Tech. Bull. Fac. Agr. Kagawa Univ., Vol. 58, 27～32, 2006

D-psicose inhibits intestinal α-glucosidase and suppresses glycemic response after carbohydrate ingestion in rats

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Abstract

D-Psicose is one of rare sugars present in small quantities in commercial carbohydrates and agricultural products. In this study, we investigate the effects of D-psicose on the activities of α-amylases and α-glucosidases in vitro, and evaluate the effects of D-psicose on postprandial glycemic response using rats in vivo. In vitro study, D-psicose potently inhibited intestinal sucrase and maltase, however, slightly inhibited intestinal and salivary α-amylase activities. Male Wistar rats (6 months old) were administrated 2 g/kg of sucrose, maltose or soluble starch together with 0.2 g/kg of D-psicose or D-fructose. D-Psicose significantly inhibited the increment of plasma glucose concentration induced by sucrose or maltose. Starch-induced glycemic response tended to be suppressed by D-psicose, however the suppression was not significant. These results suggest that D-psicose inhibits intestinal sucrase and maltase activities in an uncompetitive manner and suppresses the plasma glucose increase after sucrose and maltose ingestion. Thus, D-psicose may be useful in preventing postprandial hyperglycemia in diabetic patients when foods containing sucrose and maltose are ingested.

Key words : rare sugar, D-psicose, α-glucosidase, plasma glucose concentration, rat

Introduction

D-Psicose (*D-ribo*-2-hexulose), a C-3 epimer of D-fructose, is a "rare sugar" present in small quantities in commercial mixtures of D-glucose and D-fructose obtained from the hydrolysis of sucrose or isomerization of D-glucose.[1] Because of the very small amounts of D-psicose in natural products, few studies have examined D-psicose metabolism in animals. Recently, we developed a new method for producing D-psicose enzymatically on a large scale,[2] making it possible to conduct such studies. We have since demonstrated that D-psicose is a sweet monosaccharide that provides no energy to growing rats[3-5] and that gives little toxic effect in rats.[6,7] Thus, D-psicose may be useful as a sweetener for obese people seeking to lose weight.

On the other hand, it has been proven that strict glycemic control is associated with a low incidence of microvascular and macrovascular complications in diabetes,[8] and a delay or inhibition of carbohydrate digestion could be helpful for avoiding postprandial hyperglycemia in diabetic patients.[9,10] Specific inhibitors of α-glucosidases have shown a definite therapeutic value in suppressing the postprandial glycemic increase by delaying carbohydrate digestion.[9-11] Acarbose[9-13] and L-arabinose[14-17] are known to be competitive and uncompetitive inhibitors of the intestinal α-glucosidases, ie, sucrase, and maltase. It has also been shown that pancreatic amylase is inhibited by acarbose[13]. Although the major portion of dietary carbohydrate is starch, daily ingestion of sucrose is large in many advanced countries, however, agents that inhibit neither starch nor sucrose digestion have been used.

Recently, we reported that 5% D-psicose in diet increased liver glycogen content in rats fed a high-fat or a low-fat diet for 16 weeks.[18] We also observed that D-psicose or psico-rare sugar (mixture of 75% D-fructose and 25% D-psicose) significantly suppressed the increment of plasma glucose concentration induced by oral carbohydrate (sucrose, maltose or starch) tolerance tests.[19] These findings suggest that D-psicose may inhibit the activities of amylase and α-glucosidase. However, inhibitory effects of D-psicose on enzymes in digestive tracts are not clarified. In this study, we investigate the effects of D-psicose on the activities of α-amylases and α-glucosidases in vitro, and evaluate the effects of D-psicose on postprandial glycemic response using rats in vivo.

Materials and Methods

Inhibitory effect of D-psicose on activities of α-amylases and α-glucosidases in vitro

D-Psicose and D-allose were donated from Rare Sugar Center, Kagawa University. α-Amylases from *bacillus subtilis* and *aspergillus oryzae* were purchased from Wako Pure Chemical Industries (Osaka, Japan). Three male Wistar rats (3-week-old) obtained from Japan SLC (Shizuoka, Japan) were killed by decapitation. Small intestines and pancreases of rats were obtained immediately after death, rinsed with ice-cold saline, and stored at −40 ℃ until use. All the collected mucosa from three rats was homogenized together with 5 mmol/L EDTA-phosphate buffer (pH 7.0) and centrifuged at 4℃ for 10 min at 15000 x g. The extract was used for assay of the activities of α-amylase, sucrase, and maltase by methods of Caspary and Graf[12] and Dahlqvist.[20] For the pancreatic α-amylase assay, the tissues were homogenized together with 10 mmol/L phosphate buffer (pH 7.0), and the homogenate was used for amylase assay with the method of Whelan.[21] The standard assay mixture contained 40 μL substrate solution (20 mg/mL soluble starch, sucrose, and maltose, for assay of α-amylase, sucrase, and maltase, respectively), and 80 μL of a test carbohydrate solution (final concentration, 0.4-4.0 mg/mL D-fructose, D-psicose, and D-allose). The reaction was initiated by addition of 80 μL of appropriate dilutions of dried enzyme powders (from *bacillus subtilis* and *aspergillus oryzae*) or enzyme solutions (from small intestine and pancreas). The reaction mixture was incubated for 60 min at 37℃, and then the glucose concentration of the mixture was determined by the kits (Glucose CII-Test, Wako Pure Chemical Industries). The relative enzyme activities were calculated from glucose release. Relative rates was shown as 100% without inhibitor.

Effect of D-psicose on plasma glucose concentrations after carbohydrates loading in rats

To evaluate the potency of D-psicose in vivo, the effects of D-psicose on plasma glucose concentrations after glucose, sucrose, maltose, and soluble starch loading were examined. One hundred forty-four male Wistar rats (6 months old) were purchased from Japan SLC (Shizuoka, Japan) and acclimatized for a week under standard laboratory conditions (22±2℃, 60% humidity). The light/dark cycle was 12 h with lights on from 8:00 h to 20:00 h. Rats were randomly divided into 12 groups shown in Table 2. Twelve rats were fasted overnight for 12h before experiments. D-Psicose, or D-fructose (0.2 g/kg) was orally administered via gavage with 2g/kg glucose, sucrose, maltose, or soluble starch. At 0, 30, 60, 90, and 120 min after loading, blood was collected from the tail vein to obtain Plasma. Plasma glucose concentration was determined by the kits (Glucose CII-Test, Wako Pure Chemical Industries).

Statistical analysis.

All values are expressed as mean ± SD. Data were assessed by one-way ANOVA and Fisher's PLSD tests (In vivo experiment). Statistical significance was set at p value of <0.05. All analyses were performed with a commercially available statistical package (StatView J-5.0, SAS Institute Inc., Cary, NC).

Results

Inhibitory effect of D-psicose on activities of α-amylases and α-glucosidases in vitro

Relative enzyme activities were shown in Table 1 as 100 % without inhibitors (D-fructose, D-psicose, or D-allose). D-Fructose (0.4-4.0 mg/mL) showed no inhibition of each enzyme activity (Table 1). D-Psicose and D-allose (4.0 mg/mL) inhibited intestinal α-amylase, sucrase, maltase, and α-amylase from *aspergillus oryzae* (Table 1). Inhibitory effect of D-psicose was greater than that of D-allose (Table 1). Neither D-psicose nor D-allose inhibited pancreatic α-amylase, and α-amylase from *bacillus subtilis* activities (Table 1). D-Psicose (4.0 mg/mL) slightly inhibited salivary α-amylase activity, but D-allose did not suppress this enzyme (Table 1).

Effect of D-psicose on plasma glucose concentrations after carbohydrates loading in rats

D-Psicose significantly suppressed the increase of plasma glucose levels after sucrose and maltose loading in fasted rats, however, no suppression was found after glucose loading (Table 2). After 60 min ingestion, plasma glucose concentration was significantly lower in the Sucrose+Psicose group than in the Sucrose group, the suppression lasted from 30 to 90 min in the Sucrose+Psicose group (Table 2). At 30, 90 and 120 min after ingestion,

Table 1 Inhibitory effect of rare sugars on glucose release by a-amylase and a-glucosidase.

Enzymes	Substrate /inhibitor	D-Fructose	D-Psicose	D-Allose
		relative rate (%)		
α-Amiylase (rat small intestine)	1	100.8±1.0	83.0±1.9	89.5±1.9
	10	101.2±2.0	100.2±1.4	100.4±0.6
α-Amiylase (rat pancrease)	1	100.7±0.4	100.4±1.1	101.6±1.1
	10	98.5±1.7	98.0±1.2	101.3±2.9
α-Amiylase (human saliva)	1	101.3±1.5	96.2±3.3	103.7±3.3
	10	101.5±0.9	99.9±2.3	99.3±2.3
α-Amiylase (*bacillus subtilis*)	1	102.4±3.1	102.3±5.3	101.7±2.9
	10	99.1±0.2	99.1±0.2	98.3±0.8
α-Amiylase (*aspergillus oryzae*)	1	95.0±1.7	89.3±0.6	95.3±1.1
	10	98.8±0.5	97.5±0.3	98.0±1.0
Sucrase (rat small intestine)	1	99.6±2.6	74.5±3.9	77.9±1.3
	10	98.1±1.5	92.2±2.3	96.4±1.3
Maltase (rat small intestine)	1	99.5±3.4	85.4±2.6	90.2±5.6
	10	100.9±0.5	101.0±0.3	99.7±0.2

Values are means±SD of four experiments.
Relative rate is shown as 100% without inhibitor.

Table 2 Effects of D-psicose on plasma glucose concentrations (mg/dL) after oral carbohydrate loading in rats.

Test carbohydrates	Time after administration (min)					ΔAUC
	0	30	60	90	120	(h·mg/dL)
Glucose	99± 7	148±14	157±15	159± 7	168±18	6087±1160
Glucose+Fructose	92± 8	139±12	154±17	156±18	159±18	6530±1194
Glucose+Psicose	92±10	140±14	154±10	154±16	155±13	5866±1434
Sucrose	102± 8	135±13	151±27[a]	148±14	146±17	5235±1692
Sucrose+Fructose	102±12	134±13	142±11[a b]	146±10	144± 9	4109±1106
Sucrose+Psicose	95± 9	129±11	138±12[b]	146±16	142±17	4433±1654
Maltose	94± 9	146±12[a]	157±19	163±17[a]	159±12[a]	6855±1383
Maltose+Fructose	88±12	140±12[a b]	155±15	152± 9[a b]	147±12[a b]	6386±1430
Maltose+Psicose	87± 9	133±18[b]	149±13	147±12[b]	141±16[b]	5895±1061
Starch	98±10	131±10	133±18	130±17	125±19	4035±1233
Starch+Fructose	93± 4	128±14	134±16	127±12	126±11	3836±1330
Starch+Psicose	92± 9	124±11	125±15	121±12	120±12	3098±1138

Values are means±SD for 12 rats.
Fructose and psicose (0.2g/kg body weight) were administered with glucose, sucrose, maltose or starch (2g/kg body weight).
Means with different superscripts within a column are significantly different ($p<0.05$, one-way ANOVA and Fisher's PLSD tests).

plasma glucose concentration was significantly lower in the Maltose+Psicose group than in the Maltose group, the suppression lasted from 30 to 120 min in the Maltose+Psicose group (Table 2). Plasma glucose level was also suppressed by D-psicose after starch loading in fasted rats, however, the suppression was not significant. D-Fructose did not suppress the increase of plasma glucose levels after either carbohydrate loading (Table 2).

Dsicussion

We demonstrated in this study that D-psicose selective inhibited the intestinal α-amylase, sucrase, and maltase activities in uncompetitive manner. We also showed that D-psicose suppressed the increase of plasma glucose after ingestion of sucrose, maltose, and starch in rats, but the plasma glucose suppression was not significant after starch ingestion. Semenza and Balthazar[22], and Seri et al.[14] reported a similar inhibition of sucrase activity by L-arabinose in rabbits[22] and rats[14], however, they did not examine the intestinal α-amylase activity. We also showed that D-allose selective inhibited the activities of intestinal enzymes in vitro, but no in vivo examination was performed in this experiment. In our previous study, we

demonstrated that 5% D-psicose in diet increased liver glycogen content in rats fed a high-fat or a low-fat diet for 16 weeks.[18] We also observed that D-psicose or psico-rare sugar (mixture of 75% D-fructose and 25% D-psicose) significantly suppressed the increment of plasma glucose concentration induced by oral carbohydrate (sucrose, maltose or starch) tolerance tests.[19] The present study supports our previous findings.

Other α-glucosidase inhibitors[9-13,23] such as acarbose are recognize as potent competitive inhibitors of the activities of intestinal glucoamylase, maltase, and sucrase, and it has also been shown that acarbose has an inhibitory effect on pancreatic amylase activity.[13] In many advanced countries, starch accounts for approximately 60%, sucrose 30%, and lactose 10% of ingested carbohydrates.[12] Since the digestion of both starch and sucrose is delayed by acarbose and D-psicose, these α-glucosidase inhibitors have valuable therapeutic effect in reducing postprandial hyperglycemia in diabetic patients.

The major portion of dietary carbohydrate is starch, but sucrose is used in many foods as a sweetener or other ingredient, and its daily intake is large in many advanced countries. It has been shown that Tris competitively inhibits intestinal sucrose ingestion by rats and human subjects[24], however, Tris is of no practical use, because of its unpleasant taste and the necessity of large doses. Thus, there are known a little inhibitor of practical use such as L-arabinose that selectively inhibit intestinal sucrase and delay the ingestion of sucrose.

D-Psicose is a natural rare sugar with a sweet taste. In this study, it suppressed the increase in plasma glucose after sucrose (−15%, calculated by area under the curve, Table 2), maltose (−14%), and starch (−23%) but shown no suppression of the increase in plasma glucose after oral glucose loading in rats. These results suggest that D-psicose dose not affect the glucose absorption or gastric emptying. Among hexose structurally related to D-psicose, D-allose was equally potent in its inhibitory effect on the sucrose and maltose activities of intestinal mucosa in this study. Neither D-psicose nor D-allose inhibited amylases from rat pancreas, *bacillus subtilis*, and *aspergillus oryzae*. These results suggest that some specific interaction may exit among the enzymes, inhibitors, and the substrate to elicit the inhibitory action of D-psicose or D-allose.

D-Psicose is prevalent in nature as component of some plant and agricultural products.[25-29] It has a potent, sweet taste and low toxicity ; the LD_{50} value was 16g/kg orally in rats in our previous tests.[6] D-Psicose caused no diarrhea at a dose of 10% diet in the rat study.[6] Although a definite therapeutic value of other known α-glucosidase inhibitors in diabetic patients has been demonstrated, unpleasant side effects associated with incomplete absorption of dietary carbohydrate, ie, flatulence, abdominal discomfort, diarrhea,[9,10] and ileus-like symptoms,[30] have been reported. These side effects may be due to the potent inhibition of pancreatic amylases and many intestinal enzymes, which in turn inhibits the digestion of both sucrose and starch strongly. As shown in this study, D-psicose only inhibited intestinal enzymes and the suppression of amylase is weak, resulting in little adverse effect on the gastrointestinal tract.

In conclusion, the present study demonstrated that D-psicose inhibits intestinal sucrase and maltase activities in an uncompetitive manner and suppresses the plasma glucose increase after sucrose and maltose ingestion. Thus, D-psicose may be useful in preventing postprandial hyperglycemia in diabetic patients when foods containing sucrose and maltose are ingested. This is the first report indicating inhibition of sucrase and maltase activities by D-psicose both in vitro and in vivo.

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(Received September 30, 2005)

D-プシコースは小腸α-グリコシダーゼを阻害し，ラットにおける炭水化物摂取後の血糖値上昇反応を抑制する

松尾達博・何森　健

要　　旨

D-プシコースは市販炭水化物食品や農生産物にわずかに含まれる希少糖類の一つである．本研究では，D-プシコースがアミラーゼおよびα-グリコシダーゼ活性に及ぼす影響（In vitro試験）および炭水化物摂取後の血糖値上昇反応に及ぼす影響（ラット，In vivo試験）について検討した．In vitro試験において，D-プシコースは小腸スクラーゼおよびマルターゼを強く抑制したが，小腸アミラーゼおよび唾液アミラーゼに対する抑制効果はわずかであった．ラットIn vivo試験において，６ヶ月齢のウイスター系雄ラットに２g/kgのスクロース，マルトースおよび可溶性デンプンと共に0.2g/kgの D-プシコースあるいはD-フルクトースを経口投与した．D-プシコースはスクロースおよびマルトースによる血糖値上昇を有意に抑制した．一方，可溶性デンプンによる血糖値上昇は，D-プシコースによって抑制傾向を示したが，有意ではなかった．以上の結果から，D-プシコースは小腸スクラーゼおよびマルターゼを阻害することで，炭水化物摂取後の血糖上昇反応を抑制することが示唆された．D-プシコースは糖尿病患者の食事療法に有益である可能性が示された．