significant difference as compared with the value obtained for 28 mM dextrin at $p < 0.05$. [b] Concentration of glucose residue equivalent to 28 mM glucose.

980 times that of the crude enzyme solution. One unit (U) of α-glucosidase is defined as the activity of enzyme that can release 1 μmol of glucose from substrate in 1 min.

Assay for Hydrolytic Activities. To study the effect of palatinose and its sugar alcohol, Palatinit, on the hydrolysis of sucrose and other α-glucosylsaccharides, the enzyme preparation containing 0.26 U of sucrase activity was mixed with 28 mM each of sucrose, maltose, dextrin, or soluble starch in the absence and presence of palatinose or Palatinit. The assay mixture contained 0.1 M phosphate buffer (pH 6.8). The concentrations of dextrin and soluble starch were expressed as glucose equivalents. After incubation for 20 min at 37 °C, the reaction mixture was boiled for 3 min to stop the reaction. The released glucose concentration in the mixture was determined using a D-glucose kit (R. Biopharm GmbH, Darmstadt, Germany).

To study the inhibitory actions of palatinose and Palatinit on the hydrolysis of sucrose using the purified sucrase−isomaltase complex, the purified enzyme (0.20 U of sucrase activity) was incubated with 20 mM sucrose in the absence and the presence of either palatinose, Palatinit, or maltitol at concentrations of 5, 20, and 50 mM for 20 min at 37 °C. The released glucose and fructose concentrations in the mixture were determined using a D-glucose kit and D-fructose kit (R. Biopharm GmbH, Darmstadt, Germany), respectively.

Kinetic Analysis. In the kinetic studies, GPS and GPM were used as inhibitory substances, because the rate of hydrolysis of GPS and GPM was considered to be minimal (*7*). An enzyme preparation containing 0.25 U of sucrase activity was incubated with 10, 25, or 50 mM sucrose in the absence and the presence of 50 or 100 mM of either GPS or GPM for 10 min at 37 °C. The amount of released glucose in the mixture was determined using a D-glucose kit.

Chemicals. Palatinose (trade name of Hitsui Sugar Co., Ltd., another name is isomaltulose) and Palatinit, an equimolar mixture of GPS and GPM, were provided by Mitsui Sugar Co. Ltd. (Tokyo, Japan). Other sugars were purchased as follows: sucrose and GPM from Wako Pure Chemical Industries Ltd. (Tokyo, Japan), GPS from Carl Roth GmbH (Karlsruhe, Germany), maltose from Junsei Chemical Co., Ltd. (Tokyo,

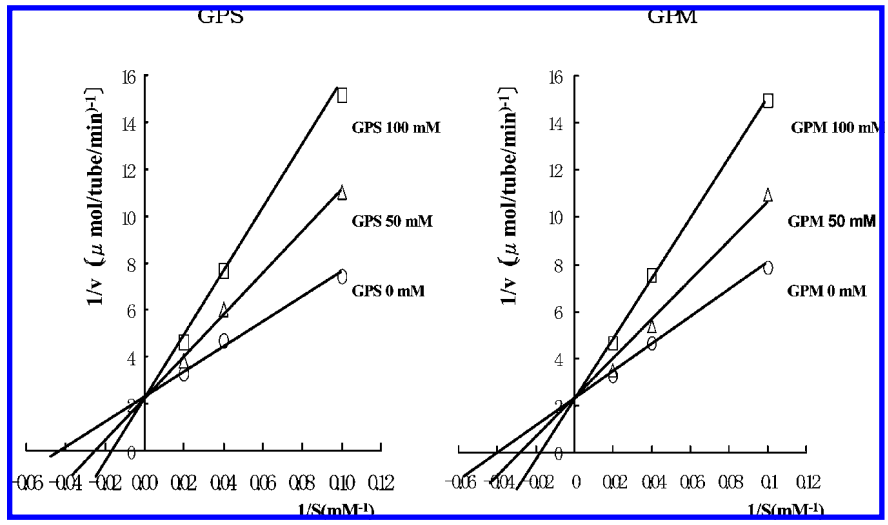

Figure 1. Lineweaver−Burk plots of sucrose hydrolytic activity in rat small intestinal extract with or without GPS and GPM.

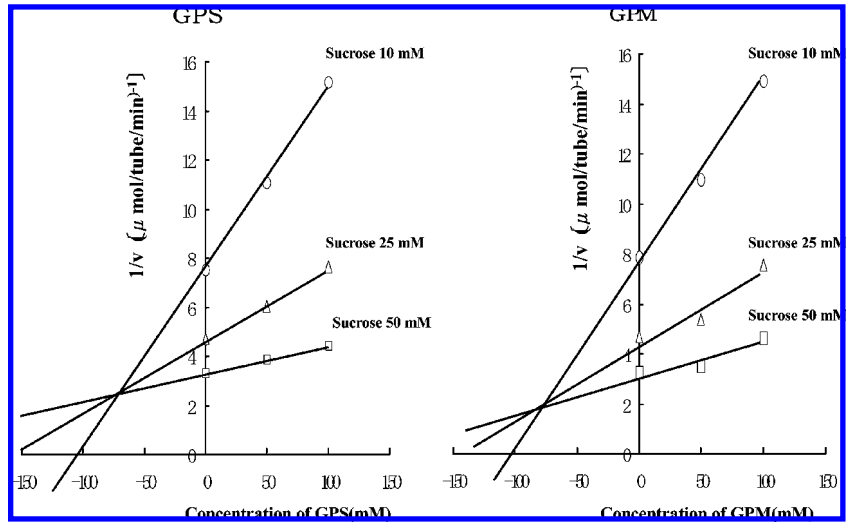

Figure 2. Dixon plots of sucrose hydrolytic activity in rat small intestinal extract with or without GPS and GPM.

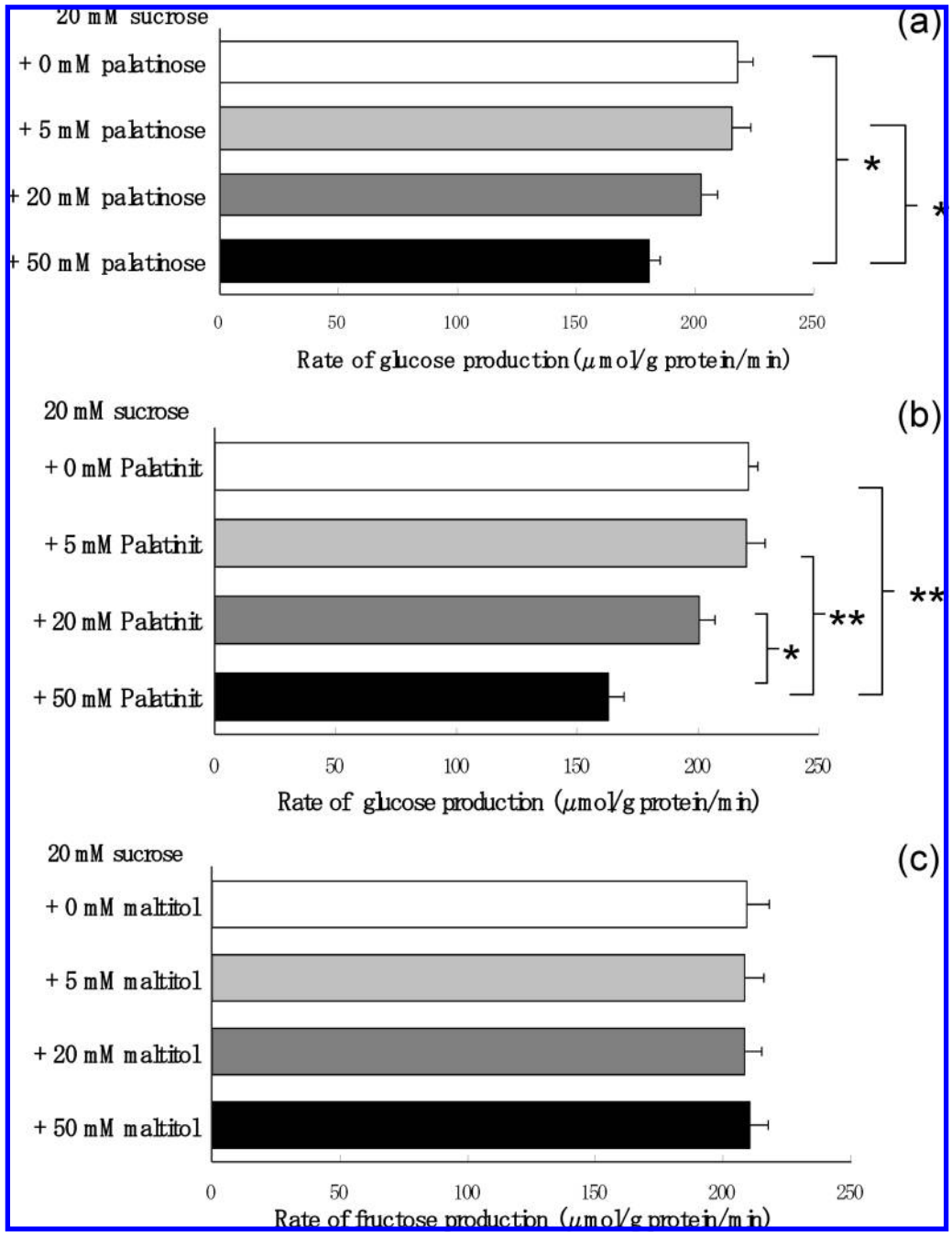

Figure 3. Inhibitory effects of palatinose (**a**), Palatinit (**b**), an equimolar mixture of GPS and GPM, and maltitol (**c**) on the hydrolysis of sucrose using a purified SI complex. Data were expressed as means ± SEM. *,** denote significant differences at $p < 0.05$ and $p < 0.01$, respectively.

Japan), dextrin from Sigma-Aldrich Inc. (Montana), and soluble starch from Merck KGaA (Darmstadt, Germany). The D-glucose kit and D-fructose kit were purchased from R.Biopharm GmbH (Dannstadt, Germany).

Data Analysis. Data were analyzed using a Student's *t* test or Bonferroni's multiple comparison test where appropriate.

RESULTS

Inhibitory Effects of Palatinose and Palatinit on the Hydrolysis of α-Glucosylsaccharides. The rate of glucose release from sucrose was significantly lower ($p < 0.01$) in the presence of palatinose than in the absence of palatinose (**Table 1**). The rate of glucose release from sucrose was also significantly lower (by 26%, $p < 0.01$) than the theoretical additive rate of glucose release from sucrose and palatinose. In the presence of Palatinit, the rates were also significantly lower ($p < 0.01$) than that in the absence of Palatinit or when compared with the theoretical additive rates (**Table 1**). The rate of glucose release from maltose was significantly lower ($p < 0.01$ and $p < 0.05$, respectively) in the presence of palatinose or Palatinit than that of maltose alone.

The rate of glucose release from dextrin was significantly lower ($p < 0.05$) in the presence of palatinose than that in the absence of palatinose (**Table 2**). The rate of glucose release from dextrin was also significantly lower (by 19.3%, $p < 0.05$) than the theoretical additive rates of glucose from dextrin and palatinose. In the presence of Palatinit, the rates were also significantly lower ($p < 0.05$) than that in the absence of Palatinit or when compared with the theoretical additive rates. The rate of glucose release from soluble starch was lower in the presence of palatinose or Palatinit than in the absence of

palatinose or Palatinit, but these differences were not significant. The results suggested that palatinose and Palatinit inhibit the hydrolysis of carbohydrates by α-glucosidases, such as maltase and glucoamylase, as well as sucrase and isomaltase.

Kinetic Analysis. The inhibition by GPS and GPM on sucrose hydrolysis is illustrated in **Figures 1** (Lineweaver−Burk plots) and **2** (Dixon plots). It is clear that GPS and GPM are competitive inhibitors of sucrose hydrolysis, as demonstrated by the inhibition patterns observed with the Lineweaver−Burk plots (**Figure 1**). There was essentially no difference between GPS and GPM in either the K_m or V_{max} values. The inhibitor constant (K_i) for sucrose hydrolysis calculated from the Dixon plots was 72 (GPS) and 74 mM (GPM), respectively (**Figure 2**).

Comparison of Disaccharide Alcohols with an α-1,6-Glucosyl Linkage vs Those with an α-1,4-Glucosyl Linkage. The inhibitory effects of palatinose, Palatinit, and maltitol on the hydrolysis of sucrose in the presence of the purified SI complex are shown in **Figure 3**. The inhibitory effects of palatinose on the hydrolysis of sucrose by the purified SI enzyme preparation appeared when 20 mM or more of palatinose was added to 20 mM sucrose. Similarly, the inhibitory effects of Palatinit on sucrose hydrolysis were also observed when 20 mM or more of Palatinit was added to 20 mM sucrose. On the other hand, maltitol did not inhibit the hydrolysis of sucrose (Figure **3**).

DISCUSSION

This study demonstrated that palatinose and its hydrogenated derivative, Palatinit, inhibited the hydrolytic activity of α-glucosidases in the small intestine. The results in this study have shown that the inhibitory effects of palatinose and Palatinit on the hydrolysis of sucrose, maltose, dextrin, and starch are characteristic properties of α-1,6-binding saccharides. We have investigated the effects of palatinose and Palatinit on the hydrolysis of trehalose and lactose and found that both have virtually no effect (unpublished data). The results of the present study suggest that palatinose, which has an α-1,6-linkage, inhibits the hydrolysis of carbohydrates normally hydrolyzable by α-glucosidases that are also present in the small intestine. Similar effects were recognized in the case of α-1,6-binding saccharides, such as palatinose and Palatinit. On the other hand, maltitol, an α-1,4-binding saccharide, did not inhibit the hydrolysis of sucrose.

There are four types of α-glucosidases (sucrase, isomaltase, maltase, and glucoamylase), which show some differences in substrate specificity, in human small intestine. Specifically, hydrolysis of dextrin and starch (polysaccharides) is mainly attributable to the action of glucoamylase and maltase, with relatively long-chain saccharides hydrolyzed by glucoamylase and short-chain saccharides by maltase. These α-glucosidases share similar properties with respect to the affinity toward their substrates, probably because these enzymes are derived from a common ancestral gene (*10*–*12*).

In this study, we have shown that both palatinose and Palatinit inhibit the activity of maltase/ glucoamylase as well as that of sucrase. Therefore, it is likely that palatinose and Palatinit inhibit the hydrolysis of sucrose, maltose, dextrin, and starch by interacting with the catalytic active sites of these α-glucosidases in the small intestine. Because maltitol, a disaccharide alcohol with α-1,4 glucosyl linkage, did not exert inhibitory effects on the hydrolysis of sucrose, maltose, dextrin, and starch, it seems likely that the inhibitory action of palatinose and Palatinit is related to its common structure, that is, α-1,6 glucosyl linkage.

It was reported that when a mixture of palatinose and sucrose was orally administered to humans, the increase in plasma glucose concentration was smaller than with the ingestion of sucrose only (*8*). The inhibitory effect of palatinose on α-glucosidases demonstrated herein explains one of the mechanisms involved in this phenomenon. Because the rates of hydrolysis of palatinose and Palatinit are low in the small intestine (*7*), it is likely that such an inhibitory effect of palatinose and Palatinit may be reflected by a reduced rate of digestion and absorption of α-glucosylsaccharides including starch and sucrose.

In conclusion, the results in this study suggest that palatinose and its hydrogenated derivatives, both with an α-1,6-glucosyl linkage, competitively inhibit intestinal α-glucosidases and may reduce the rate of hydrolysis of sucrose and other α-glucosylsaccharides.

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