Caffeoylsophorose, a New Natural α-Glucosidase Inhibitor, from Red Vinegar by Fermented Purple-Fleshted Sweet Potato

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The suppressive effect on the postprandial blood glucose rise through α-glucosidase (AGH) inhibition was investigated in this study in order to clarify an antihyperglycemic function of 6-O-caffeoylsophorose (CS) from diacylated anthocyanin. The administration of CS (100 mg/kg) following maltose (2 g/kg) to Sprague-Dawley rats resulted in the maximal blood glucose level after 30 min being significantly decreased by 11.1% compared to the control. A reduction in the serum insulin secretion was also observed in parallel to the decrease in blood glucose level. No blood glucose change was apparent when sucrose or glucose was ingested, suggesting that the antihyperglycemic effect of CS was achieved by maltase inhibition, rather than by sucrase or glucose transport inhibition. An AGH inhibitory assay demonstrated that the non-competitive maltase inhibition of CS was partly due to acylation by phenolic acid with sugar, the presence of hydroxyl groups in the aromatic ring, and the presence of an unsaturated alkyl chain in the acylated moiety.

Key words: α-glucosidase; caffeoylsophorose; diacylated anthocyanin; antihyperglycemic effect; noninsulin-dependent diabetes mellitus

The incidence of noninsulin-dependent diabetes mellitus (NIDDM), called type 2 diabetes, is reportedly due to the worldwide spread of the western diet. The disease is closely associated with the onset of dyslipidemia and arterial hypertension, as well as hyperglycemia.1) The key player in this onset is insulin resistance in the skeletal muscle, and fat and liver cells. Reduced insulin secretion from the pancreatic β-cells is also a candidate. The effect is compensated for by hyperglycemia or a high blood glucose level (BGL), this being caused by reduced uptake of glucose in the peripheral tissues, elevation of glucose release from the liver, and, in particular, a continuously excessive uptake of glucose from food.

A prominent pathway for exogenous glucose production from food is the hydrolysis of carbohydrates and/or sucrose by amylases or glycoside hydrolases in the gut. Thus, preventing an excessive postprandial BGL rise or maintaining BGL within the normal range, i.e., controlling glucose production from food, would be effective management for NIDDM and/or borderline patients. Acarbose or voglibose, therapeutic drugs, that inhibit α-glucosidase (AGH, EC 3.2.1.20) present in the epithelium of the small intestine, have been demonstrated to decrease postprandial BGL and improve impaired glucose metabolism without promoting insulin secretion in NIDDM patients.2) In a recent report on the STOP-NIDDM trial study,3) long-term acarbose treatment was also effective in borderline subjects, indicating that appropriate postprandial BGL control seems to be of benefit for preventing the development of hyperglycemia.

In our series of studies, we have focused on screening antihyperglycemic natural compounds with AGH inhibitory activity4–7) to develop an alternative medicinal antidiabetic food, as have other researchers.8–10) Among our results, anthocyanins have been found to inhibit the digestion of carbohydrates via maltase inhibition.9) In particular, a major diacylated anthocyanin of YGM-6 (peonidin (Pn) 3-O-(2-O-(6-O-E-feruloyl (Fer)-β-D-glucopyranosyl)-6-O-E-caffeoyl (Caf)-β-D-glucopyranoside)-5-O-β-D-glucopyranoside) in Ipomoea batatas cv Ayamurasaki11,12) was identified as a potent maltase inhibitor (IC50 of 200 μM).13) The beneficial antihyperglycemic effects of acylated anthocyanins have already...
been clarified when 100 mg/kg of YGM-6 was administered after maltose (2 g/kg) to male 8-week-old Sprague-Dawley (SD) rats, a significant BGL reduction of 16.5% after 30 min being observed against a glycemic rise in the control rats. Contrary to this prevalence, no further study has been performed concerning which part of their structure was essential for exerting the anti-hyperglycemic effect of diacylated anthocyanins. In our preliminary in vitro experiment using an immobilized AGH assay system, deacylation of the anthocyanins resulted in a serious loss of AGH inhibitory action; i.e., Pn 3-sophoroside-5-glucoside did not show any maltase inhibitory action at all. This finding strongly suggested that the remaining moieties of the anthocyanins, acylating Caf or Fer residues, may be of greatest significance in the expression of AGH inhibition.

We have recently identified a new compound with the structure of 6-O-(E)-caffeoyl-2-O-β-D-glucopyranosyl-α-D-glucopyranoside (cafeoylsophorose) from red-colored vinegar which had been developed via fermentation with the storage root paste of purple-fleshed sweet potato (I. batatas cv Ayamurasaki). We named this new caffeoylsophorose compound as CS. Fortunately, CS showed high antioxidative activity in partial accordance with the acylated sugar moiety of the YGM-6 structure (as depicted in Table 1), being matched by our subsequent approach to elucidating the origin of the antihyperglycemic effect of acylated anthocyanins. Thus, in this present study, we examined the antihyperglycemic function of CS derived from YGM-6 in vitro and in vivo. In addition, the structural factors of CS for eliciting AGH inhibition were also examined in a comparative study with phenolic acids.

### Materials and Methods

**Materials.** α-Glucosidase (AGH, EC 3.2.1.20) from rat intestinal acetone powder and human saliva α-amylase (EC 3.2.1.1) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and CNBr-activated Sepharose 4B was purchased from Pharmacia Biotech AB (Upsala, Sweden). The phenolic acids used in this study (caffeic acid, ferulic acid, p-coumaric acid, 3-hydroxy-4-methoxycinnamic acid, chlorogenic acid, dihydrocaffeic acid, 3,4-dihydroxyphenyl acetic acid, protocatechuic acid, and gallic acid) were purchased from Wako Pure Chemical Industries, Co. (Osaka, Japan). Storage roots of the purple-fleshed sweet potato cv Ayamurasaki were obtained from National Agricultural Research Center for Kyushu Okinawa Region in Miyazaki prefecture. Other reagents were of analytical grade and used without further purification. Fermented red vinegar (RV) was produced according to our previous report. Ethanol (200-liter) was mixed with water (2,600-liter), seed vinegar (800-liter), and steamed and mashed storage root of the purple-fleshed sweet potato (400 kg). The mixture was fermented for 3 months with acetic bacteria in the seed vinegar, the filtered and heat-sterilized product being used for the source of CS.

**Preparation of diacylated anthocyanin and CS.** Crude anthocyanin pigments were extracted from the storage roots of the purple-fleshed sweet potato cv Ayamurasaki. The preparation procedures for the anthocyanin extract and YGM-6 were the same as previously reported. Isolation was done by applying the extract to preparative ODS-HPLC (L-6200 intelligent pump system, Hitachi).  

### Table 1. Inhibitory Activities of Caffeoylsophorose and Its Related Compounds toward α-Glucosidase and α-Amylase

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ value (µM)</th>
<th>Compound</th>
<th>IC₅₀ value (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maltase Sucrase α-Amylase</td>
<td></td>
<td>Maltase Sucrase α-Amylase</td>
</tr>
<tr>
<td>YGM-6</td>
<td>200 ± 4.1 NF 813 ± 22.6</td>
<td>6-O-Caffeoylsophorose</td>
<td>699 ± 17.1 874 ± 39.0 25200 ± 11500</td>
</tr>
<tr>
<td>6-O-Caffeoylsophorose</td>
<td>17200 ± 14000 3500 ± 400</td>
<td></td>
<td></td>
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</table>

*An α-glucosidase inhibitory assay was performed by an immobilized assay system with 10 mM maltose or 45 mM sucrose as described in the Materials and Methods section.  
YGM-6, peonidin 3-O-(2-O-(6-O-E-feruloyl-β-D-glucopyranosyl)-6-O-E-caffeoyl-β-D-glucopyranoside)-5-O-β-D-glucopyranoside.  
NI, no inhibition.  
Not measured. Each value is the mean ± SEM (n = 3).
Co., Japan) in a column (Inertsil ODS 5, φ 20 × 250 mm, GL Sciences, Japan) with a linear gradient solvent system of A (15% AcOH):B (15% AcOH and 30% acetonitrile) = 70:30–50:50, v/v for 60 min at 520 nm. The YGM-6 fraction was evaporated to dryness, dissolved in a minimum amount of TFA, and precipitated by adding an excess of diethyl ether, the precipitate being collected and dried in vacuo. YGM-6 was obtained as a purple powder of the TFA salt.

CS was prepared according to the method in our previous report. Briefly, freeze-dried powder (4.9 g) from 3-liter of the red vinegar (RV) produced through a fermentation with purple-fleshed sweet potato was dissolved in methanol and centrifuged. The supernatant was precipitated with an excess of diethyl ether, and the resulting precipitate was applied to the foregoing preparative HPLC analysis with an isocratic solvent system of A (15% AcOH):B (15% AcOH and 30% acetonitrile) = 90:10 at room temperature with a flow rate of 7.0 ml min⁻¹ and monitoring at 310 nm with an L-4200 UV–VIS detector (Hitachi, Japan). A peak with the retention time of 12.9 min was collected and evaporated to dryness. As a result of the HPLC preparation, a slightly reddish powder of CS (ca. 0.1 g) was obtained from the RV powder for subsequent use in this study.

**AGH inhibitory assay.** The AGH inhibitory assay was performed according to our proposed immobilized AGH (rAGH) assay system. The immobilization of AGH partially purified from rat acetone powder on CNBr-activated Sepharose 4B (Pharmacia Biotech AB, Uppsala, Sweden) has been described in detail in our previous paper. In the rAGH assay, the rAGH support (10 mg of wet gel, 4.1 mL/mg of wet gel) was taken in an end-capped Assist mini-column with a 45–90-μm polyethylene filter (CC-07, 5 mL, Assist, Tokyo, Japan), and the assay was started after adding 100 μL of an inhibitor solution and 900 μL of a model intestinal fluid (0.1 M phosphate buffer (pH 6.8) as described in the Japanese Pharmacopoeia (JP XIII)) containing maltose (10 mM) or sucrose (45 mM). After incubating in a rotating cultivator (4 rpm, RT-5, Taitec, Saitama, Japan) at 37°C for 30 min (maltase assay) or for 60 min (sucrase assay), the reaction was stopped by filtering the solution in the column. The maltase activity was determined by measuring the glucose liberated from maltose in the filtrate by a Glucose F-kit (Roche Diagnostics Co., Tokyo, Japan). The inhibitory assay for human saliva α-amylase was done with Amylase-Test Wako (Wako Pure Chemical Industries, Tokyo, Japan), the decrease in starch by the action of α-amylase being measured at 660 nm. One unit of AGH or α-amylase activity is defined as the amount of enzyme required to hydrolyze 1 μmol of substrate per min under the above-mentioned assay conditions. The concentration of an inhibitor required to inhibit 50% of the enzyme activity under the assay conditions is defined as the IC₅₀ value.

**Kinetic study.** A kinetic study was performed under the same conditions as those just described, except for using an incubation time of 10 min. The concentration range of maltose as a substrate was 0.5–10 mM. The inhibitory mode of YGM-6 and CS in the presence of 0.2 mM and 0.7 mM, respectively, of maltose was determined by Lineweaver–Burk plots.

**Animal experiments with SD rats.** Four male 7-week-old Sprague-Dawley (SD) rats (SPF/VAF Crj:SD; Charles River Japan, Kanagawa, Japan) in each rat experiment were fed on a laboratory diet (MF, Oriental Yeast, Tokyo, Japan) and given water ad libitum. All rats were housed for 1 week at 21 ± 1°C and 55 ± 5% humidity under controlled lighting from 8:30 to 20:30. Each rat (n = 4, 275.4 ± 2.2 g) was starved for 16 h before a single oral administration of the CS sample by gavage. Five min after the administration, 1 ml of 2 g/kg of a substrate (maltose, sucrose or glucose) solution was administered to each rat. The control rats were administered with the same volume of the substrate solution without a sample. At each time point up to 120 min, about 20 μl of blood was collected from the tail vein, being immediately subjected to BGL measurement with a disposable glucose sensor (Glutest Pro; Sanwa Chemical Research Co., Tokyo, Japan). The remaining blood (serum) in the sample was subjected to an insulin assay (Rat Insulin EIA Biotrak system, Amersham Pharmacia Biotech UK, Little Chalfont, Buckinghamshire, England). All measurements were taken three times.

**Data analyses.** Each result for the administration study is expressed as the mean of BGL (mg/dl of blood) ± SEM (%). Statistical differences of BGL between the control and sample groups at each administration time were evaluated by using a two-factor analysis of variance (ANOVA) and followed by the Tukey–Kramer t-test for a post hoc analysis. Other statistical evaluations were performed by Student’s t-test. P < 0.05 was considered statistically significant. Analyses were performed with Stat View J 5.0 (SAS Institute, Cary, NC, U.S.A.).

**Results**

**AGH inhibitory activity of CS**

The rAGH inhibition study of CS was performed by using a substrate of maltose or sucrose, because AGH occurs in the epithelium of the small intestine as an SI (sucrase–isomaltase) complex. As shown in Table 1, CS inhibited the maltase action with an IC₅₀ value of 699 ± 17.1 μM. CS was also a potent sucrase inhibitor, rather than other natural inhibitors such as D-xylose (IC₅₀ of 1.19 mM). On the other hand, CS did not affect the α-amylase action (IC₅₀ of >25 mM). The maltase inhibition profile of the YGM-6 fragment family shown in Table 1 reveals that the activity of CS was 1/3.5-fold lower than that of the precursor (YGM-6, IC₅₀
of 200 μM), but still remained potent maltase inhibiting power. In contrast, sophorose and caffeic acid from CS were no longer maltase inhibitors, indicating that acylation was probably essential for eliciting potent maltase inhibitory power.

**Kinetic study**

Figure 1 presents Lineweaver–Burk plots for the hydrolysis of maltose by iAGH (maltase) in the absence or presence of CS (0.7 mM) or YGM-6 (0.2 mM). As a result, both compounds inhibited maltase action non-competitively (Km, 1.6 mM; vmax, 0.47 μmol/ml/min). This implies that the inhibitory action of CS on the SI complex of AGH was the same as that of its precursor. The apparent Ki values of CS and YGM-6 against maltase were estimated to be 979 and 210 μM, respectively, almost the same as their IC50 values.

**CS structure-iAGH inhibitory activity relationship**

As shown in Table 1, YGM-6 had other acylated moieties such as ferulic acid, feruloylsophorose and chlorogenic acid. Thus, to clarify the structural factors attributable to the powerful iAGH (maltase) inhibition of CS and YGM-6, their analogues were subjected to an iAGH assay. As summarized in Table 2, chlorogenic acid (3-O-caffeoylquinic acid) had weak maltase inhibition like caffeic acid, whereas no inhibition was observed for the other phenolic acids. Sucrase inhibition by the active phenolic acids was 5–6 times stronger than maltase inhibition. Additional experiments on iAGH inhibition were performed by using caffeic acid analogues (Table 2), because methoxylated or mono hydroxyl phenolic acids in the aromatic ring did not inhibit maltase. The concentration of each analogue was respectively set at 18 mM and 8 mM for the maltase and sucrase inhibition assays. For both inhibition studies, the iAGH inhibitory activity of the caffeic acid analogues was in descending order of potency as follows: maltase inhibition, gallic acid and caffeic acid > 3,4-dihydroxyphenyl acetic acid > dihydrocaffeic acid >> protocatechuic acid; sucrase inhibition, caffeic acid > dihydrocaffeic acid >> 3,4-dihydroxyphenyl acetic acid, protocatechuic acid and gallic acid.

**Change in blood glucose level after a single oral administration of CS to SD rats**

Based on the result that CS showed potent maltase inhibitory activity (Table 1), the change in BGL after administering CS with maltose to SD rats was examined. As shown in Fig. 2, a significant reduction of glycemic response (P < 0.01 vs. control) with a dose of 100 mg/kg to SD rats at 30 min (BGLcs{30 min, 153 ± 6.0 mg/dl of blood) was observed against the control rats that ingested maltose alone (BGLcontrol{30 min, 172 ± 2.8 mg/dl of blood). Although the lowering effect was transient at a dose of 100 mg/kg, we found for the first time that CS had latent physiological capability for a postprandial antihyperglycemic effect like that of YGM-6. The area under the curve (AUC0–120 min) for CS ingestion (100.6 ± 6.7 mg/h/dl of blood) also showed a significant reduction of 22.9% compared with that of the control (130.5 ± 4.8 mg/h/dl of blood). Figure 3 shows the AUC change of dose-dependent experiments for CS ranging from 50 mg/kg to 200 mg/kg to SD rats after maltose ingestion. As a result of the linear relationship between the dose and reduction ratio of AUC0–120 min for CS against that of the control, the ED20 value (the effective dose required to achieve 20% suppression of the BGL rise) was interpolatively estimated to be 117 mg/kg, the extrapolative ED50 value being 248 mg/kg.

As shown in Fig. 4, CS at a dose of 100 mg/kg significantly reduced (P < 0.05 vs. control) the serum
### Table 2. Inhibitory Activities of Phenolic Acids and Caffeic Acid Analogues toward α-Glucosidase

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>IC₅₀ value (mM)</th>
<th>Inhibitory ratio⁺ (%)</th>
<th>Maltase</th>
<th>Sucrase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeic acid</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td>17.2 ± 4.1</td>
<td>59.0 ± 4.4</td>
<td>93.0 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>OH</td>
<td>OCH₃</td>
<td>OH</td>
<td>NI</td>
<td>20.0 ± 7.1</td>
<td>76.0 ± 8.6</td>
<td></td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
<td>NI</td>
<td>45.0 ± 3.1</td>
<td>4.8 ± 3.3</td>
<td></td>
</tr>
<tr>
<td>3-Hydroxy-4-methoxy cinnamic acid</td>
<td>OCH₃</td>
<td>OH</td>
<td>OH</td>
<td>NI</td>
<td>7.2 ± 4.2</td>
<td>4.4 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>OH</td>
<td>OH</td>
<td>Glc</td>
<td>18.9 ± 2.2</td>
<td>71.0 ± 5.5</td>
<td>9.7 ± 2.0</td>
<td></td>
</tr>
</tbody>
</table>

*The α-glucosidase inhibitory assay was performed by an immobilized assay system with 10 mM maltose or 45 mM sucrose as described in the Materials and Methods section.*

*The concentrations of caffeic acid analogues were set at 18 mM and 8 mM for the maltase and sucrase inhibition study, respectively. The obtained data are presented as the inhibitory ratio (%) under a fixed sample concentration.*

*NI, no inhibition. Each value is the mean ± SEM (n = 3).*

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**Fig. 2.** Effect of Caffeoylsophorose on the Blood Glucose Level after a Single Oral Administration of 2 g of Maltose/kg to SD Rats.

One ml of 100 mg of caffeoylsophorose/kg (○) was dosed to male 8-week-old SD rats. After 5 min, 1 ml of 2 g of maltose/kg was administered to each rat. The control (□) was administered with the same volume of the substrate solution without an inhibitor. At each time point to 120 min, about 22 l of blood was collected from the tail vein, being immediately subjected to a blood glucose level measurement by a disposable glucose sensor. Each value is the mean (mg/dl of blood) ± SEM. Significant difference between a sample and control group was examined with the Tukey–Kramer t-test (n = 4, §§p < 0.01).

**Fig. 3.** Dose-Dependence of Caffeoylsophorose (○) on the Reduction of Glycemic Response after Maltose Ingestion (2 g/kg dose) by SD Rats.

AUC₀₋₁₂₀min is the area under the curve of incremental blood glucose level up to 120 min. The reduction ratio (%) of AUC₀₋₁₂₀min for caffeoylsophorose against AUC₀₋₁₂₀min for the control was used for estimating either each ED₂₀ or ED₅₀ value. Each plot shows the mean ± SEM (n = 4).
insulin level after 30 min (insulin_{control-30 min} \pm 6.7 \pm 0.7 \text{ ng/ml of serum}; \text{insulin}_{CS-30 \text{ min}} \pm 3.5 \pm 0.5 \text{ ng/ml of serum}) in response to the maltose ingestion, supporting the notion that CS suppressed the BGL rise in SD rats by lowering the glucose absorption, and not by promoting the insulin secretion. Similar rat experiments were performed by using sucrose (Fig. 5) and glucose (Fig. 6) as a substrate sugar (2 g/kg). The result shows that neither sucrose nor glucose ingestion with CS affected the postprandial BGL curve (AUC_{0-120 \text{ min}} of sucrose: control, 93.6 \pm 6.8 \text{ mg-h/dl of blood}; \text{CS}, 78.0 \pm 7.3 \text{ mg-h/dl of blood}; AUC_{0-120 \text{ min}} of glucose: control, 103.5 \pm 8.3 \text{ mg-h/dl of blood}; \text{CS}, 114.5 \pm 13.5 \text{ mg-h/dl of blood}). Although the in vitro iAGH

study of CS (Table 1) showed sucrase inhibition with an IC_{50} value of 874 \mu M, CS was found to have a poor antihyperglycemic effect through sucrase inhibition throughout our rat experiments (Fig. 5). The lack of BGL reduction in the glucose-ingesting rats (Fig. 6) demonstrates that the antihyperglycemic effect induced by CS was achieved by restrictive maltase inhibition, and not by inhibiting glucose transport in the small intestinal membrane via the Na^+/glucose co-transporter.

Discussion

Many natural food resources^8–10^ have been examined for their AGH inhibitory activity. Among them, a human study reported by Fujita et al.\(^9\) has pointed out that, even if an AGH inhibitor was from a natural food component, efficient postprandial BGL control could be achieved, in which 0.3 g of inhibitor ingestion after 200 g of cooked rice resulted in a 40% reduction in BGL rise in diabetic subjects. We have clarified in the present study for the first time that a newly identified natural compound, CS, from diacylated anthocyanin had the ability to retard the action of AGH (Table 1), as well as the antioxidation (radical scavenging) activity.\(^16\) This new physiological function of CS was also demonstrated in rat experiments, in which 100 mg/kg dose of CS apparently suppressed the postprandial BGL rise at 30 min in maltose-ingesting SD rats (Fig. 2). This finding indicates that substantial benefit for normotensive or borderline NIDDM subjects would result by developing a functional food involving CS with an antihyperglycemic effect against maltose and dietary carbohydrate intake. Subsequent animal experiments on glucose-ingesting SD rats (Fig. 6) imply that the antihyperglycemic effect was achieved by the intestinal maltase inhibition of CS, and not by its glucose transport inhibition. As Kobayashi et al.\(^18\) have reported, a
polyphenolic structure like that of esterified catechins (e.g., epicatechin gallate) would be needed for competitive glucose transport inhibition, and not being matched with the CS structural property.

CS had a 1/1.7-fold lower antihyperglycemic activity with ED$_{50}$ of 117 mg/kg than that of YGM-6 (ED$_{50}$ of 69 mg/kg), and was also much weaker than the therapeutic drug, acarbose (ED$_{50}$ of 2.2 mg/kg). This demonstrates that the BGL reduction capability of CS in SD rats was about 50 times lower than that of acarbose. In addition, although there has been no comparable study on the BGL reduction of natural active components in maltose or carbohydrate ingesting rats, CS seemed to have weak antihyperglycemic potency compared to that of L-arabinose (ED$_{50}$ of 18.5 mg/kg) or a Sophora plant extract (ED$_{50}$ of 6.3–8.0 mg/kg). However, a daily intake of CS would be acceptable for preventing a postprandial BGL rise due to food carbohydrates because of its moderate and beneficial inhibitory effect against maltase. The STOP-NIDDM randomized human trial also supports the effectiveness of a daily intake of the AGH inhibitor, by which 50–100 mg intake of acarbose three times daily improved or delayed the impaired glucose tolerance.

Evidence for improved hyperglycemia in spontaneous diabetic GK (Goto–Kakizaki) rats by showing down the intestinal AGH activities provides further evidence that long-term AGH inhibition by any natural inhibitor would be of significant benefit to non-obese NIDDM subjects.

The iAGH inhibitory experiments (Table 1) show that CS preferably inhibited AGH rather than α-amylase, suggesting that the antihyperglycemic effect of CS would be achieved through the retardation of AGH activity at the small intestine. The inhibition mode of CS was noncompetitive, like that of YGM-6 (Fig. 1). Non-competitive inhibition has also been observed for such other natural compounds as fructose and thio-fructofuranoside (against sucrase, maltase or both). As Hauri et al. have reported, sucrase and isomaltase formed a dimeric SI complex at the intestinal membrane, to which only the C-terminal of the isomaltase subunit was anchored, and the sucrase subunit was present in a complex form with the isomaltase subunit. Thus, the non-competitive AGH inhibition of CS would have resulted from its binding to the SI complex region.

Our next strategy was to clarify the structural relationship between CS and the AGH inhibitory activity. We have already pointed out that the potent iAGH (maltase) inhibition capability was elevated by acylated anthocyanins with phenolic acids, but not by their aglycons. In the present study, only an acylated moiety (CS) of diacylated anthocyanin (YGM-6) was found to retain some degree of maltase inhibitory activity, as YGM-6 did inhibit maltase (Table 1). This indicates that the acylated moieties of YGM-6 with Caf and Fer were involved in the expression of AGH inhibition. Similar AGH inhibitory expression by acylation has also been reported for sulfoquinovosyldiacylglycerol from edible brown algae and esterified catechins from tea polyphenols. Thus, the iAGH inhibition study of phenolic acids was primarily done to clarify the potential inhibition capability of CS. As summarized in Table 2, among the phenolic acids, but excepting the caffeic acid derivative, i.e., chlorogenic acid (3-cafeoylquinic acid), inhibition behavior against iAGH was only observed for caffeic acid. This result indicates that substitution of the hydroxyl group to both the R$_1$ and R$_2$ positions would be required to elicit this activity. Similar inhibition behavior of chlorogenic acid also supports the importance of substitution in the aromatic ring. However, considering the fact that the iAGH (maltase) inhibitory activity of chlorogenic acid (IC$_{50}$ of 18.9 mM) was much lower than that of CS (IC$_{50}$ of 699 µM), the sugar moiety was an alternative candidate for maltase inhibition. According to the maltase inhibition study by caffeic acid analogues (Table 2), the inhibition activity was, in descending order of potency, caffeic acid > 3,4-dihydroxyphenyl acetic acid > dihydrocaffeic acid > protocatechuic acid. The markedly lower maltase inhibitory activity of protocatechuic acid than that of other caffeic acids suggests that the longer, unsaturated alkyl chain of phenolic acid would be needed for maltase inhibition. A comparable result that the maltase inhibitory activity of gallic acid was much higher than that of protocatechuic acid also reveals that multi-substitution of the hydroxyl group on the aromatic ring seems favorable for eliciting maltase inhibition.

The results of the present study reveal for the first time that CS derived from diacylated anthocyanin possessed a new physiological function regarding its postprandial antihyperglycemic effect on SD rats through the retardation of maltase activity. AGH (maltase) inhibition of CS is concluded to have been due to such structural properties as 1) the acylation of phenolic acid with sugar, 2) the presence of a hydroxyl group at both the R$_1$ and R$_2$ positions on the aromatic ring, and 3) the presence of an unsaturated alkyl chain in the acylated moiety.

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