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# α-Glucosidase Inhibitory Profile of Catechins and Theaflavins

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To clarify the postprandial glucose suppression effect of flavonoids, the inhibitory effects of catechins and theaflavins against α-glucosidase (AGH) were examined in this study. It was initially demonstrated that theaflavins and catechins preferentially inhibited maltase rather than sucrase in an immobilized AGH inhibitory assay system. For the maltase inhibitory effects of theaflavins, the effects were observed in descending order of potency of theaflavin (TF)-3-*O*-gallate (Gal) > TF-3,3′-di-*O*-Gal > TF-3′-*O*-Gal > TF. This suggests that the AGH inhibition induced by theaflavins is closely associated with the presence of a free hydroxyl group at the 3′-position of TF as well as the esterification of TF with a mono-Gal group. In addition, the *R*-configuration at the 3′-position of TF-3-*O*-Gal showed a higher inhibitory activity than the *S*-configuration. As a result of a single oral administration of maltose (2 g/kg) in rats, a significant reduction in blood glucose level was observed at a dose of 10 mg/kg of TF-3-*O*-Gal, demonstrating for the first time that TF-3-*O*-Gal can suppress glucose production from maltose through inhibition of AGH in the gut.

**KEYWORDS: α-Glucosidase; theaflavin; catechin; non-insulin-dependent diabetes mellitus; inhibition; antihyperglycemic effect**

## INTRODUCTION

Diabetes mellitus is a chronic disease caused by inherited and/or acquired deficiency in insulin secretion and/or by decreased responsiveness of the organs to secreted insulin (insulin resistance). Such a deficiency results in increased blood glucose levels (BGL), which in turn can damage many of the body's systems, including blood vessels and nerves (*1*). In particular, non-insulin-dependent diabetes mellitus (NIDDM), which is closely related to diet or lifestyle, may lead to the onset of dyslipidemia and arterial hypertension, as well as hyperglycemia (*2*). The effect results in hyperglycemia, as a consequence of reduced uptake of glucose in the peripheral tissues, elevation of glucose release from the liver, and, in particular, excessive food intake.

The treatment goal for NIDDM patients is, thus, generally agreed to be to maintain near-normal levels of glycemic control, in both the fasting and postprandial states. Although diet and exercise are the first steps toward achieving this treatment goal, an alternative treatment or improvement in functional food intake is also in great demand to prevent the loss of quality of life. The STOP-NIDDM trial study, which examined the effectiveness of a daily intake of a therapeutic drug, acarbose, has demonstrated that preventing the excessive rise in postprandial BGL is effective in improving hyperglycemia at an early or borderline stage (*3*). The evidence strongly led us to examine the anti-hyperglycemic potential of natural compounds as alternative medicinal foods. Many natural resources have been investigated with respect to the suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine (*4*, *5*). One successful approach to achieve optimal BGL is through retardation of α-glucosidase (AGH, EC 3.2.1.20) activity (*1*), in a fashion similar to that of acarbose [a membrane-bound sucrase−isomaltase (SI) complex] in the small intestine (*6*). Clinical indications of some anti-hyperglycemic foods have demonstrated the efficacy of food intake on postprandial BGL control (*7*, *8*), all of which involve the inhibition or delay of glucose production from carbohydrates.

In the course of our studies on bioactive compounds with AGH inhibitory activity, we have successfully identified several AGH inhibitors from natural plants (*9*−*13*). Among them, acylated anthocyanins were found to be beneficial toward the

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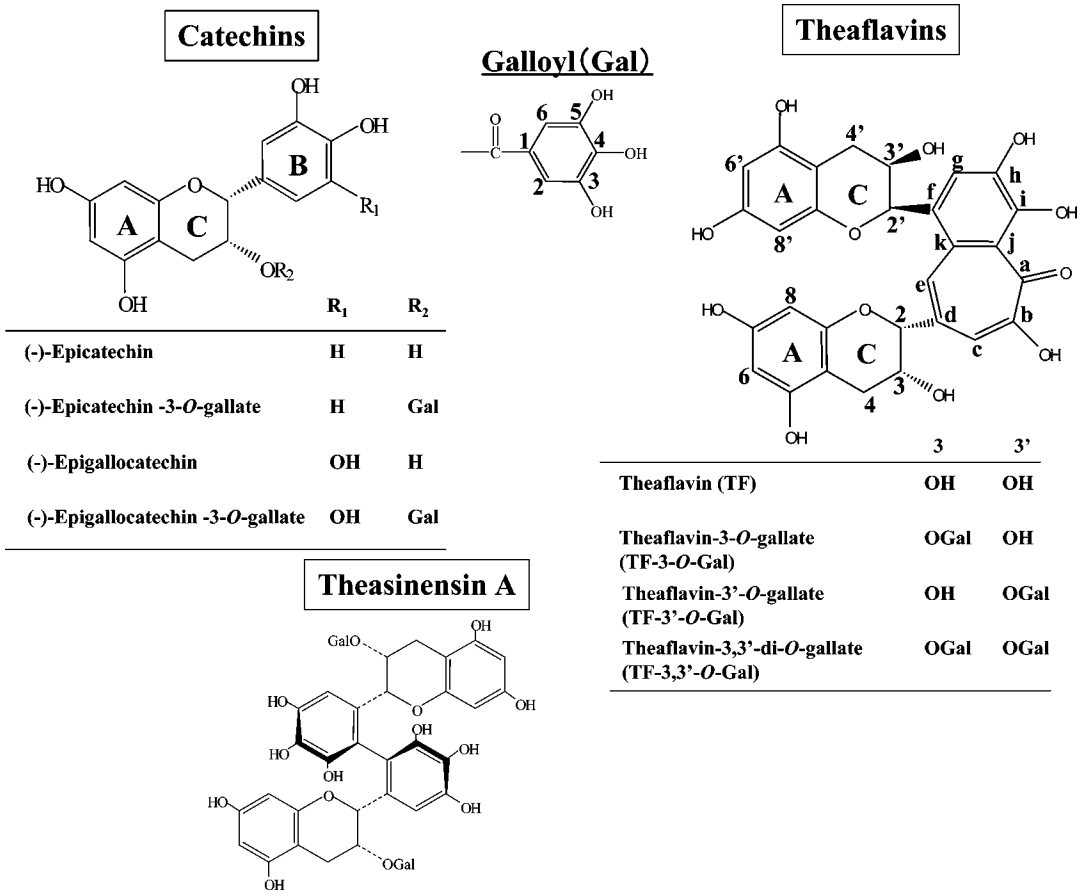


**Figure 1.** Structures of catechins and theaflavins used in this study.

inhibition of glucose production from dietary carbohydrates (*10*). In addition, the caffeoyl group (or acylated moiety) plays an important role in exerting AGH inhibitory activity, and an increasing number of caffeoyl groups of acylated anthocyanins and caffeoly quinic acid analogues increases the effectiveness (*11*−*13*). Potent inhibition by caffeoyl quinic acid analogues [particularly 3,4,5-tri-*O*-caffeoylquinic acid (*12*)] and poor inhibition by aglycones (*10*) also support the importance of acylation or esterification in eliciting AGH inhibition. Accordingly, as an ongoing part of our screening for AGH inhibitors, we focused our attention on acylated or esterified polyphenols. In the present study, we evaluated the AGH inhibitory action of catechins and their oxidative coupling product, theaflavines, because of an apparent anti-hyperglycemic effect of esterified catechins in previous findings (*14*, *15*) and because there has been no comparable study of the AGH inhibitory effect of theaflavins. To clarify their bioactivity, we applied our immobilized AGH assay system (*16*), which mimics the intestinal membrane-bound state of the SI complex (*6*).

## MATERIALS AND METHODS

**Materials.** α-Glucosidase (EC 3.2.1.20) from rat intestinal acetone powder was purchased from Sigma Chemical Co. (St. Louis, MO). CNBr-activated Sepharose 4B was purchased from Pharmacia Biotech AB (Uppsala, Sweden). Catechins [(+)-catechin, (+)-epicatechin, (−)-epicatechin, (−)-epicatechin-3-*O*-gallate, (−)-epigallocatechin, and (−)-epigallocatechin-3-*O*-gallate] were purchased from Nacalai Tesque Co. (Kyoto, Japan). (−)-Catechin and theaflavins [theaflavin (TF), TF-3-*O*-gallate (TF-3-*O*-Gal), TF-3′-*O*-Gal, TF-3,3′-di-*O*-Gal, and theasinensin A] were purchased from Wako Pure Chemical Institute, Co. (Osaka, Japan). The stereoisomer of TF-3-*O*-Gal [(2′*R*,3′*R*)-TF-3-*O*-Gal], (2′*R*,3′*S*)-TF-3-*O*-Gal, was synthesized as described below. Structures of catechins and theaflavins used in this study are depicted in **Figure 1**. Other reagents were of analytical grade and were used without further purification.

**Synthesis of (2′*R*,3′*S*)-TF-3-*O*-Gal.** The method of Collier et al. (*17*) was used to synthesize (2′*R*,3′*S*)-TF-3-*O*-Gal. Briefly, (+)-catechin (200 mg) and (−)-epigallocatechin-3-*O*-gallate (200 mg) were dissolved in water (5 mL) and cooled in an ice bath. An aqueous solution (2 mL) of potassium ferricyanide (300 mg) and $NaHCO_3$ (78 mg) was added to the solution, in a dropwise manner, with stirring for 15 min. The mixture was acidified by the addition of 1 M HCl and extracted with ethyl acetate. The ethyl acetate layer was concentrated and subjected to a Sephadex LH-20 column chromatography eluted with 35% acetone to yield (2′*R*,3′*S*)-TF-3-*O*-Gal (18.5 mg). The $^1$H NMR spectral data were identical to those described in the literature (*18*).

**NMR Measurement.** One-dimensional difference nuclear Overhauser enhancement (NOE) spectra were measured on a JNM A400 instrument (JEOL, Tokyo, Japan). The sample was dissolved in methanol-$d_4$ (MeOD) (isotopic purity of both: 99.80 atom % D, Nacalai Tesque Inc.). The NOE experiment was performed for 64 scans by irradiating a specific proton with an irradiation power of 200 and duration of 10 s against off-resonance spectrum. Three-dimensional structural analysis was performed using Chem 3D software (Ultra, version 8.0, Cambridge Soft Co., Cambridge, MA), with which a structure was optimized by minimizing energy by an AM1 theory with a closed shell function of a semiempirical MOPAC calculation method.

**Immobilized AGH (iAGH) Assay System.** The immobilization of AGH partially purified from rat acetone powder onto CNBr-activated Sepharose 4B has been described in detail previously (*16*). The iAGH assay was performed as follows: the iAGH support (10 mg of wet gel) was placed in an end-capped ASSIST mini-column with a 45−90 μm polyethylene filter (CC-07, 5 mL, ASSIST, Tokyo, Japan). The assay was initiated after the addition of 100 μL of inhibitor solution and 900 μL of the model intestinal fluid, containing 10 mM maltose

or 45 mM sucrose, to the column. After incubation on a rotating cultivator (4 rpm, RT-5, TAITEC, Saitama, Japan) at 37 °C for 30 min (maltase assay) or 60 min (sucrase assay), the reaction was terminated by filtration of the solution through the column. Maltase or sucrase activity was evaluated by determining the liberated glucose from the substrate in the filtrate by an F-kit glucose (Roche Diagnostics, Co., Tokyo, Japan). Briefly, 50 μL of the filtrate was diluted with 950 μL of distilled water, followed by the addition of 500 μL of reagent I, containing 1.22 mg of NADP and 2.89 mg of ATP, and 10 μL of reagent II, containing 2.9 units of hexokinase and 1.45 units of G6PDH. After the incubation at 37 °C for 20 min, absorbance at 340 nm was measured. The samples assayed in this system were dissolved in 10% dimethyl sulfoxide (DMSO) solution, and no effect (inactivation) of the solvent system to iAGH activity onto the support was confirmed before the inhibition assay. One unit of maltase or sucrase activity was defined as the amount of enzyme that hydrolyzed 1 μmol of substrate per minute under the above-described assay conditions. The $IC_{50}$ value was defined as the concentration of AGH inhibitor required to inhibit 50% of the AGH activity under the assay conditions.

**Animal Experiments in SD Rat.** Male 6-week-old Sprague−Dawley rats (SPF/VAF Crj: SD, Charles River Japan, Kanagawa, Japan) were fed a laboratory diet (CE-2, Clea Japan, Tokyo, Japan) and provided with water ad libitum. All rats were housed for 1 week at 21 ± 1 °C and 55 ± 5% humidity under controlled lighting from 8:30 a.m. to 8:30 p.m. A single oral administration of 1 mL of distilled water with 10 mg/kg theaflavin was performed by gavage in SD rats (220.6 ± 5.4 g) that had been fasted for 16 h. After 5 min, 1 mL of a 2 g/kg of substrate (maltose) solution was administered to each rat. Control rats were administered the same substrate solution after the administration of 1 mL of distilled water solution without inhibitor. At each time point up to 120 min after the ingestion of maltose solution, 20 μL blood samples were collected from the tail vein and immediately subjected to a BGL measurement by a disposable glucose sensor (Glutest Pro, Sanwa Chemical Research, Co., Tokyo, Japan). Rat experiments in this study were carried out under the guidance of the Animal Experiment in the Faculty of Agriculture and in the Graduate Course of Kyushu University and the Low (No. 105, 1973) and Notification (No. 6, 1980 of the Prime Minister's Office) of the Japanese government.

**Data Analysis.** The results for the administration study are expressed as the mean of BGL (mg/dL) ± SEM. Statistical differences of BGL between control and sample groups at each administration time point were evaluated using a two-factor analysis of variance (ANOVA) followed by Tukey−Kramer's *t* test for post hoc analysis. $P < 0.05$ was considered to be statistically significant. The analysis was performed with Stat View J5.0 (SAS Institute Inc., Cary, NC).

## RESULTS

**iAGH Inhibition by Catechins.** As summarized in **Table 1**, (−)-epicatechin-3-*O*-gallate and (−)-epigallocatechin-3-*O*-gallate exhibited an apparent inhibitory effect against iAGH−maltase activity compared with (−)-epicatechin and (−)-epigallocatechin: the magnitude of maltase inhibition induced by the esterified catechins was 15−20 times higher than that of the nonesterified ones. The overall iAGH inhibitory profile demonstrated that the inhibitory activity of catechins was greater against maltase activity compared to sucrase activity.

**iAGH Inhibition by Theaflavins.** Investigation of the iAGH inhibitory effects of theaflavins provided a characteristic inhibition profile, in which TF-3-*O*-Gal was the most potent maltase inhibitor among the theaflavins and catechins, with an $IC_{50}$ value of 10 μM (**Table 1**). For even the strongest inhibitory compound among the compounds, the inhibitory potency was still ca. 20-fold lower than that of the therapeutic drug acarbose [$IC_{50}$ for maltase = 430 nM (*16*)]. It was apparent that the attachment of the Gal moiety to theaflavin was responsible for the potent iAGH inhibition. However, the number of Gal moieties was no longer a candidate for iAGH inhibition, as disubstituted TF-3,3′-di-*O*-Gal exerted an unexpected lowering of iAGH inhibitory activity compared with monosubstituted TF-3-*O*-Gal. **Table 1** also provides an interesting observation that the position of the Gal moiety greatly affects the enhancement of maltase inhibitory activity; TF esterified with gallate at the 3-position was 14-fold more effective than TF esterified at the 3′-position. These findings suggest that iAGH inhibition by theaflavins is closely associated with the presence of a free hydroxyl group at the 3′-position of TF as well as esterification of TF with a mono-Gal group. As a result of the Lineweaver−Burk plot, both monosubstituted TF-*O*-Gal inhibitors were found to be competitive maltase inhibitors, with $K_i$ values of 48 and 250 μM for TF-3-*O*-Gal and TF-3′-*O*-Gal, respectively (**Figure 2**). Theaflavins preferentially inhibited maltase compared to sucrase, similar to the inhibitory behavior of the catechins.

**Table 1.** α-Glucosidase (AGH) Inhibitory Activity of Catechins and Theaflavins Evaluated by an Immobilized AGH Assay System[a]

| inhibitor | $IC_{50}$ (μM) maltase[b] | sucrase[b] |
|---|---|---|
| catechins | | |
| (−)-epicatechin | 770 | 1080 |
| (−)-epicatechin-3-*O*-gallate | 53 | 172 |
| (−)-epigallocatechin | 1260 | 921 |
| (−)-epigallocatechin-3-*O*-gallate | 40 | 169 |
| theaflavins | | |
| theaflavin | 500 | >10000 |
| theaflavin-3-*O*-gallate | 10 | 1024 |
| theaflavin-3′*O*-gallate | 136 | 573 |
| theaflavin-3,3′-di-*O*-gallate | 58 | 159 |
| theasinensin A | 142 | 286 |
| acarbose[c] | 0.43 | 1.2 |

[a] Immobilized AGH assay was performed at 37 °C for 30 or 60 min for maltase or sucrase inhibitory assay, respectively. [b] A 10 mM concentration of maltose or 45 mM sucrose was used as a substrate. [c] Reference *16*.

**iAGH Inhibition Behavior of Monosubstituted TF-O-Gal Isomers.** To ascertain the importance and potential role of the free hydroxyl group at the 3′-position in monosubstituted TF-*O*-Gal in exerting potent maltase inhibition, conformational analysis of two monosubstituted TF-*O*-Gal inhibitors was conducted by measurement of NOE-NMR. When the 2-positioned proton of the Gal group was irradiated, an apparent NOE of the e-positioned proton of the benzotropolone in TF-3′-*O*-Gal was observed, whereas no NOE was observed in TF-3-*O*-Gal. When the e-positioned proton of the benzotropolone ring was irradiated, an apparent NOE of the 8-positioned proton of TF-3-*O*-Gal, as well as the 2-positioned proton of the Gal group in TF-3′-*O*-Gal, was observed. According to the NOE observations, we proposed a conformational structure of two monosubstituted TF-*O*-Gal inhibitors tentatively as illustrated in **Figure 3**; that is, TF-3-*O*-Gal exhibited potent maltase inhibitory activity due to an expanded structure, whereas an opposite structural conformation for the folded structure was obtained for a weaker inhibitor, TF-3′-*O*-Gal.

**Table 2** presents the effect of configuration of TF-3-*O*-Gal isomers on iAGH (maltase) inhibitory activity. As shown in the table, the *S*-configuration at the 3′-position of TF-3-*O*-Gal resulted in an 8-fold lower maltase inhibitory activity ($IC_{50}$ = 83 μM) compared with the *R*-configuration ($IC_{50}$ = 10 μM). This finding strongly suggested that the free hydroxyl group at the 3′-position of TF-3-*O*-Gal was responsible for eliciting the potent maltase inhibition, matched with the result of more potent inhibition of (−)-epicatechin, that is, (2*R*,3*R*)-catechin ($IC_{50}$ = 770 μM) compared with that of the *S*-configuration at the

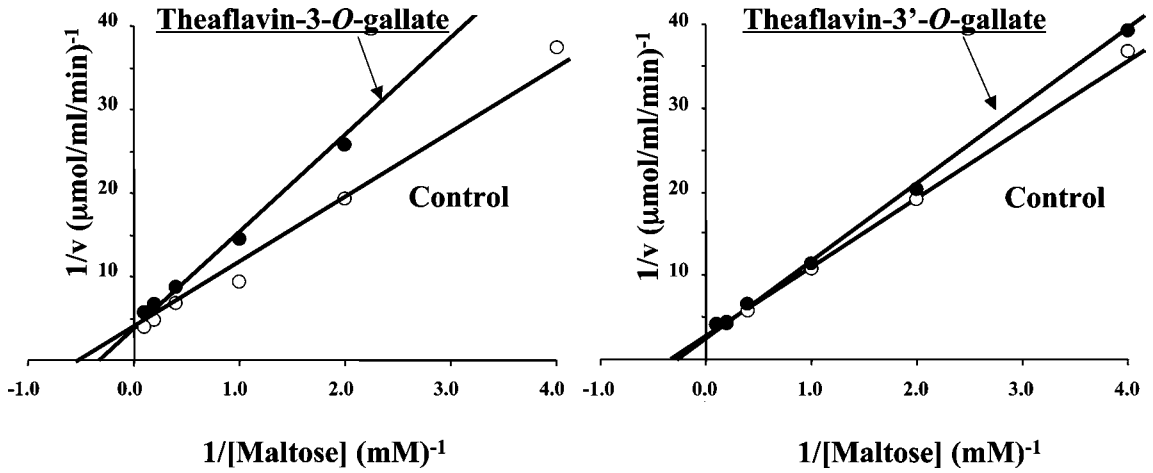


**Figure 2.** Lineweaver–Burk plots of theaflavin-3-*O*-gallate and theaflavin-3′-*O*-gallate in the immobilized AGH assay system. Experiments with and without inhibitor were conducted at 37 °C for 30 min at maltose concentrations of 0.25, 0.5, 1.0, 2.5, 5.0, and 10 mM. Each value is the mean of three measurements.

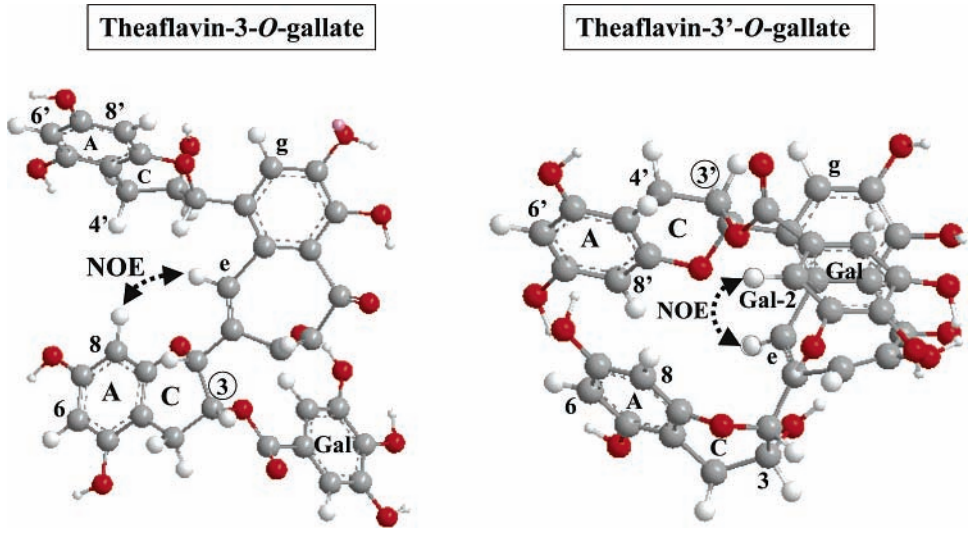


**Figure 3.** Estimated conformational structures of theaflavin-3-*O*-gallate and theaflavin-3′-*O*-gallate. Important NOE correlations are represented by arrows.

**Table 2.** Maltase Inhibitory Activity of Stereoisomers of Catechins and Theaflavins Evaluated by an Immobilized AGH Assay System[a]

| | *R*,*S*-expression | | | | |
|---|---|---|---|---|---|
| position | 2 | 3 | 2 | 3′ | $IC_{50}$ ($\mu$M) |
| catechins | | | | | |
| (−)-catechin | *S* | *R* | | | 1890 |
| (+)-catechin | *R* | *S* | | | 4320 |
| (−)-epicatechin | *R* | *R* | | | 770 |
| (+)-epicatechin | *S* | *S* | | | 1320 |
| theaflavins | | | | | |
| theaflavin-3-*O*-gallate | *R* | *R* | *R* | *R* | 10 |
| theaflavin-3-*O*-gallate | *R* | *R* | *R* | *S* | 83 |

[a] Immobilized AGH assay was performed at 37 °C for 30 min using 10 mM maltose as a substrate.

3-position of (−)-catechin [(2*R*,3*S*)-catechin, $IC_{50}$ = 1,890 $\mu$M] (**Table 2**). Additionally, the *R*-configuration at the 2-position of catechins may also be required for potent maltase inhibition.

**Changes in BGLs after a Single Oral Administration of Monosubstituted TF-*O*-Gal Isomers to SD Rats.** On the basis of the result that TF-3-*O*-Gal exhibited potent iAGH (maltase) inhibitory activity among the theaflavins (**Table 1**), we examined the changes in BGL after the administration of either TF-3-*O*-Gal or TF-3′-*O*-Gal (10 mg/kg) with maltose in SD rats. As presented in **Figure 4**, a significant reduction of BGL (22.6 mg/dL) 30 min after the administration ($P < 0.05$ vs control) was observed for TF-3-*O*-Gal, whereas TF-3′-O-Gal did not exhibit any reduction at the dosage of 10 mg/kg, as predicted from their iAGH inhibitory activities (**Table 1**). The area under the curve (AUC) demonstrated that at least 10 mg/kg of TF-3-*O*-Gal, at which a significant ($P < 0.01$) AUC reduction of 46.8% ($AUC_{0-120\ min}$ = 81.7 ± 12.4 mg·h/dL) was observed compared to controls (145.7 ± 3.0 mg·h/dL), was effective in eliciting the anti-hyperglycemic effects.

## DISCUSSION

Theaflavins, which are characteristic polyphenols in black tea that are formed by the oxidative condensation of catechins (*19*), have proven to exhibit physiological functional effects, including anti-inflammatory and cytotoxic effects (*20*). An antidiabetic effect of theaflavins and catechins has also been reported by Honda and Hara (*21*), who suggested that the possible mechanism was via inhibition of intestinal glucosidases. In addition, Matsumoto et al. (*15*) reported a significant BGL reduction role of catechins in sucrose- or starch-loaded rats.

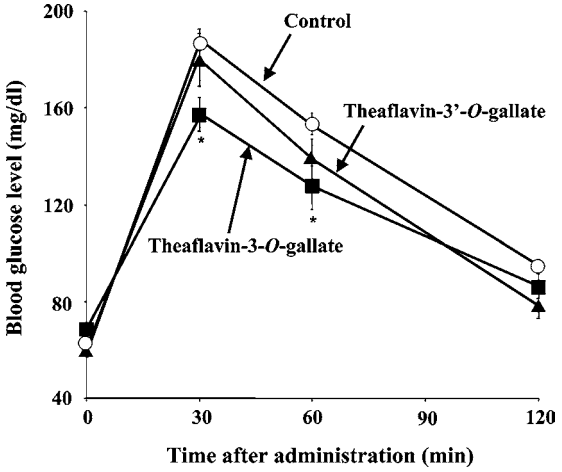


**Figure 4.** Effect of theaflavin-3-*O*-gallate and theaflavin-3′-*O*-gallate on blood glucose levels after a single oral administration of 2 g/kg maltose in SD rats. A 10 mg/kg dose of theaflavin-3-*O*-gallate (■) or theaflavin-3′-*O*-gallate (▲) was administered to male 7-week-old SD rats. After 5 min, 2 g/kg of maltose solution was administered to each rat. Control rats (○) were administered the same volume of substrate solution without inhibitor. At each time point up to 120 min after the ingestion of maltose solution, blood samples of about 20 μL were collected from the tail vein and immediately subjected to the blood glucose level measurement by a disposable glucose sensor. Data are presented as the mean (mg/dL) ± SEM. Significant difference versus control was examined with the Tukey–Kramer *t* test ($n = 4$, *, $P < 0.05$).

However, these researchers did not describe any potential mechanisms of theaflavins with respect to an in vivo antidiabetic effect or structure−activity relationship. In this respect we demonstrated that monosubstituted theaflavin-3-*O*-gallate (TF-3-*O*-Gal) is one of the strongest natural competitive maltase inhibitors with an $IC_{50}$ value of 10 μM and that acute administration of TF-3-*O*-Gal (10 mg/kg) to maltose-loaded SD rats exhibits a significant anti-hyperglycemic effect. Although the inhibitory potency was still lower than that of the therapeutic drug, acarbose [$IC_{50}$ for maltase = 430 nM (*16*)], this is the first report of an anti-hyperglycemic effect induced by theaflavins. The anti-hyperglycemic potential of TF-3-*O*-Gal (10 mg/kg) was consistent with other natural inhibitors, such as 3,4,5-tri-*O*-caffeoylquinic acid (20 mg/kg), but was still weaker than that of acarbose (3 mg/kg) (*12*). The mechanism of the anti-hyperglycemic effect in the maltose-loaded SD rats, however, remains unclear, because the effect may be achieved by either restrictive maltase inhibition or glucose transport inhibition in the small intestinal membrane via the $Na^+$/glucose cotransporter or both. In the case of catechins, Kobayashi et al. (*22*) reported catechins inhibited the glucose transport competitively in a rat everted jejunal sac experiment, and the esterified galloyl group was a candidate responsible for the glucose transport inhibition. In contrast, we have demonstrated that diacylated anthocynanins with maltase inhibitory effect gave no influence on the postprandial BGL in glucose-loaded SD rats (*23*). Therefore, a further in vivo study, for example, a glucose-loaded rat experiment, is needed to clarify the mechanism of anti-hyperglycemic effect of TF-3-*O*-Gal and is now in progress.

Our previous findings on natural AGH inhibitors using a proposed iAGH assay system (*16*) that mimics the membrane-bound condition of AGH, and thus provides a substantially useful prediction of the in vivo suppression of glucose absorption, may be helpful for understanding the structural relationship of theaflavins as potent AGH inhibitors. As we have already reported, a strong iAGH (maltase) inhibitory potency was increased by acylation of anthocyanins with phenolic acids, but not by their aglycones (*10, 11*). A multisubstitution of the free hydroxyl group, but not the methoxyl group, to the aromatic ring in phenolic acids, such as caffeic acid and gallic acid, was found to contribute to the enhancement of activity (*13*). The importance of the multihydroxyl substitution on AGH inhibition was also observed in flavones, in which methylation of the hydroxyl group on the B-ring of trihydroxyflavone resulted in a marked decrease of AGH inhibitory activity (*24*). The decreasing activity of methoxyl group-substituted flavones was reported to be due to its repulsion with AGH, but such details remain unclear. In contrast, Adisakwattana et al. (*25*) demonstrated a 10-fold higher AGH inhibitory activity of 4-methoxy-*trans*-cinnamic acid compared with 4-hydroxy-*trans*-cinnamic acid; this discrepancy with our results likely arose from the difference in AGH species between baker's yeast AGH for cinnnamic acid assay and rat intestinal AGH for our study.

In addition to the importance of multisubstitution of the hydroxyl group, we revealed the importance of the esterified moiety as a prominent structural factor; the potency of AGH inhibition apparently depends on the esterification of caffeic acid with quinic acid and an increasing number of caffeoyl groups in caffeoylquinic acid analogues (*12*). In the present study, the strong AGH inhibitory effect by (−)-epigallocatechin-3-*O*-gallate as well as (−)-epigallocatechin among the catechins provides substantial evidence that both esterification and hydroxyl-substitution play an important role in eliciting AGH inhibition.

In contrast, theaflavins exhibit a characteristic inhibitory profile as shown in **Table 1**: (1) An increasing number of galloyl groups attached to TF is not necessarily a determining factor for AGH inhibition, unlike caffeoylquinic acid analogues. (2) Differences in the substituted position with a monogalloyl moiety (3- or 3′-position) greatly affect the AGH inhibitory potential. Both findings indicate that the hydroxyl group at the 3′-position as a free form (or no replacement of hydroxyl group at this position) may play a key role in enhancing the AGH inhibitory effect of theaflavins. This speculation would be supported by the results that luteolin with no replacement of hydroxyl group at the 3-position of flavan did not show any inhibitory effect (*26*) and that theasinensin A, which possesses two galloyl moieties, similar to TF-3,3′-di-*O*-Gal (**Figure 1**), inhibited maltase activity to a lesser extent, with an $IC_{50}$ value of 142 μM, compared with that of monosubstituted TF-3-*O*-Gal ($IC_{50}$ = 10 μM). The demonstration that only a configuration change of *R* to *S* at the 3′-position resulted in a marked decrease of AGH inhibitory activity (**Table 2**) strongly indicates that the hydroxyl group at the 3′-position is significantly associated with the approach or attachment of theaflavins to the AGH enzyme. A recent report by Gamberucci et al. (*27*) also provides useful information that the configuration of the gallo moiety of catechins largely influenced inhibition of rat liver microsomal α-glucosidase II, which catalyzes the hydrolysis of α-1,3-linkage of glycoproteins, and the importance of the galloyl group in this effect, similar to the iAGH inhibition profile of catechins. Although AGH and α-glucosidase II have different catalytic actions, it is remarkable that both enzymes substantially recognize any conformational difference of catechins (or theaflavins).

According to the estimated conformational structures of two monosubstituted TF-*O*-Gal inhibitors (**Figure 3**), the resulting folded structure in TF-3′-*O*-Gal might affect the enhancement of AGH inhibitory activity by shielding the approach of the TF

to the active site of the AGH enzyme. Theaflavin has a benzotropolone ring stacked orthogonally with two flavan rings, and the electron-deficient tropolone ring may interact favorably with the electron-rich ring of a galloyl group in the molecular environment (*28*). Thus, the *R*-configuration at the 2′- and 3′-positions in TF-3′-*O*-Gal, and the strained tropolone ring may enable a conformational approach of the galloyl moiety to the benzotropolone ring and lead to a stable folded structure. In contrast, the expanded structure of TF-3-*O*-Gal may result from the difficult conformational approach of the galloyl moiety to the benzotropolone ring by the strained tropolone ring and the *R*-configuration at the 2- and 3-positions. However, further investigations, including three-dimensional structure analysis, are needed to clarify the relationship between AGH activity and the structure of theaflavins.

In conclusion, our results indicate that theaflavins possess a potent anti-hyperglycemic effect compared with catechins. The mechanism involves the delay or inhibition of glucose production at the intestine through the inhibition of AGH (maltase) activity. Other possible mechanisms might be also involved in the anti-hyperglycemic effect in vivo, because catechins have been proven to elicit the effect through diverse physiological actions, such as promotion of hepatic glycogen synthesis (*29*) and inhibition of intestinal glucose transport (*22*). Further investigation is necessary to clarify the underlying anti-hyperglycemic mechanisms, but the strong AGH (maltase) inhibitory action of theaflavins (in particular, TF-3-*O*-Gal) must be added to their physiological functions.

## ABBREVIATIONS USED

AGH, α-glucosidase; NIDDM, non-insulin-dependent diabetes mellitus; BGL, blood glucose level; TF, theaflavin; Gal, gallate; SD, Sprague−Dawley; DMSO, dimethyl sulfoxide.

## NOTE ADDED AFTER ASAP PUBLICATION

The original posting of December 16, 2006, contained a minor error in Table 1. This has been corrected with the posting of December 21, 2006.

## LITERATURE CITED


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