

Durable Antihyperglycemic Effect of 6-*O*-Caffeoylsophorose with α -Glucosidase Inhibitory Activity in Rats

Tomoko FUJISE^{1,4*}, Norihiko TERAHARA², Keiichi FUKUI³, Koichi SUGITA³, Hideaki OHTA⁴, Toshiro MATSUI¹ and Kiyoshi MATSUMOTO¹

¹ Department of Bioscience and Biotechnology, Division of Bioresource and Bioenvironmental Sciences, Faculty of Agriculture, Graduate School of Kyushu University, 6-11-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan

² Department of Food Science for Health, Faculty of Health and Nutrition, Minami-Kyushu University, 5-1-2 Kirishima, Miyazaki 880-0032, Japan

³ Miyazaki JA Food Research & Development Inc., Ikimedai, Miyazaki 880-0943, Japan

⁴ Department of Nutritional Sciences, Faculty of Nutritional Sciences, Nakamura Gakuen University, 5-7-1 Befu, Jonan-ku, Fukuoka 814-0198, Japan

Received January 31, 2008; Accepted May 23, 2008

To evaluate the duration of antihyperglycemic effects of 6-*O*-caffeoylsophorose (CS), a newly identified natural α -glucosidase inhibitor from fermented purple-sweet potato, a single oral administration of CS was given to maltose-loaded Sprague-Dawley rats. Administration of CS (200 mg/kg) 30 min or 60 min before maltose administration produced an elevation of blood glucose level by administration of 2 g/kg of maltose in rats that was significantly lower than for no administration (control). In contrast, simultaneous or pro-administration of CS with maltose eliminated the antihyperglycemic effect. CS significantly reduced rat intestinal α -glucosidase activity in all of the small intestinal mucosal regions with a maximal reduction ratio of ca. 40% up to 60 min after CS administration. Thereafter, the intestinal α -glucosidase activity tended to return to basal level. These findings suggest that the antihyperglycemic effect of CS is restricted to pre-administration within 60 min.

Keywords: α -glucosidase, caffeoylsophorose, antihyperglycemic effect, diabetes

Introduction

Anthocyanins, one of the families of polyphenol compounds found in fruits and plants, are valued for their beneficial health effects. Pharmacologically, anthocyanins are reputed to be antimutagenic (Yoshimoto *et al.*, 2001) and to have antioxidative effects (Oki *et al.*, 2002). Crude anthocyanin extract of *Ipomoea batatas* cv Ayamurasaki has also been demonstrated to have considerable antihyperglycemic effect in Sprague Dawley (SD) rats (Matsui *et al.*, 2002). In our previous reports, (Matsui *et al.*, 2001a; Matsui *et al.*, 2001b; Matsui *et al.*, 2002) the main compounds showing antihyperglycemic effects in the extract were found to be acylated anthocyanins, both of which possess α -glucosidase (AGH, EC 3.3.1.20) inhibitory activity: YGM-3 (Cyanidin;

3-*O*-(2-*O*-(6-*O*-*E*-feruloyl- β -D-glucopyranosyl)-6-*O*-*E*-caffeoyl- β -D-glucopyranoside)-5-*O*- β -D-gluconopyranoside) and 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-gluconopyranoside). Further study regarding the structure-AGH inhibition relationship revealed that the acylated moieties (e.g., 6-*O*-*E*-caffeoyl- β -D-glucopyranoside) were responsible for eliciting AGH inhibition (Matsui *et al.*, 2001b; Terahara *et al.*, 2003). 6-*O*-Caffeoylsophorose (CS), which is a constituent of YGM-3 and YGM-6, was successfully produced by fermentation of purple-fleshed sweet potato and was identified for the first time as a natural compound and proven to be a potent AGH inhibitor (Matsui *et al.*, 2004; Terahara *et al.*, 2003).

Recently, AGH inhibitors have attracted much attention as being effective drugs for noninsulin-dependent diabetes mellitus (NIDDM), as they can suppress or delay excessive

*To whom correspondence should be addressed.

Email: tfujise@nakamura-u.ac.jp

postprandial elevation of blood glucose level (BGL) without promoting insulin secretion. In addition, the effective use of long-term therapeutic drug acarbose treatment in the prevention of NIDDM was reported (Chiasson *et al.*, 2002). Clinical indications of some AGH inhibitory foods have also demonstrated the efficacy of food intake on postprandial BGL control (Deguchi *et al.*, 1998; Fujita *et al.*, 2001). This evidence prompted us to examine the antihyperglycemic potential of CS as a new alternative medicinal food compound.

However, aside from information on the inhibition potency or mode of natural AGH inhibitors, there are few reports on the duration of the AGH inhibition effect; information on the duration of the antihyperglycemic effect would be of great benefit for evaluating whether these AGH inhibitors exert acute or long-term improvement of postprandial BGL and the timing of their administration. Catechin extract has been reported to show at least a 2 h-suppression effect of sucrase activity at the intestinal mucosa of Wistar rats in response to suppression of BGL elevation (Matsumoto *et al.*, 1993). Therapeutic AGH inhibitors, acarbose and voglibose, have been shown to have the ability to suppress elevation of BGL by sucrose for 2 h and >3 h, respectively (Matsuura *et al.*, 2001). In this study, we examined the duration of AGH inhibition in the small intestinal mucosal membrane after a single oral administration of CS to SD rats. The effect was also evaluated by checking BGL change.

Materials and Methods

Materials α -Glucosidase (AGH) 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 (Uppsala, Sweden). *p*-Nitrophenyl α -D-glucopyranoside (PNP-G) as a substrate was obtained from E. Merck (Darmstadt, Germany). Acarbose as a synthetic AGH inhibitor was obtained from Bayer Medical Co. (Leverkusen, Germany). 6-*O*-Caffeoylsophorose (CS; 6-*O*-*E*-caffeoyl-(2-*O*- β -D-glucopyranosyl)-D-glucopyranose) was obtained from red-colored vinegar that had been developed *via* fermentation of the storage root paste of purple-fleshed sweet potato (*I. batatas* cv Ayamurasaki) with acetic bacteria in the seed vinegar (Terahara *et al.*, 2003). Briefly, Red vinegar (3 L) was evaporated and freeze-dried under reduced pressure. The dried powder of vinegar (4.9 g) was dissolved in methanol and centrifuged. The supernatant was precipitated with an excess of diethyl ether, and the resulting precipitate was applied to preparative ODS-HPLC analysis (L-6200 intelligent pump system, Hitachi Co., Tokyo, Japan) on a column (Inertsil ODS 5, ϕ 20 \times 250 mm, GL Sciences, Tokyo, Japan) 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, Tokyo, Japan). A peak with a retention time of 12.9 min was collected and evaporated to dryness (0.11 g, 2.2% yield from the red vinegar crude powder).

Oral Maltose Tolerance Test Male 7-week-old Sprague-Dawley (SD) rats (SPF/VAF Crj:SD, Charles River Japan, Kanagawa, Japan) in each rat experiment were fed a laboratory diet (MF, Oriental Yeast, Tokyo, Japan) and given water *ad libitum*. All rats were housed for 1 week at 23 \pm 1°C and 55 \pm 5% humidity under controlled lighting from 8:30 to 20:30. Each rat was starved for 16 h before a single oral administration of AGH inhibitor (200 mg/kg CS or 3 mg/kg acarbose) by gavage. Acarbose, which is a therapeutic AGH inhibitor, was used as a positive control throughout the animal experiments. At 0, 30, 60, and 120 min after, or 5 min before the administration of AGH inhibitor solution, 1 ml of 2 g/kg of substrate (maltose) solution was administered to SD rats. The control rats were administered the same volume of maltose solution without AGH inhibitor. At each time point up to 120 min after the substrate administration, about 20 μ l of blood was collected from the tail vein, and immediately subjected to a BGL measurement using a disposable glucose sensor (Glutest Pro, Sanwa Chemical Research, Co., Tokyo, Japan). All measurements were done in triplicate.

Measurement of rat intestinal AGH activity Male 8-week-old SD rats starved for 16 h were used for measurement of rat intestinal AGH activity. Rats were sacrificed at 0 (control), 30, 60, or 120 min after CS administration (200 mg/kg). As rat AGH activity changes greatly during a day and rat intestinal AGH activity shows circadian rhythms (Saito, 1972), all the sacrifice experiments were performed at 11:00. The small intestine was taken immediately and cut transversely into three segments (upper, middle, and lower parts) of roughly equal length, which were about 5, 20, 35 cm from the stomach, respectively. Each segment was flushed with ice-cold phosphate buffered saline. The mucosa of 1 cm of each segment was collected by scraping with a glass slide and homogenized in 0.5 ml of 100 mM sodium phosphate buffer (pH 6.8) using a micro-homogenizer (Iuchi S-203, Tokyo, Japan) for 30 s with cooling on ice. After centrifugation at 3,000 \times g for 10 min, the supernatant was used as a crude enzyme solution.

Overall rat intestinal AGH activity was determined according to our previous report (Oki *et al.*, 1999) using *p*-nitrophenyl- α -D-glucopyranoside (PNP-G) as a nonspecific synthetic AGH substrate. Briefly, the reaction was started by adding 20 μ l the enzyme solution to 980 μ l of 0.7 mM PNP-G solution (100 mM sodium phosphate buffer, pH 6.8),

followed by incubation at 37°C for 20 min. After stopping the reaction by adding 250 μ l of 0.5 M Tris solution, AGH activity was determined by monitoring the PNP released from PNP-G at 400 nm using a Shimadzu 1200 UV-Vis spectrophotometer (Kyoto, Japan). Protein content of the crude enzyme solution was measured using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, CA, USA) based on the Lowry method with bovine serum albumin as a standard. Rat experiments in this study were carried out under the guidelines of the Animal Experiment Committee of the Faculty of Agriculture and the Graduate Course of Kyushu University and the Law (No. 105, 1973) and Notification (No. 6, 1980 of the Prime Minister's Office) of the Japanese Government.

Data Analysis Each result for the administration study is expressed as the mean of BGL (mg/dl) \pm SEM. Statistical differences between the control and AGH inhibitor groups were evaluated by two-factor analysis of variance (ANOVA) followed by Tukey-Kramer's *t*-test for post hoc analysis using Stat View J5.0 (SAS Institute Inc., Cary, NC, USA). $P < 0.05$ was considered to be statistically significant.

Results

Antihyperglycemic effect of CS and acarbose in SD rats To evaluate the durable antihyperglycemic effects of CS in SD rats, we performed single oral experiments of CS administered at 0, 30, 60, and 120 min before or 5 min after maltose (2 g/kg) administration. The dosage of 200 mg/kg of CS was selected on the basis of reported BGL lowering effective dosage (ED)₅₀ of 248 mg/kg (Matsui *et al.*, 2004). In addition, since at this dosage of CS resulted in no significant reduction of BGL in 2 g/kg sucrose-loaded SD rats (Matsui *et al.*, 2004), maltose-loaded rat experiments were carried out in this study. Fig. 1a shows incremental BGL curves for CS administered 30, 60, or 120 min before maltose administration. Administration of CS at 30 and 60 min before maltose administration effectively suppressed BGL elevation by maltose. In 30-min and 60-min pre-administration experiments of CS, significant BGL reductions of 32.7 mg/dl and 43.3 mg/dl, respectively, induced by CS were observed at 30 min after maltose administration. In addition, significant reduction of the area under the curve (AUC_{0-120min}) (87.4 \pm 5.6 mg \cdot h/dl for 30-min pre-administration, 87.7 \pm 10.8 mg \cdot h/dl

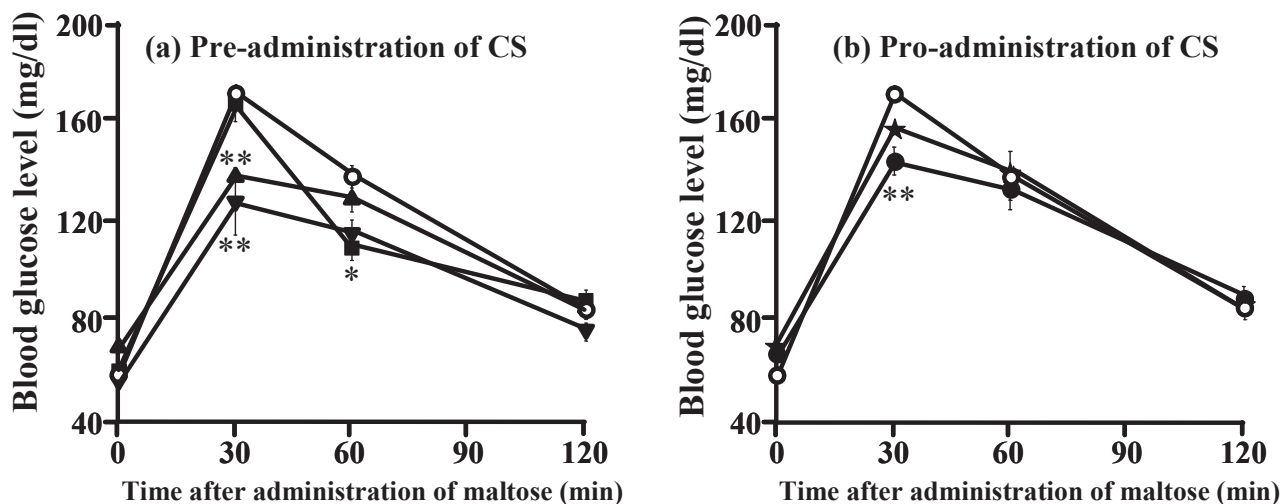


Fig. 1. Effect of caffeoylsophorose (CS) on blood glucose levels after single oral administration of 2 g/kg maltose to 8-week-old SD rats. (a) Pre-administration experiments of CS at a dose of 200 mg/kg. At 30 min (▲), 60 min (▼), and 120 min (■) after CS administration, 1 ml of maltose solution was administered to each rat. (b) pro-administration experiments of CS at a dose of 200 mg/kg. At 0 min (●) and 5 min (★) before CS administration, 1 ml of maltose solution was administered to each rat. Control rats (○) were administered the same volume of maltose solution without CS. At each time point up to 120 min after maltose administration, 20- μ l blood samples were collected from the tail vein and immediately subjected to blood glucose level measurement using a disposable glucose sensor. Data are presented as the mean (mg/dl) \pm SEM. Significant differences versus the control were examined with Tukey-Kramer's *t*-test ($n=3$, * $P < 0.05$; ** $P < 0.01$).

for 60-min pre-administration) compared to the control (130.5 ± 4.8 mg·h/dl) revealed the antihyperglycemic potential of CS when pre-administered within 60 min of maltose administration. CS administered simultaneously with maltose (Fig. 1b), showed a significant ($P < 0.05$), but less extensive AUC reduction of 98.9 ± 13.2 mg·h/dl. CS administration at 5 min after or 120 min before maltose administration did not produce a significant effect. This indicates that the appearance of the antihyperglycemic effect of CS is restricted to simultaneous or to pre-administration of CS within 60 min.

The same administration experiments as for CS were conducted with acarbose. A 3 mg/kg dose was given based on the acarbose ED_{50} value of 3.1 mg/kg (Matsui *et al.*, 2004). As shown in Figs. 2a and 2b the antihyperglycemic effect was more durable than that of CS; a significant ($P < 0.01$) $AUC_{0-120\text{min}}$ reduction of 66.8 mg·h/dl was observed even when acarbose was given 120 min before maltose administration. No effects of acarbose-induced BGL lowering in SD rats were observed when acarbose was simultaneously or pro-administered (5 min) with maltose, revealing that pre-administration of acarbose within 120 min was effective in

producing an antihyperglycemic effect within the present SD rat experiments.

Changes in intestinal AGH activity after CS or acarbose ingestion in SD rats To clarify the durable inhibition effect of CS on AGH activity in the small intestinal mucosal membrane, changes in AGH activity after CS administration (200 mg/kg) were examined in three parts (upper, middle, and lower) of the intestine over time. Acarbose (3 mg/kg) was used as the positive control. Although CS showed antihyperglycemic effects only in maltose-loaded rats at a dose of 200 mg/kg (Matsui *et al.*, 2004), we used a nonspecific synthetic substrate of PNP-G to examine the overall effect of CS on intestinal AGH activity, including those of maltase, sucrase, isomaltase, trehalase, and lactase, since CS inhibited maltase as well as sucrase to a lesser extent (Matsui *et al.*, 2004). As depicted in Fig. 3a, the middle part of the small intestine was found to possess the highest AGH activity with 24.8 ± 2.4 mU/mg-protein in the descending order of middle > upper > lower parts, but CS gave an overall suppression of AGH activity for all parts of the small intestine up to 60 min. Thereafter, the intestinal AGH activity for each part

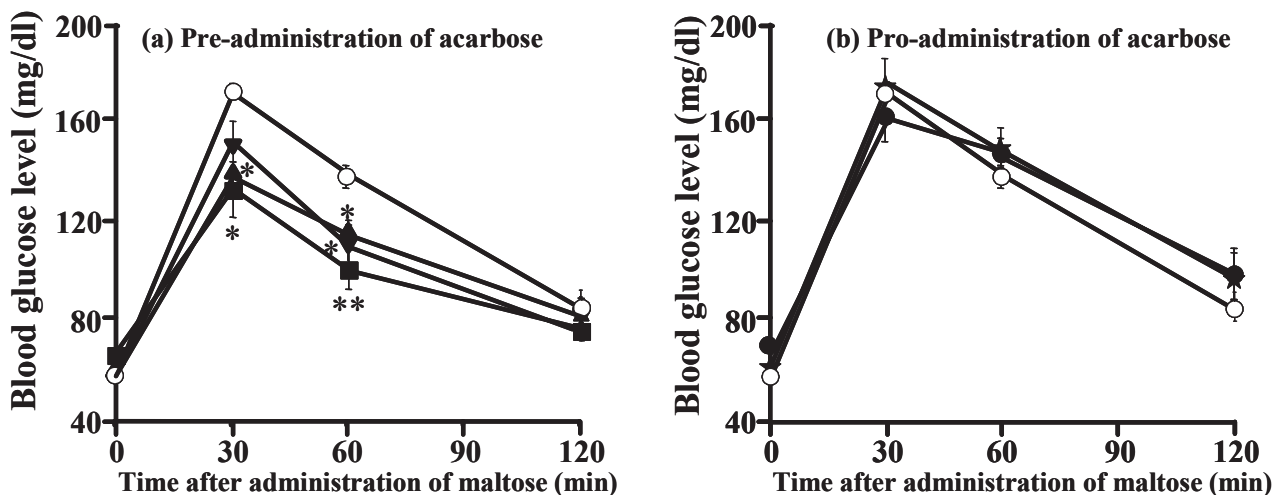


Fig. 2. Effect of acarbose on blood glucose levels after single oral administration of 2 g/kg maltose to 8-week-old SD rats. (a) Pre-administration experiments of acarbose at a dose of 3 mg/kg. At 30 min (▲), 60 min (▼), and 120 min (■) after acarbose administration, 1 ml of maltose solution was administered to each rat. (b) pro-administration experiments of acarbose at a dose of 3 mg/kg. At 0 min (●) and 5 min (★) before acarbose administration, 1 ml of maltose solution was administered to each rat. Control rats (○) were administered the same volume of maltose solution without CS. At each time point up to 120 min after maltose administration, 20- μ l blood samples were collected from the tail vein and immediately subjected to blood glucose level measurement using a disposable glucose sensor. Data are presented as the mean (mg/dl) \pm SEM. Significant differences versus the control were examined with Tukey-Kramer's *t*-test ($n=3$, * $P < 0.05$; ** $P < 0.01$).

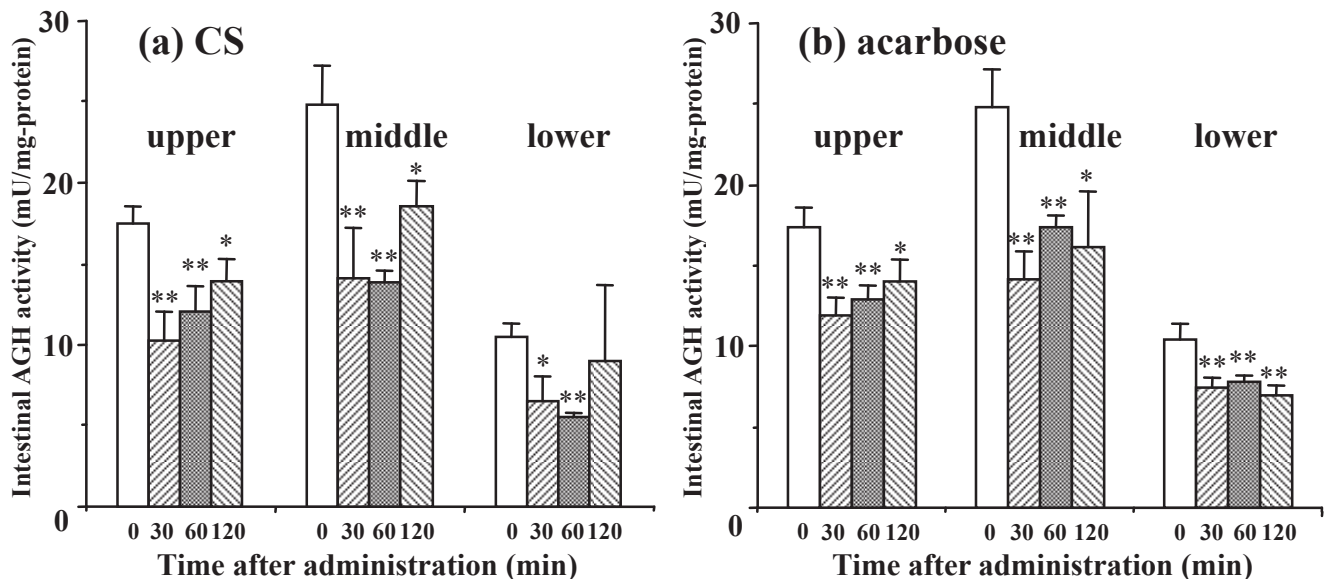


Fig. 3. Change in α -glucosidase activity of rat intestinal mucosa after the administration of caffeoylsophorose (a) or acarbose (b) to 8-week-old SD rats. At a fixed time points of 0, 30, 60, and 120 min after the administration of caffeoylsophorose (200 mg/kg) or acarbose (3 mg/kg), the upper, middle, and lower parts of the small intestine were taken from sacrificed rats and each part was subjected to an α -glucosidase activity assay using PNP-G as a substrate. Data are presented as the mean (mU/mg-protein) \pm SEM. Significant differences versus 0 min (before administration) were examined with Tukey-Kramer's *t*-test ($n=3$, * $P<0.05$; ** $P<0.01$).

tended to return to the basal level. These findings suggest that the antihyperglycemic effect of CS, as shown in Figs. 1a and 1b, could be partly due to a durable (at least within 60 min after the administration) retardation of AGH action in the entire small-intestinal mucosa.

Contrary to the suppression behavior of AGH activity induced by CS, acarbose was much more effective for longer retardation of intestinal AGH activity. As shown in Fig. 3b, even at 120 min after administration acarbose adequately suppressed overall AGH activity in all parts of the intestine with a suppression ratio of 20% to 35%.

Discussion

To date, many natural AGH inhibitors have been identified (Du *et al.*, 2006; Iwai *et al.*, 2006; Matsui *et al.*, 2007; Matsumoto *et al.*, 1993; shim *et al.*, 2003), some of which possess strong antihyperglycemic effects via the inhibition of disaccharidases responsible for glucose production. Among them, a human study reported by Fujita *et al.* (2001) has demonstrated that touchi extract shows efficient postprandial BGL control with a 40% reduction in BGL rise in diabetic subjects. Polyphenols, including cyanidin derivatives, caf-

feic acid derivatives (Iwai *et al.*, 2006; Matsui *et al.*, 2006), catechins, theaflavins (Matsui *et al.*, 2007), and anthocyanins (Matsui *et al.*, 2001b), were also reported to be excellent candidates for inhibiting disaccharidase enzymes and proven to have an *in vivo* antihyperglycemic effect in rats or mice. A typical evaluation test for the effect is performed by simultaneously administering a given inhibitor with carbohydrates to animals and monitoring the resulting BGL change. Sim *et al.* (2003) demonstrated the acute antihyperglycemic effect of *Rhus chinensis*, a herbal alternative medicine, by simultaneous administration with sucrose to SD rats. Our previous animal studies (Matsui *et al.*, 2002; Matsui *et al.*, 2004; Matsui *et al.*, 2006; Matsui *et al.*, 2007) used pre-administration of inhibitors (5 min before carbohydrate administration). However, neither protocol gave information on the duration of the antihyperglycemic effects of the inhibitors.

In this study, we clarified that the antihyperglycemic effect induced by CS in SD rats was achieved only when CS (200 mg/kg) was pre-administered with maltose. Pre-administration of CS with maltose could not produce an effect similar to acarbose, a therapeutic AGH inhibitor. In addition, single administration of CS to SD rats demonstrated that

overall AGH activity from rat intestinal mucosal membranes was significantly lower in all three areas of the intestine compared to those from control rats and that the reduction continued for at least 60 min after administration, which was similar to acarbose. These findings suggest that in taking CS to improve postprandial BGL as an alternative antihyperglycemic functional food, pre-intake of CS as well as acarbose would be highly effective. In addition, it seems likely that the maximal antihyperglycemic effect of CS can be achieved when pre-administered at 30-60 min before maltose administration in SD rats, while acarbose (3 mg/kg) continued to have an ameliorating effect on BGL rise even when pre-administered at 120 min before maltose administration (Fig. 2a). Either inhibitor had little or no antihyperglycemic ability when given simultaneously or pre-administered with maltose. In contrast, Matsuura *et al.* (2001) revealed that even in continuous intragastric infusion of 15% maltose solution to SD rats, portal serum glucose level was suppressed by acarbose (24 mg/kg) for 30 min. The discrepancy was possibly due to large differences in dosages of acarbose (3 mg/kg in the present study) between the two studies.

There have been few comparative studies on the duration of intestinal AGH inhibition by natural inhibitors in animal intestines. Matsumoto *et al.* (1993) reported that crude catechins suppressed rat intestinal mucosal sucrase activity for 120 min after sucrose administration in response to apparent BGL reduction. The long-term reduction behavior was in good agreement with our findings (Fig. 3a). Similarly, Yasuda *et al.* (2003) pointed out that overall AGH activity, including those of sucrase, maltase, and isomaltase except for trehalase and lactase, remained lower in all three parts (upper, middle and lower) of the small intestine throughout 6-month voglibose treatment in Goto-Kakizaki rats. This demonstrates that CS as well as other such AGH inhibitors could produce an equivalent effect on the whole small intestinal mucosa.

A newly identified natural compound, CS, an acylated moiety of anthocyanin YGMs, is formed by the condensation of caffeic acid with sophorose (Terahara *et al.*, 1999). As clarified in our previous report, CS preferentially inhibited maltase (IC_{50} : 699 μ M) rather than sucrase (IC_{50} : 874 μ M) and α -amylase (IC_{50} : 25.2 mM) and did not show significant antihyperglycemic effect in sucrose-loaded SD rats for doses of 200 mg/kg or less, while a significant reduction of BGL rise in maltose-loaded rats was observed. CS showed a much weaker antihyperglycemic effect compared to acarbose. The BGL reduction potency of CS was about 50-70 times lower than that of acarbose (Matsui *et al.*, 2004). Few comparable studies on the natural constituents which suppress the BGL rise after maltose load have been reported: the methanol extraction of ranawana (ED_{50} of 4.9 mg/kg) (Abesundara K. J.

M. *et al.*, 2004), 3,4,5-tri-caffeoylquinic (ED_{50} of ~20 mg/kg) (Matsui *et al.*, 2004) acid and theaflavins (ED_{50} of ~10 mg/kg) (Matsui *et al.*, 2007). CS has a weaker antihyperglycemic potency compared with these compounds, however, daily intake of CS would be useful for preventing a postprandial BGL rise corresponding to the consumption of carbohydrate. In addition, the present study proved that CS exerts a durable antihyperglycemic effect similar to acarbose, and CS seems to be practical as an alternative medicine food. CS also showed non-competitive inhibition against maltase with a K_i of 1.0 mM and did not inhibit glucose transport (SGLT1) as has been observed for esterified catechins (Kobayashi *et al.*, 2000) and naringenin (Li *et al.*, 2006). Thus, the long-term suppression of BGL rise by CS in maltose-loaded rats (Fig. 1a) may be due to the interaction of CS with the sucrase-isomaltase (SI) complex (Hauri *et al.*, 1982) of the AGH enzyme. Competitive (acarbose, Fig. 3b) or non-competitive (CS, Fig. 3a) inhibition mode may not affect the durability of suppression of overall intestinal AGH activity within the present experimental conditions. However, degradation of components in the gut should be considered for their long-term AGH inhibition. In human studies, acarbose was reported to be very poorly absorbed, and was partially absorbed after degradation by intestinal microflora. In the present study, acarbose exerted a durable antihyperglycemic effect lasting 2 hours after administration. This result shows that the durability of the antihyperglycemic effect of acarbose is partly responsible for the less absorbent property. Chlorogenic acid, a caffeic acid derivative similar to CS with an antihyperglycemic effect, was reported to reach the colon intact in a human study and then was degraded by colonic microflora, resulting in the production of caffeic acid (Gonthier *et al.*, 2003). Our study revealed the lowering AGH activity in the lower part of the small intestine by CS administration, suggesting that CS may possess degradative resistance against intestinal microflora similar to chlorogenic acid and reach the lower part of the small intestine in an intact form with AGH inhibitory activity. These possibilities support the durable antihyperglycemic effect of CS; however, further metabolic studies are needed to elucidate the interaction of CS with the SI complex.

In conclusion, the present study revealed that CS (6-*O*-caffeoylsophorose) shows an antihyperglycemic effect in rats when simultaneously or pre-administered with maltose (within 60 min before maltose administration), and the durable suppression of rat intestinal AGH activity in the whole of the small intestine was observed. These results indicate that the intake of CS as an alternative medicinal food to maintain improved postprandial BGL should be restricted to pre-intake of diet.

Acknowledgements The authors thank Ms. Satomi Tamura and Ms. Mayu Shimada for their technical assistance.

References

- Abesundara, K. J. M., Matsui, T., Matsumoto, K., (2004). α -Glucosidase inhibitory activity of some Sri Lanka plant extracts, one of which, *Cassia auriculata*, exerts a strong antihyperglycemic effect in rats comparable to the therapeutic drug acarbose. *J. Agric. Food Chem.*, **52**, 2541-2545.
- Chiasson, J.L., Josse, R.G., Gomis, R., Hanefeld, M., Karasik, A., Laakso, M. (2002). Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomized trial. *Lancet*, **359**, 2072-2077.
- Deguchi, Y., Osada, K., Uchida, K., Kimura, H., Yoshikawa, M., Kudo, T., Yasui, H. (1998). Effects of extract of Guava leaves on the development of diabetes in the db/db mouse and on the postprandial blood glucose of human subjects. *Nippon Noeikagaku Kaishi*, **72**, 923-931.
- Du, Z.Y., Liu, R.R., Shao, W.Y., Mao, X.P., Ma, L., Gu, L.Q., Huang, Z.S., Chan, A.S.C. (2006). α -Glucosidase inhibition of natural curcuminoids and curcumin analogs. *Eur. J. Med. Chem.*, **41**, 213-218.
- Fujita, H., Yamagami, T., Ohshima, K. (2001). Fermented soybean-derived water-soluble Touchi extract inhibits α -glucosidase and is antiglycemic in rats and humans after single oral treatments. *J. Nutr.*, **131**, 1211-1213.
- Gonthier, M.P., Verny, M.A., Besson, C., Remesy, C., Scalbert, A. (2003). Chlorogenic acid bioavailability largely depends on its metabolism by the gut microflora in rats. *J. Nutr.*, **133**, 1853-1859.
- Hauri, H.P., Wacker, H., Rickli, E.E., Meier, B.B., Quaroni, A., Semenza, G. (1982). Biosynthesis of sucrase-isomaltase. *J. Biol. Chem.*, **257**, 4522-4528.
- Iwai, K., Kim, M.Y., Onodera, A., Matsue, H. (2006). α -Glucosidase inhibitory and antihyperglycemic effects of polyphenols in the fruit of *Viburnum dilatatum* thunb. *J. Agric. Food Chem.*, **54**, 4588-4892.
- Kobayashi, Y., Suzuki, M., Satsu, H., Arai, S., Hara, Y., Suzuki, K. (2000). Green tea polyphenols inhibit the sodium-dependent glucose transporter of intestinal epithelial cells by a competitive mechanism. *J. Agric. Food Chem.*, **48**, 5618-5623.
- Li, J.M., Che, C.T., Lau, C.B.S., Leung, P.S., Chen, C.H.K. (2006). Inhibition of intestinal and renal Na⁺-glucose cotransporter by naringenin. *Int. J. Biochem. Cell Biol.*, **38**, 985-995.
- Matsui, T., Ebuchi, S., Fukui, K., Matsugano, S., Terahara, N., Matsumoto, K. (2004). Caffeoylsophorose, a new natural α -glucosidase inhibitor, from red vinegar by fermented purple-fleshed sweet potato. *Biosci. Biotechnol. Biochem.*, **68**, 2239-2246.
- Matsui, T., Ebuchi, S., Fujise, T., Abesundara, K. J. M., Doi, S., Yamada, H., Matsumoto, K. (2004). Strong antihyperglycemic effects of water-soluble fraction of Brazilian Propolis and its bioactive constituent, 3,4,5-tri-O-caffeoylquinic acid. *Biol. Pharm. Bull.*, **27**, 1797-1803.
- Matsui, T., Ebuchi, S., Kobayashi, M., Fukui, K., Sugita, K., Terahara, N., Matsumoto, K. (2002). Anti-hyperglycemic effect of diacylated anthocyanin derived from *Ipomoea batatas* cultivar Ayamurasaki can be achieved through the α -glucosidase inhibitory action. *J. Agric. Food Chem.*, **50**, 7244-7248.
- Matsui, T., Ogunwande, I.A., Abesundara, K.J.M., Matsumoto, K. (2006). Anti-hyperglycemic potential of natural products. *Mini-Reviews in Med. Chem.*, **6**, 109-120.
- Matsui, T., Tanaka, T., Tamura, S., Tushima, A., Tamaya, K., Miyata, Y., Tanaka, K., Matsumoto, K. (2007). α -Glucosidase inhibitory profile of catechins and theaflavins. *J. Agric. Food Chem.*, **55**, 99-105.
- Matsui, T., Ueda, T., Oki, T., Sugita, K., Terahara, N., Matsumoto, K. (2001a). α -Glucosidase inhibitory action of natural acylated anthocyanins. 1. Survey of natural pigments with potent inhibitory activity. *J. Agric. Food Chem.*, **49**, 1948-1951.
- Matsui, T., Ueda, T., Oki, T., Sugita, K., Terahara, N., Matsumoto, K. (2001b). α -Glucosidase inhibitory action of natural acylated anthocyanins. 2. α -Glucosidase inhibition by isolated acylated anthocyanins. *J. Agric. Food Chem.*, **49**, 1952-1956.
- Matsumoto, N., Ishigaki, F., Ishigaki, A., Iwashina, H., Hara, Y. (1993). Reduction of blood glucose levels by tea catechin. *Biosci., Biotechnol. Biochem.*, **57**, 525-527.
- Matsuura, T., Horina, E., Kishimoto, M., Ichikawa, T. (2001). α -Glucosidase inhibitory activity of various sugars in rats with portal vein catheterization. *J. Jpn. Nutr. Food Sci.*, **54**, 155-160 (in Japanese).
- Oki, T., Masuda, M., Furuta, S., Nishiba, N., Terahara, N., Suda, I. (2002). Improvement of anthocyanins and other phenolic compounds in radical-scavenging activity of purple-fleshed sweet potato cultivars. *J. Food Sci.*, **67**, 1752-1756.
- Oki, T., Matsui, T., Osajima, Y. (1999). Inhibitory effect of α -glucosidase inhibitors varies according to its origin. *J. Agric. Food Chem.*, **47**, 550-553.
- Saito, M. (1972). Daily rhythmic changes in brush border enzymes of the small intestine and kidney in rat. *Biochim. Biophys. Acta.*, **286**, 212-215.
- Shim, Y.J., Doo, H.K., Ahn, S.Y., Kim, Y.S., Seong, J.K., Park, O.S., Min, B.H. (2003). Inhibitory effect of aqueous extract from the gall of *Rhus chinensis* on alpha-glucosidase activity and postprandial blood glucose. *J. Ethnopharmacol.*, **85**, 282-287.
- Terahara, N., Kato, Y., Nakamura, M., Maitani, T., Yamaguchi, M., Goda, Y. (1999). Six diacylated anthocyanins from the storage roots of purple sweet potato, *Ipomoea batatas*. *Biosci. Biotechnol. Biochem.*, **63**, 1420-1424.

- Terahara, N., Matsui, T., Fukui, K., Matsugano, K., Sugita, K., Matsumoto, K. (2003). Caffeoylsophorose in a red vinegar produced through fermentation with purple sweetpotato. *J. Agric. Food Chem.*, **51**, 2539-2543.
- Yasuda, K., Shimowada, K., Uno, M., Odaka, H., Adachi, T., Shihara, N., Suzuki, N., Tamon, A., Nagashima, K., Hosokawa, M., Tsuda, K., Seino, Y. (2003). Long-term therapeutic effects of voglibose, a potent intestinal alpha-glucosidase inhibitor, in spontaneous diabetic GK rats. *Diabetes Res. Clin. Prac.*, **58**, 113-122.
- Yoshimoto, M., Okuno, S., Yamaguchi, M., Yamakawa, O. (2001). Antimutagenicity of deacylated anthocyanins in purple-fleshed sweetpotato. *Biosci. Biotechnol. Biochem.*, **65**, 1652-1655.