Anti-hyperglycemic Potential of Natural Products

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Abstract: In order to evaluate the anti-hyperglycemic effect of natural compounds *via* the inhibition of α-glucosidase (AGH), the potential inhibitory effect of anthocyanins, caffeic acid analogs, and caffeoylquinic acid analogs have been reviewed. A new AGH assay system to mimic the membrane-bound AGH at the small intestine was proposed and evaluated.

Keywords: Diabetes, α-glucosidase, maltase inhibition, anti-hyperglycemic effect.

INTRODUCTION

Among the life style related diseases, diabetes is a serious condition for the individual and on the global scale, given its rapidly increasing prevalence. According to the recently compiled data from WHO, approximately 150 million people have diabetes worldwide, and that this number may well double by the year 2025 [1] due to population growth, ageing, unhealthy diets, obesity and sedentary lifestyles. Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [2]. The long-term manifestation of diabetes can result in the development of some complications, broadly classified as microvascular or macrovascular disease. Microvascular complications include neuropathy (nerve damage), nephropathy (renal disease) and vision disorders (retinopathy, glaucoma, cataract and corneal diseases). Macrovascular complications include heart disease, stroke and peripheral vascular disease, which can lead to ulcers, gangrene and amputation [3]. There are two types of diabetes, Type 1 diabetes mellitus, typically diagnosed during childhood, is characterized by destruction of β-cells caused by an autoimmune process, usually leading to absolute insulin deficiency.Insulin resistance in peripheral tissue and an insulin secretive defect of the β-cells characterizes type 2 diabetes mellitus (NIDDM). It is the most common form of diabetes mellitus and highly associated with a family history of diabetes, older age, obesity and lack of exercise [3].

Several studies have proven that early intervention in subjects at increased risk of developing Type 2 diabetes may be beneficial in delaying or preventing the onset of the disease [4, 5] and that the prevention of hyperglycemia could reduce the risk of micro- and macro-vascular complications as well. Persons with fasting blood glucose levels ranging from 110 to 126 mg/dl (6.1 to 7.0 mmol/l) are said to have impaired fasting glucose (IFG), while those with a 2 h-postprandial blood glucose level (BGL) between 140 mg/dl and 200 mg/dl (7.75 mmol/l to 11.1 mmol/l) are said to have impaired glucose tolerance (IGT) [6]. Both impaired fasting glucose and impaired glucose tolerance are associated with an increased risk of developing Type 2 diabetes mellitus and therefore form an important target group for interventions aimed at preventing diabetes [7].

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IMPROVEMENT OR TREATMENT OF HYPERGLYCEMIA

It is widely believed that postprandial hyperglycemia plays an important role in the development of Type 2 diabetes and complications associated with the disease such as micro- and macro-vascular diseases [8, 9]. Most anti-diabetic agents that are currently available reduce fasting blood glucose levels, but have little impact on postprandial glycemic excursions and thus do not normalize postprandial hyperglycemia. Therefore, agents that reduce postprandial hyperglycemia have a key role in the treatment of Type 2 diabetes and pre-diabetic states [8]. Furthermore, such agents have a potential to reduce the progression of diabetes as well as micro- and macro-vascular complications [8]. Therefore, new agents, α-glucosidase inhibitors, which control postprandial hyperglycemia, have been developed [9]. AGH (EC 3.2.1.20), exo-type α-D-glucopyranoside *O*-linkage hydrolase, a membrane bound enzyme located in the epithelium of the small intestine, catalyzes the cleavage of glucose from disaccharides and oligosaccharides [10, 11]. Hence, AGH inhibition is effective in the prevention or treatment of diabetes mellitus and therefore AGH inhibitors, such as acarbose [12] and voglibose [13] has received considerable attention in the past two decades [10-14]. In addition, since the mechanism of action of AGH inhibitors is different from that of other oral agents, their effects on glycemic control are additive when used in combination. This is important, as most patients with Type 2 diabetes will require combination therapy to reach an acceptable level of glycemic control [15].

It is a known fact that natural agents that retard or delay the absorption of carbohydrate may mimic the therapeutic drugs [16, 17]. In addition, from ages, patients with diabetes have been treated orally in folk medicine. Therefore, evaluation of natural resources, for their active natural principles will be a logical way of searching for new pharmacological agents to prevent or treat Type 2 diabetes. Furthermore, the relatively reduced cases of adverse reaction

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to plant preparations, as compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging both the consuming public and national health care institutions to consider natural medicinal products as alternatives to synthetic drugs [17]. In addition, the prevention of progression to diabetes by taking daily foods with specific functionality would be a preferable option for people suffering from the disease and likely to be practicable than the use of synthetic drugs. This review thus, focused on the screening of potential AGH inhibitors from natural medicinal resources underlying the development of functional foods, for the prevention and/or treatment of diabetes.

AGH INHIBITORY ASSAY SYSTEM

In order to evaluate the antihyperglycemic potentials of natural resources or compounds through a retardation of AGH activity, an adequate and reliable assay system for AGH inhibition is required. The present assay system, which is a spectrophotometer technique involving a pseudo-substrate, *p*-nitrophenyl-α-D-glucopyranoside, and free AGH from baker's yeast [18, 19], was recently shown to be unreliable. Chiba *et al.* [20] have pointed out, the catalytic property of AGH greatly differs based on its origin, *i.e.* type I from baker's yeast, and type II from mammals. As summarized in (Table **1**), all the inhibitors gave a variety of AGH inhibition behavior according to its origin (baker's yeast, rat, rabbit, and porcine small intestines).

Voglibose and acarbose showed 10^{-4} to 10^{-2} times lower ability to inhibit AGH from baker's yeast than that from small intestines. Cogoli and Semenza [21] obtained a similar result, for glucono-1, 5-lactone, which exhibited a strong inhibitory activity to mammalian AGH. This finding, in which the inhibitors exhibited a relatively weak effect to baker's yeast AGH as compared to mammalian AGHs agreed with the results of aminocyclitols from *Streptomyces hygroscopicus* fermentation broth, which showed more potent porcine AGH inhibitory activity than baker's yeast [22]. Among the mammalian AGHs, porcine AGH was

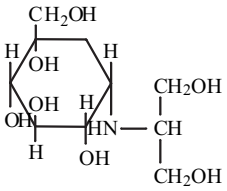
Table 1. AGH Inhibitory Profile of Natural and Synthetic Inhibitors with p-Nitrophenyl-α-D-Gluconopyranoside as a Substrate

		IC50 (μM)		
origin	voglibose	acarbose	(+)-catechin	glucono-1,5-lactone
Baker's yeast	26	NI	130	NI
Rat	0.073	63	NI	2,500
Rabbit	0.14	62	NI	950
Pig	0.0017	87	NI	140

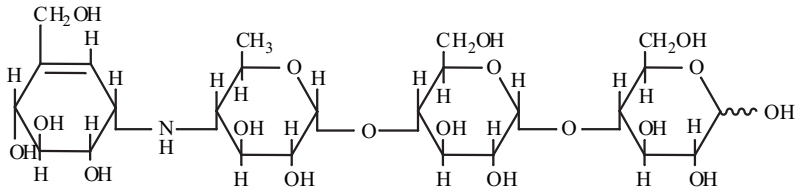
NI: no inhibition

Structures of inhibitors:

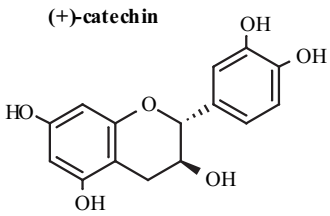
Voglibose



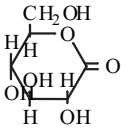
Acarbose



(+)-catechin



Glucono-1, 5-lactone



strongly inhibited by voglibose and glucono-1, 5-lactone except for acarbose with a 20 to 100-fold higher sensitivity than other AGHs. These findings, followed by the fact that the oral administration of acarbose in non-insulin-dependent diabetes subjects was made to moderate the postprandial blood glucose level [12] strongly suggested that the conventional AGH inhibitory study against baker's yeast might not give any practical information concerning suppression of glucose production from carbohydrates in the gut. Although the tested inhibitors displayed significant potent activities to porcine AGH, rat's AGH was used because of its easy development for *in vivo* study rather than that of porcine [10].

As illustrated in (Fig. **1**), AGH in mammalian intestine is anchored in the membrane by the polypeptide chain spanning the bilayer only once in an N (in)/C (out) orientation [23]. This indicates that AGH inhibition studies should involve membrane bound or immobilized AGH assay system and not with free AGH. Hence, we newly proposed an immobilized AGH assay system to mimic the membrane-bound condition [24]. AGH from rat intestinal acetone powder was homogenized with 1% papain in PCC buffer (100 mM potassium phosphate, 50 mM potassium citrate, and 154 mM potassium chloride, pH 7) containing 5 mM EDTA and 10 mM L-cysteine to obtain an outer part of AGH protein from the membrane. After the purification with $(NH_4)_2SO_4$, immobilization onto CNBr-activated Sepharose 4B was performed at 20˚C for 2 h. β-alanine was added as a blocking reagent to induce a negative charge onto the surface as shown in (Fig. **1**). It is known that glycocalyx at the intestinal epithelium microvilli consists of glycoprotein containing acid mucopolysaccharides, such as sialic acid, uronic acid and so on [25]. Therefore, βAla-*i*AGH may be a good tool to mimic membrane-bound AGH or to evaluate AGH catalysis in the small intestine. *i*AGH activity was then assayed as follows. The *i*AGH support (10 mg-wet gel) put in an end-capped column with 45-90 mm of polyethylene. The assay commenced after the addition of 1.0 ml of the model intestinal fluid containing 10 mM maltose or 45 mM sucrose into the column. After incubation (rotating cultivator, 4 rpm) at 37˚C for 30 min (maltase assay) or 60 min (sucrase assay), the reaction was stopped by filtration of the solution. Activity was determined by measuring the liberated glucose from maltose or sucrose in the filtrate. Inhibitory activity was estimated by the difference in the amounts of glucose in the filtrate with or without inhibitor. The concentration of AGH inhibitor required to inhibit 50% of the AGH activity under the assayed condition was defined as the IC_{50} value. The prepared AGH support possessed a 55 mU-maltase activity and 16 mU-sucrase activities. In an actual *i*AGH assay, AGH (maltase) inhibitory activity of acarbose estimated by *i*AGH assay was 1/30- to 1/40-fold lower than that by free AGH assay (IC_{50} = 11 nM) (Table **2**). Consequently, inhibition power of an AGH inhibitor may vary greatly depending upon whether the AGH was in a free or immobilized state. Odaka *et al*. [26] reported that the ED_{50} value of acarbose, was 14.6-fold higher than that of voglibose after the oral administration of sucrose in Sprague-Dawley (SD) rats (ED_{50}: acarbose; 5.42 mmol/kg, voglibose; 0.37 mmol/kg). In this study, the *in vitro* IC_{50} value of acarbose (890 nM) by free AGH-sucrase assay was estimated to be 125-fold higher than that of voglibose (7.1 nM) (Table **2**). This revealed that *in vitro* free AGH inhibitory assay system gave little information on *in vivo* suppression effect of glucose absorption. On the other hand, under the βAla-*i*AGH assay system, the ratio of IC_{50} values of acarbose against voglibose was found to be 19.4 (IC_{50}: acarbose; 1,200 nM, voglibose; 62 nM), and almost agreed with the ED_{50} ratio of 14.6. Based on this finding, the present βAla-*i*AGH assay system appeared to mimic *in vivo* membrane-bound AGH hydrolysis reaction in the small intestine. A conflicting result was reported by Kim *et al.* [27], which showed that luteolin had a strong AGH inhibition rather than acarbose. However, according to our *i*AGH assay experiment, the inhibitory activity of luteolin (IC_{50}; 2.3 mM) was lower than that of acarbose [28]. In addition, luteolin does not produce a significant change in the blood glucose level [28], supporting the validity of our proposed *i*AGH assay system.

INHIBITION OF GLUCOSE PRODUCTION IN THE GUT BY NATURAL AGH INHIBITORY RESOURCES

In a recent report on the STOP-NIDDM trial study [12], long-term acarbose treatment was effective in borderline subjects, indicating that an appropriate postprandial BGL control by AGH inhibitors seems to be of benefit for preventing the development of hyperglycemia. (Table **3**) lists the natural AGH inhibitors evaluated by *i*AGH assay. The

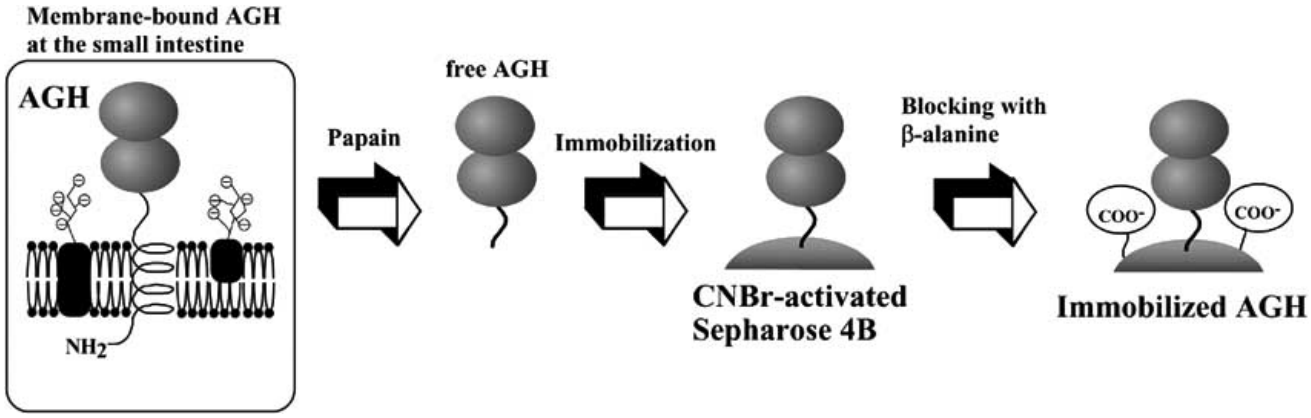
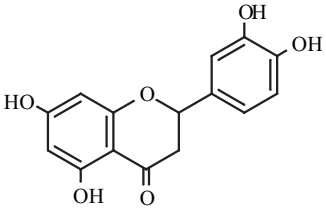


Fig. (1).

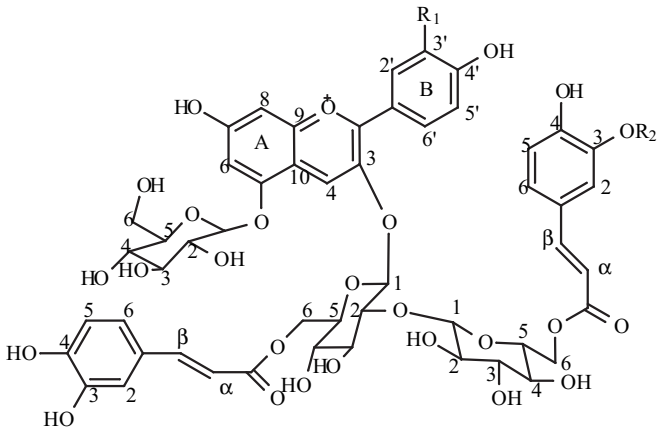
Table 2. AGH Inhibition Profile of Synthetic Inhibitors Evaluated by Free and Immobilized AGH Assay Systems

	IC50 (nM)		IC50 (nM)		IC50 (mM)
	voglibose		acarbose		luteolin
Assay system	maltase	sucrase	maltase	sucrase	maltase
Free AGH	8.9	7.1	11	890	Not measured
Immobilized AGH	5.5	62	430	1200	2.3

Structure of luteolin



*i*AGH inhibition study demonstrated that even a natural plant extract shows an apparent AGH inhibitory potential. postprandial BGL rise from carbohydrates in borderline NIDDM subjects. As shown in (Fig. **2**), colored plants are



	R_1	R_2	IC_{50} = (μM)
SOA-4	H	Glc	60
SOA-6	H	H	107
YGM-3	OH	Me	193
YGM-6	OMe	Me	200
Pg3S5G			4610
Pn3S5G			14100
Cy3S5G			18200

Fig. (2).

The listed plant extracts are specific inhibitors against maltase, not against sucrase or both [29-32].

Since sucrase is not directly involved in the dietary BGL rise, an inhibition of glucose production from maltose or isomaltose seems to be of great benefit for controlling a good resources for providing AGH inhibitors. Anthocyanins and their derivatives have a potential for retarding the action of intestinal maltase. Such compounds includes; SOA-4 (pelargonidin (Pg) 3-*O*-(2-*O*-(6-*O*-(*E*-3-*O*-(β-D-glucopyranosyl) caffeoyl (Caf))-β-D -glucopyranosyl)-6-*O*-*E*-Caf-β-D-glucopyranoside)-5-*O*-β-D-glucopyranoside) from

Table 3. AGH Inhibitory Activity of Some Natural Resources by Using *i*AGH Assay System

	IC50 (mg/ml)	
Extract	maltase	sucrase
Morning glory	0.17	NI
Purple sweet potato	0.26	NI
Green tea	0.22	NI
Propolis (50% MeOH eluate on an Amberlite XAD-2 column)	0.049	0.52
Ranawara (MeOH extract)	0.023	0.398

NI: no inhibition

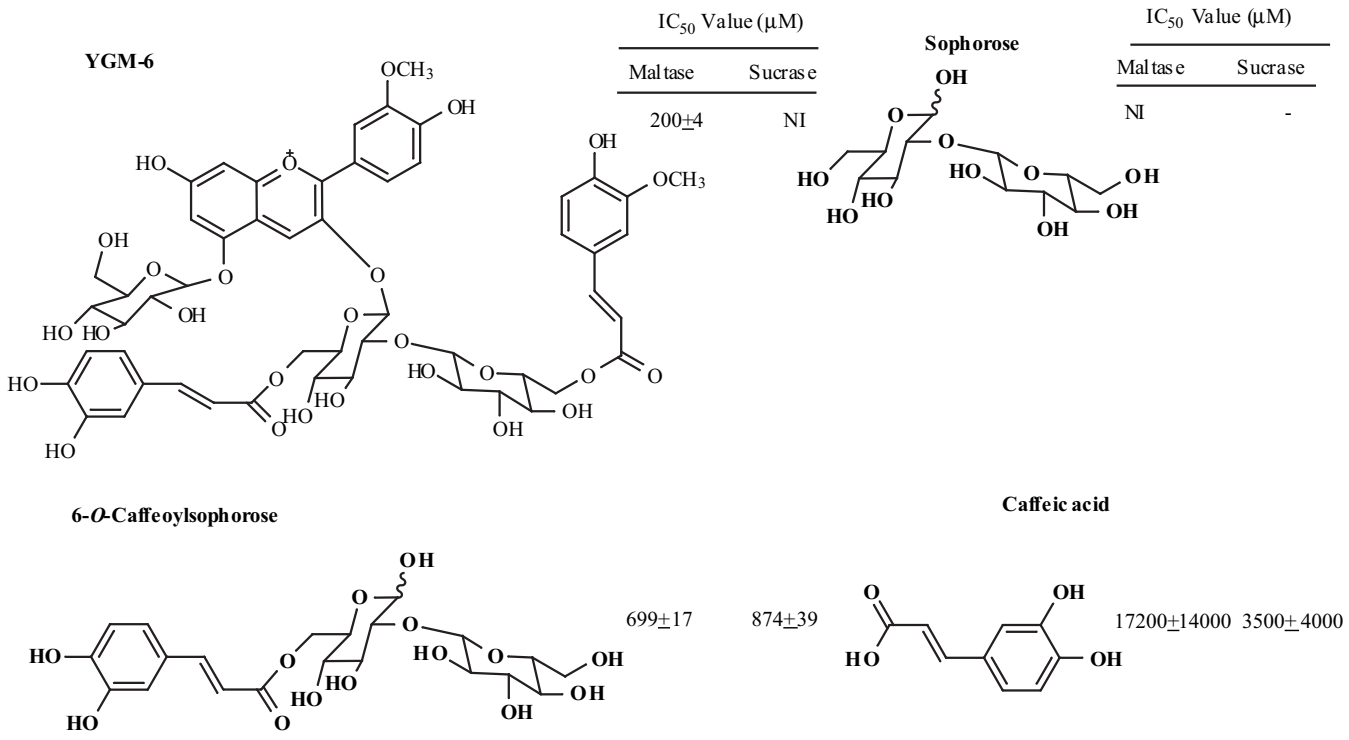


Fig. (3).

Phabilis nil cv Scarlet O'Hara (IC_{50}; 60 μM); and YGM-6 (peonidin (Pn) 3-*O*-(2-*O*-(6-*O*-*E*-feruloyl (Fer)-β-D-glucopyranosyl)-6-*O*-*E*-Caf-β-D-glucopyranoside) -5-*O*-β-D-glucopyranoside from storage roots of purple sweet potato *(Ipomoea batatas* cv Ayamurasaki) (IC_{50}; 200 μM)) [29, 30]. Structure-activity relationship indicated that in any case, acylation of anthocyanin must be important in the expression of *i*AGH (maltase) inhibition. For sucrase inhibition study, esterified catechins such as epicatechin gallate exhibited stronger activity than the non-esterified ones [33]. Similar AGH inhibitory expressions by acylation were observed in sulfoquinovosyldiacylglycerol from edible brown algae [34]. Therefore, the stronger potent *i*AGH inhibition of acylated SOA and YGM anthocyanins than the corresponding deacetylated ones would be due to a higher affinity with the enzyme. Among the three deacylated anthocyanins, Pg-based anthocyanin (Pg3S5G) showed the most potent maltase inhibition with an IC_{50} value of 4.6 mM, and the effect was in the descending order of potency of Pg3S5G > Cy3S5G ⇌ Pn3S5G (Table **3**). Interestingly, the order was almost similar with the case of acylated anthocyanins (SOA-4 (Pg type) > SOA-6 (Pg type) > YGM-3 (Cy type) ⇌ YGM-6 (Pn type)). Therefore, these findings strongly suggested that maintaining the 3' (5')-position of the B-ring would be essential for *i*AGH action.

Further experiment was carried out to clarify the structural factors attributable to the powerful *i*AGH (maltase) inhibition of diacylated anthocyanins. As described above, a potent *i*AGH (maltase) inhibition capability was elevated by acylated anthocyanins with phenolic acids, but not by their aglycons. As shown in (Fig. **3**), only an acylated moiety (6-*O*-caffeoylsophorose, CS) [35] of diacylated anthocyanin (YGM-6) was found to retain some degree of maltase inhibitory activity [36], as YGM-6 did inhibit maltase. 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 was also reported for esterified catechins from tea polyphenols [33] as well as sulfoquinovosyldiacylglycerol [32]. Thus, the *i*AGH inhibition study of phenolic acids was carried out to ascertain the potential inhibition capability of CS. As summarized in (Fig. **4**), among the phenolic acids, but with the exception of caffeic acid derivative (*i.e.* chlorogenic acid; 3-caffeoylquinic acid), inhibition behavior against *i*AGH were observed for caffeic acid [36]. This result indicates that substitution of the hydroxyl group of 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 *i*AGH (maltase) inhibitory activity of chlorogenic acid (IC_{50}; 18.9 mM) was much lower than that of CS (IC_{50}; 699 μM), the sugar moiety was an alternative candidate for maltase inhibition. For caffeic acid analogs (Fig. **4**), the inhibition activity was in the descending order of caffeic acid> 3,4-dihydroxyphenyl acetic acid> dihydrocaffeic acid >> protocatechuic acid. The markedly reduced maltase inhibitory activity of protocatechuic acid than that of other caffeic acids suggests that the longer, unsaturated alkyl chain of phenolic acid is a requirement for potent maltase inhibition. The fact that the maltase inhibitory activity of gallic acid is higher than that of protocatechuic acid reveals that a multi-substitution of the hydroxyl group on the aromatic ring seems favorable for eliciting maltase inhibition.

As shown in (Fig. **5**), 3,5-di-*O*-caffeoylquinic acid (3,5-di-CQA), 3,4-di-*O*-caffeoylquinic acid (3,4-di-CQA), and 3,4,5-tri-*O* -caffeoylquinic acid (3,4,5-tri-CQA) were found to be an AGH inhibitor for the first time. Potent and

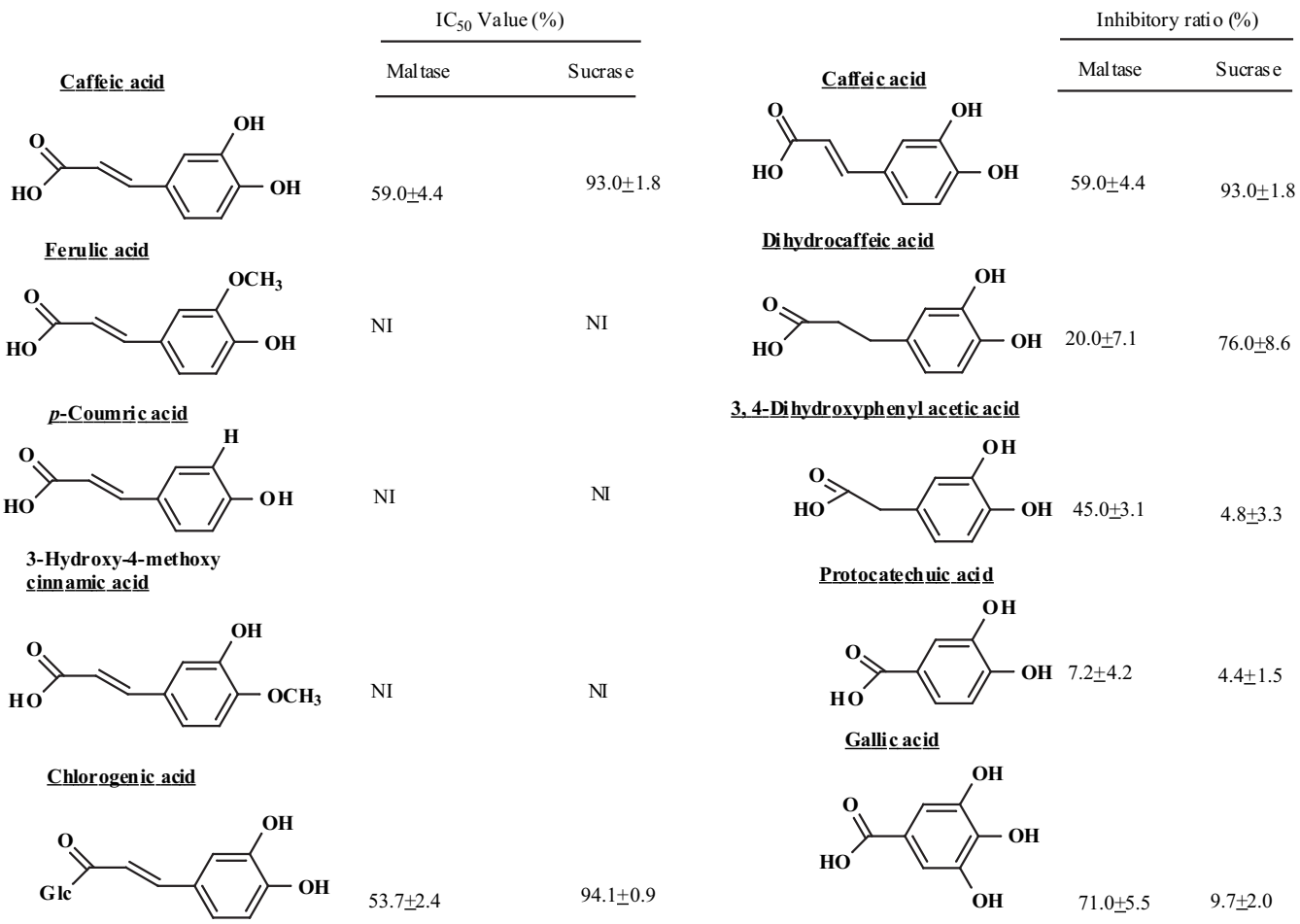


Fig. (4).

preferential maltase inhibition was observed only for 3, 4, 5-tri-CQA (IC_{50}, 24 μM) among the CQA family, being comparable to that of acarbose. Although chlorogenic acid (3-CQA) acts as an antagonist of the intestinal glucose transporter [37], the acid had little influence on intestinal AGH. Caffeic acid or quinic acid alone did not show any potent *i*AGH inhibitory activity; 3-CQA and two di-CQAs showed significant activity, but they were less active than tri-CQA. These findings clearly indicate that the caffeoyl group plays an important role in exerting *i*AGH-inhibitory activity and that an increasing number of caffeoyl group enhances the maltase-inhibitory activity.

The Lineweaver-Burk plots for the hydrolysis of maltose by *i*AGH (maltase) in the presence or absence of the natural inhibitors including diacylated anthocyanins, CS and tri-CQA (e.g., tri-CQA in (Fig. **5**), gave rise to an interesting result. The inhibition potentials towards maltase were noncompetitively (e.g., Ki of tri-CQA; 21 μM). A noncompetitive inhibitory action has been a feature in other natural AGH inhibitors such as fructose [38], and thio-fructofuranoside [39]. As Hauri *et al*. [23] had reported, sucrase and isomaltase form a dimeric complex at the intestinal membrane, into which only the C-terminal of the isomaltase subunit is anchored, and the sucrase subunit occurs as a complex form with the isomaltase subunit. Thus, one reason why they showed maltase-specific action would be due to their binding to the sucrase subunit region in the sucrase-isomaltase complex.

ANTIHYPERGLYCEMIC EFFECT OF NATURAL *I*AGH INHIBITORS

The natural inhibitors mentioned above were tested for their ability to effect a change in the BGL (*in vivo* acute antihyperglycemic effect). For instance, a significant reduction of glycemic response ($P<0.01$ *vs*. control) with a dose of 100 mg/kg of CS to SD rats at 30 min ($BGL_{cs\text{-}30min}$; 153.0±6.0 mg/dl) was observed against the control rats that ingested maltose alone ($BGL_{control\text{-}30min}$; 172.2±2.8 mg/dl) [36]. This demonstrates that even a natural inhibitor exerts a latent physiological capability for postprandial antihyperglycemic effect. The area under the curve ($AUC_{0\text{-}120min}$) for CS ingestion (100.6±6.7 mg•h/dl) also showed a significant reduction of 22.9% compared with that of the control (130.5±4.8 mg•h/dl). CS at a dose of 100 mg/kg significantly reduced ($P<0.05$ vs. control) the serum insulin level after 30 min ($insulin_{control\text{-}30min}$; 6.7±0.7 ng/ml of serum, $insulin_{cs\text{-}30min}$; 3.5±0.5 ng/ml of serum), supporting the notion that CS suppressed the BGL rise in SD rats by lowering the glucose absorption, and not by promoting the insulin secretion. It should be noted that no change was observed in the postprandial BGL when sucrose and glucose were used as substrate sugars ($AUC_{0\text{-}120min}$ of sucrose: control, 93.6±6.8 mg•h/dl; CS, 78.0±7.3 mg•h/dl; $AUC_{0\text{-}120min}$ of glucose: control, 103.5±8.3 mg•h/dl; CS, 114.5±13.5 mg•h/dl). These results lend credence to the fact that the antihyperglycemic effect induced by CS was restrictive to maltase inhibition, and not by inhibiting

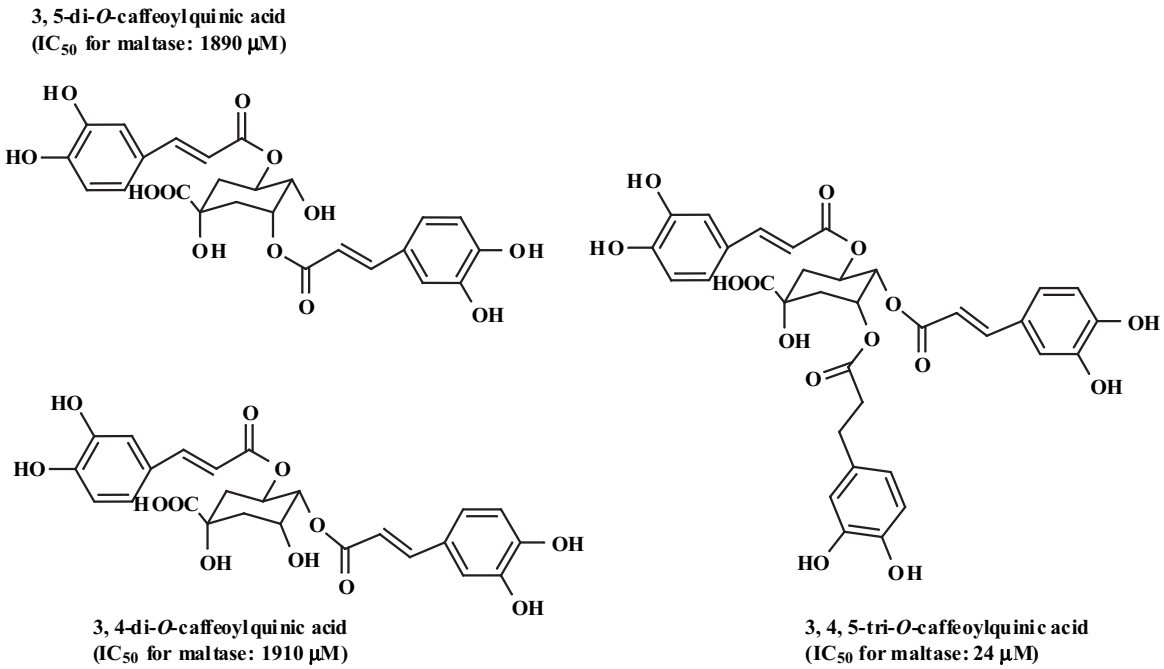


Fig. (5).

glucose transport in the small intestinal membrane *via* the Na^+/glucose co-transporter as illustrated in (Fig. **6**). Similar results were achieved with diacylated anthocyanin (YGM-6) and tri-CQA, which was restrictive to maltose-loaded rats [31, 34, 40]. Overall, both CS and YGM-6 have a much lower anti-hyperglycemic activity (ED_{20} of 117 mg/kg and 69 mg/kg respectively) than the therapeutic drug, acarbose (ED_{20}; 2.2 mg/kg) [13]. On the other hand, a 10 mg dosage of tri-CQA ($AUC_{0\text{-}120\ min}$; 100.4±4.5 mg•h/dl) was enough to lower the BGL rise when compared to that of control (130.7±8.3 mg•h/dl).

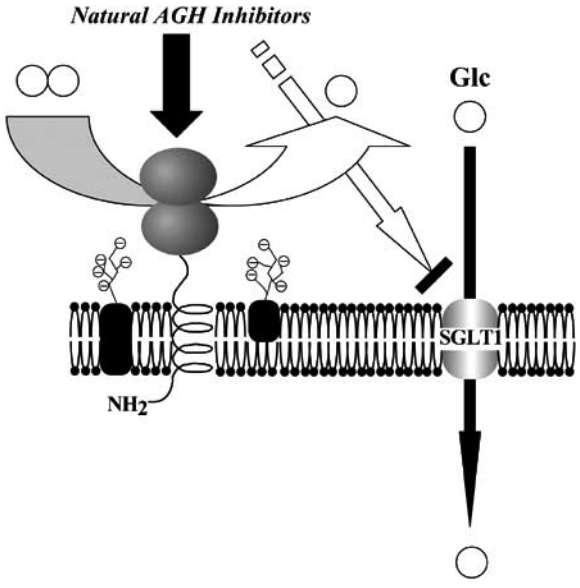


Fig. (6).

From these *in vivo* experiments of the active inhibitors, it can be deduced that (1) the antihyperglycemic effect induced by natural inhibitors seems to be weak, (2) the magnitude of the acute antihypertensive effect is dependent on the *in vitro* inhibitory potential evaluated by *i*AGH assay, and (3) the effect is achieved only by specific inhibition of intestinal membrane bound AGH, not by the promotion of insulin secretion and inhibition of glucose transporter (SLGT1). The weak antihyperglycemic effect of natural inhibitors would be, however, acceptable for preventing a postprandial BGL rise induced by 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 [12]. Evidence for improved hyperglycemia in spontaneous diabetic GK (Goto-Kakizaki) rats by slowing down the intestinal AGH activities [41] provide support for the fact that long-term AGH inhibition by any natural inhibitor would be of significant benefit to non-obese NIDDM subjects.

CONCLUSION

Natural products obtained from medicinal or non-medicinal plants may be of benefit in the prevention of Type 2 diabetes through the inhibition of intestinal AGH. Further study is required concerning safety (toxic effect) and human trial to develop an anti-hyperglycemic food.

LIST OF ABBREVIATIONS

AGH	=	α-glucosidase
*i*AGH	=	Immobilized AGH
BGL	=	Blood glucose level
IFG	=	Impaired fasting glucose
IGT	=	Impaired glucose tolerance
SLGT1	=	Glucose transporter

NIDDM	=	Non-insulin dependent diabetes mellitus
Caf	=	Caffeoyl
Fer	=	Feruloyl
CS	=	Caffeoylsophorose
SD	=	Sprague-Dawley
CQA	=	Caffeoylquinic acid
AUC	=	Area under the curve

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Received: May 26, 2005 Revised: July 11, 2005 Accepted: July 12, 2005

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