Persimmon-Tannin, an α-Amylase Inhibitor,
Retards Carbohydrate Absorption in Rats

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Summary Inhibitors of carbohydrate-hydrolyzing enzymes play an important role in controlling postprandial blood glucose levels. Thus the effect of persimmon tannin on pancreatic α-amylase and intestinal α-glucosidase has been investigated. Persimmon tannin inhibits pancreatic α-amylase and intestinal α-glucosidase in a concentration-dependent manner with the 50% inhibition concentration (IC_{50}) for amylase, maltase and sucrase being 1.7 μg/mL, 632 μg/mL and 308 μg/mL, respectively. The effect of persimmon-tannin extract on carbohydrate absorption in rats has also been investigated. Oral administration of persimmon tannin to normal rats fed cornstarch (2 g/kg body weight) significantly suppressed the increase in blood glucose levels and the area under the curve (AUC) after starch loading in a dose-dependent manner. The effective dose of persimmon tannin required to achieve 50% suppression of the rise in blood glucose level was estimated to be 300 mg/kg body weight. Administration of persimmon tannin to rats fed maltose or sucrose delayed the increase of blood glucose level and slightly suppressed AUC, but not significantly. These results suggest that persimmon tannin retards absorption of carbohydrate and reduces post-prandial hyperglycemia mainly through inhibition of α-amylase.

Key Words persimmon tannin, amylase inhibitor, blood glucose, oral carbohydrate tolerance test

α-Amylase is a key enzyme in dietary carbohydrate absorption in mammals, catalyzing the first step in carbohydrate digestion by transforming starch to oligosaccharides, which are then further hydrolyzed by α-glucosidases such as maltase. After these steps, the resulting product “glucose” is absorbed into the small intestine and the blood glucose concentration is increased. If these enzymes are inhibited, dietary carbohydrate absorption is also inhibited and the subsequent increase in blood glucose concentration is prevented. Thus, many inhibitors of carbohydrate digestion enzymes have been marketed for the regulation of blood glucose concentration, for example wheat protein, kidney bean protein and polyphenolic compounds (1–4). Polyphenolic compounds are widely distributed in plants and are not essential for survival of the plant. The role of these compounds may be to defend plants against a variety of herbivores and pathogenic microbes. They may produce an unpalatable bitter taste to prevent plant organs from being eaten and also inhibit digestive enzymes which prevents the plant organs from being digested (5). They are also produced to enhance the plant’s ability to fight disease or damage, such as oxidation. Matsuo and Ito reported that persimmon fruits, especially young fruits, contain abundant tannins (6). Persimmon tannins have been studied and found to possess many bioactivities such a hypolipidemic effect (7), anti-oxidant properties (8), cardioprotection effect (9), anti-diabetic effect (10) and detoxification effect (11). However, there are no reports about amylase inhibition by persimmon tannin. In this study, I discovered that the persimmon tannin obtained from young persimmon fruits strongly inhibits α-amylase.

Persimmon is a deciduous timber tree of the genus Diospyros, in the Ebenaceae family. Persimmons are cultivated over large areas in warm and dry regions, especially in Japan, China, Korea, Myanmar, Northern India and California. Persimmon trees can be classified broadly into two general categories: those producing astringent and non-astringent fruits. An astringent cultivar is high in tannins and bitter taste, and must be insolubilized before eating by treating the fruit with carbon dioxide or alcohol. A non-astringent persimmon contains less tannin and can be eaten due to its sweet taste. In the present study, persimmon tannin from dried young fruits of an astringent type persimmon (“Atago”) was prepared and shown to be a strong inhibitor of α-amylase, which may retard absorption of carbohydrate and reduce post-prandial hyperglycemia in rats.

MATERIALS AND METHODS

Materials. α-Amylases from porcine pancreatic and human saliva were obtained from Sigma-Aldrich Japan (Tokyo, Japan). Soluble starch was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Preparation of persimmon tannin. Persimmon tannin was extracted from dried young persimmon (Diospyros Kaki ‘Atago’) fruits according to the procedure

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Abbreviations: AUC, area under the curve; IC_{50}, concentration of inhibitor to inhibit 50% of its activity.
**Table 1. Preparation of persimmon tannin.**

<table>
<thead>
<tr>
<th>Dry weight</th>
<th>Amylase inhibition</th>
<th>Polyphenol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total kU</td>
<td>IC\textsubscript{50} µg/mL</td>
</tr>
<tr>
<td>Extract I</td>
<td>8.19</td>
<td>68.8</td>
</tr>
<tr>
<td>Extract II</td>
<td>7.65</td>
<td>1.950</td>
</tr>
<tr>
<td>Inner dialysate</td>
<td>3.50</td>
<td>2.060</td>
</tr>
<tr>
<td>Outer dialysate</td>
<td>2.41</td>
<td>0</td>
</tr>
<tr>
<td>Precipitate</td>
<td>6.08</td>
<td>156</td>
</tr>
</tbody>
</table>

described by Matsumoto et al. (7). Twenty-five grams of the dried and powdered young persimmon fruits was added to 500 mL of 70% v/v aqueous methanol, followed by mixing at 80°C for 20 min. The mixture was then centrifuged and the supernatant was evaporated, lyophilized and designated extract I. The precipitate was extracted with 500 mL of acidified methanol (70% methanol contained 1% HCl (v/v)) at 80°C for 30 min. The mixture was then centrifuged and the supernatant was evaporated, lyophilized and designated extract II. Extract II was dissolved in 140 mL of water and dialyzed against 2 L of water using a Spectra/Pro membrane (MWCO=10,000 Da, Spectrum Japan, Otsu, Japan.). The inner and outer dialyzed materials were concentrated and lyophilized. The lyophilized inner dialysate was designated persimmon tannin.

**Assay methods.** α-Amylase activity was determined by measuring the reducing power of oligosaccharides released from soluble starch by the method of Miller (12) with the following minor modifications. The assay system comprised the following components in a total volume of 1 mL: 100 mM sodium phosphate, pH 7.0, 17 mM NaCl, 5 mg soluble starch, 100 µL of inhibitor solution, and 10 µL of enzyme solution. After incubation at 37°C for 10 min, the reaction was stopped by the addition of 0.1 mL 2 N NaOH and 0.1 mL color reagent (4.4 µmol of 3,5-dinitrosalicylic acid, 106 µmol of potassium sodium (+)-tartrate tetrahydrate and 40 µmol of NaOH), followed by a 3-min incubation, at 100°C and subsequent A\textsubscript{540} measurement.

Maltase and sucrase activities were determined using maltose and sucrose as substrates, and glucose produced in the reaction was measured with a commercial assay kit (Glucose C II-test, Wako Pure Chemical Industries, Ltd.) (13, 14). Pancreatic lipase activity was determined by measuring the rate of release of oleic acid from trioleoylglycerol (15). The rate of enzyme inhibition was calculated as a percentage of the control (without inhibitor) using the formula

\[
\text{Enzyme inhibition (％)} = \left(\frac{(A_i - A_s)}{A_i}\right) \times 100
\]

where \(A_i\) = activity with inhibitor and \(A_s\) = control activity (activity without inhibitor).

One unit of enzyme inhibition (U) was expressed by the weight of IC\textsubscript{50} (concentration of inhibitor to inhibit 50% of its activity) value per mg.

Polyphenols (total phenolics) were determined by measuring the rate of release of oleic acid from trioleoylglycerol (15). The rate of enzyme inhibition was calculated as a percentage of the control (without inhibitor).

Statistical analysis. Results are expressed as the mean±SE. The statistical significance of differences with and without (control) persimmon tannin was assessed using the paired Student’s t-test.

**RESULTS**

**Preparation of persimmon tannin from young persimmon fruits**

The dried and powdered young persimmon fruits were extracted with 70% v/v aqueous methanol (extract I). The dry weight of extract I was about 33% of dry fruits, but total α-amylase inhibitory activity was very little (about 3.5% of extract II) (Table 1). Most α-amylase inhibitory activity was recovered in the acidic aqueous methanol extract (extract II). When extract II was dialyzed against water, all α-amylase inhibitory activity was recovered in the inner dialysate and no α-amylase inhibitory activity was observed in the outer dialysate (Table 1). Most of total polyphenol (Folin-Ciocalteu method), flavan-3-ol (vanillin method) and procyanidins (butanol-HCl method) contents were also recovered in the inner dialysate (Table 1). Therefore, the inner dialysate was designated persimmon tannin and its properties were characterized.

**Properties of persimmon tannin**

Matsuo and Ito reported that the main components of persimmon tannin obtained from immature persimmon
fruit were the thioether of catechin, catechin-3-gallate, gallocatechin and gallocatechin-3-gallate (21). Figure 1 shows the \( \text{H9251} \)-amylase inhibitory activity of persimmon tannin, and typical polyphenols such as gallic acid, catechin and epigallocatechin gallate (EGCG). These polyphenols inhibited \( \text{H9251} \)-amylase in a concentration-dependent manner. Persimmon tannin strongly inhibited \( \text{H9251} \)-amylase and the IC50 value was 1.70 g/mL. However, the IC50 values for gallic acid, catechin and EGCG were about 580, 1,500 and 290 times higher than that of the persimmon tannin, respectively. Table 2 shows enzyme inhibition values obtained with persimmon tannin. Persimmon tannin strongly inhibited porcine pancreatic and human saliva \( \text{H9251} \)-amylase. Persimmon tannin also inhibited \( \alpha \)-amylase from Bacillus sp. in a concentration-dependent manner (data not shown). However, the IC50 value was about 5 times higher than that for porcine pancreatic \( \alpha \)-amylase. Inhibition of carbohydrate-hydrolyzing enzymes, other than \( \alpha \)-amylase, was weak; the IC50 values for persimmon tannin with intestinal \( \alpha \)-glucosidase (maltase and sucrase) and porcine pancreatic lipase were about 370,180 and 240 times higher than that with porcine pancreatic \( \alpha \)-amylase, respectively (Table 2). Persimmon tannin did not inhibit bacterial glucoamylase or pancreatic proteases such as trypsin and chymotrypsin activities up to concentrations of 1,000 \( \mu \)g/mL. These results suggest that persimmon tannin specifically and strongly inhibits mammalian \( \alpha \)-amylase.

The optimum pH for the starch-hydrolytic activity of pig pancreatic \( \alpha \)-amylase is at pH 7.5, and persimmon tannin inhibited this activity at all pH values between 5.0 and 8.5. However, \( \alpha \)-amylase inhibitory activity was strongly influenced by pH: persimmon tannin more strongly inhibited at acidic pH (Fig. 2A) with the IC50 value at pH 9.0 being about 40-fold higher than that at pH 5.5 (Fig. 2B).

### Table 2. Enzyme inhibition by persimmon tannin.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>IC50 value, ( \mu )g/mL</th>
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<tbody>
<tr>
<td>( \alpha )-Amylase from porcine pancreas</td>
<td>1.70±0.178</td>
</tr>
<tr>
<td>( \alpha )-Amylase from human saliva</td>
<td>1.78±0.121</td>
</tr>
<tr>
<td>( \alpha )-Amylase from Bacillus sp.</td>
<td>9.21±0.304</td>
</tr>
<tr>
<td>Glucoamylase from ( Rizopus ) sp.</td>
<td>&gt;1.000</td>
</tr>
<tr>
<td>Maltase from rat intestine</td>
<td>632±57.3</td>
</tr>
<tr>
<td>Sucrase from rat intestine</td>
<td>308±4.85</td>
</tr>
<tr>
<td>Lipase from porcine pancreas</td>
<td>402±16.6</td>
</tr>
<tr>
<td>Trypsin from porcine pancreas</td>
<td>&gt;1.000</td>
</tr>
<tr>
<td>Chymotrypsin from bovine pancreas</td>
<td>&gt;1.000</td>
</tr>
</tbody>
</table>

Results are expressed as the mean±SE of 4 assays.

Figure 4A shows maltose administration. In normal rats blood glucose levels increased from a baseline of 54.4±1.38 mg/dL at 0 min to a peak of 116.8±5.14 mg/dL (increased blood glucose value 63.5±5.22 mg/dL) at 30 min after maltose administration (2 g/kg body weight). At 30 min the rise in blood glucose was significantly suppressed when per-
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simmon tannin (300 mg/kg body weight) was given with maltose, while the level was increased at 90 and 120 min. AUC0–180 min for persimmon tannin administration was slightly decreased, but not significantly, compared to administration of maltose alone (Fig. 4B). Similar results were observed with sucrose administration (Fig. 4C). Blood glucose levels increased from a baseline of 52.8 ± 2.15 mg/dL at 0 min to a peak of 84.1 ± 1.78 mg/dL (increased blood glucose value 36.7 ± 2.90 mg/dL) at 60 min after sucrose administration (2 g/kg body weight). The rise in blood glucose was suppressed at 30 and 60 min when persimmon tannin (300 mg/kg body weight) was given, while the level was increased at 120 and 180 min. AUC0–180 min for persimmon tannin administration was slightly decreased, but not significantly, compared to administration of sucrose alone (Fig. 4D).

DISCUSSION

Immature persimmon fruit contains abundant polyphenols such as condensed tannin and these compounds are involved in various physiological functions. Matsumoto et al. reported that persimmon tannin was able to bind bile acids and exert a hypolipidemic effect by increasing fecal bile acid excretion (7). Gu et al. reported that persimmon tannin has powerful antioxidant properties which had protective effects against chronic diseases such as cardiovascular diseases and certain types of cancers (9). Matsumoto and Yokoyama reported that persimmon tannin prevented dyslipidemia, hyperinsulinemia and fatty liver in type 2 diabetic mice by activation of brown adipose tissue (22). Li et al. reported a detoxification effect on snake venom (11) and Achiwa et al. reported inhibitory effects on human lymphoid leukemia cells (23).

Matsuo and Ito reported that persimmon tannin obtained from immature persimmon fruit comprised the thioether of catechin, catechin-3-gallate, gallocatechin and gallocatechin-3-gallate and they were mainly highly polymerized or condensed polyphenols (21). In this paper, the effect of persimmon tannin on pancreatic α-amylase activity has been investigated. We previously reported that high-polymerized polyphenols were found to strongly inhibit mammalian α-amylase activity (4, 24). Gu et al. reported that there was a strong relationship between α-amylase inhibition and the degree
of polymerization of procyanidins (25). Therefore, it is estimated that persimmon-tannin might have high-
amylase inhibitory activity. Indeed, persimmon tannin was found to strongly inhibit pig pancreatic /H9251-
amylase activity (Fig. 1): a concentration of 1.7 /H9262 g/mL was determined to result in 50% inhibition. However, the IC50 values for monomeric polyphenols such as gallic acid, catechin and EGCG were about /H11022 500 /H9262 g/mL (Fig. 1). Similar IC50 values for monomeric polyphenols were reported by other studies (26–30).

Examination of whether the /H9251-amylase inhibition observed in vitro could exert an inhibitory effect on carbohydrate absorption in vivo was performed. In vitro, persimmon tannin strongly inhibits /H9251-amylase activity, with the IC50 value for monomeric polyphenols such as gallic acid, catechin and EGCG were about >500 μg/mL (Fig. 1). Similar IC50 values for monomeric polyphenols were reported by other studies (26–30).

Fig. 4. Effect of persimmon tannin on increased blood glucose concentration (A and C) and AUC0–180 min (B and D) in normal rats. Rats were fasted for 15 h and persimmon tannin (300 mg/kg body weight (●)) and maltose (A, B) or sucrose (C, D) were administered at 2 g/kg body weight. As a control (○), rats were given carbohydrate and water. The results are expressed as means±SE, n=5, *p<0.05 vs. control.

Diabetes is a disease in which the amount of blood glucose is too high because the body cannot use glucose properly. Diabetes is a major worldwide public health problem with the number of patients increasing greatly in the last 50 y in both developed and developing nations (31). In Japan, about 9.5 million people are diabetic patients and about 11.0 million people are prediabetic patients (from the actual conditional report of 2013 from the Ministry of Health, Labor and Welfare of Japan). For diabetic patients, maintenance of healthy blood glucose levels is important and blood glucose concentration is greatly affected by dietary carbohydrates. Recent studies suggest that postprandial hyperglycemia is an important contributing factor to the development of atherosclerosis and cardiovascular disease (32–34). Control of postprandial plasma glucose levels is important and various /H9251-glucosidase or /H9251-amylase inhibitors have been used to inhibit excess energy supply, to control blood glucose levels and to prevent or treat obesity and diabetes (35–37).

In conclusion, this investigation suggests that persimmon tannin might exert an anti-diabetic effect by inhibiting /H9251-amylase and suppressing carbohydrate absorption from the intestine, thereby reducing post-prandial increase in blood glucose. Therefore, persimmon tannin may be useful as a potential additive to foods and beverages to inhibit carbohydrate adsorption.

REFERENCES

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do not hallucinate.


